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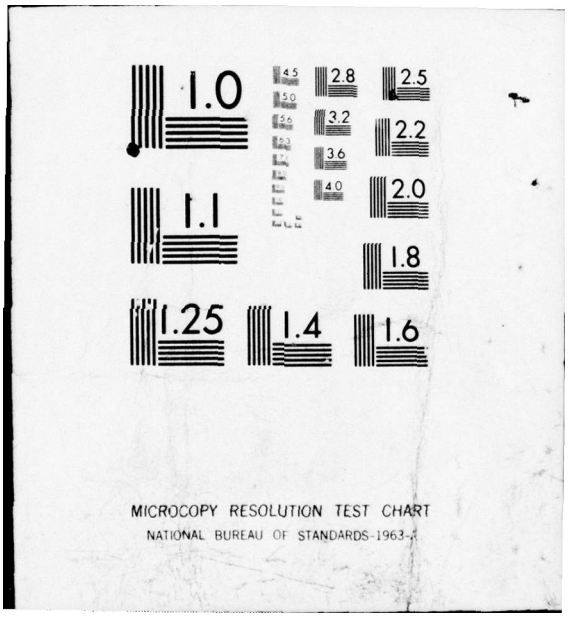
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DREDGED MATERIAL RESEARCH PROGRAM

TECHNICAL REPORT D-77-3

BIOLOGICAL ASSESSMENT OF THE SOLUBLE FRACTION OF THE STANDARD ELUTRIATE TEST

by

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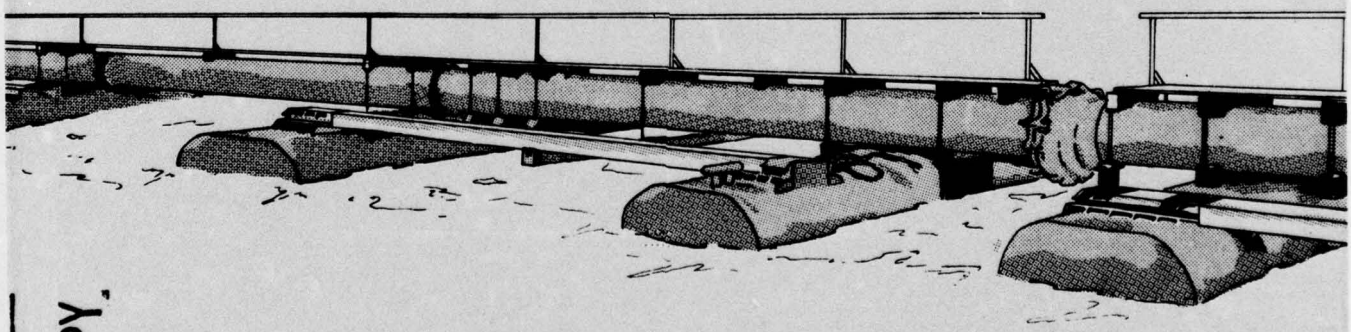

Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Under DMRP Work Unit No. 1E06

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DEPARTMENT OF THE ARMY
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IN REPLY REFER TO: WESYV

31 March 1977

SUBJECT: Transmittal of Technical Report D-77-3

TO: All Report Recipients

1. The Dredged Material Research Program (DMRP) is a broad, multifaceted investigation of the environmental impacts of dredged material disposal that includes the development of new or improved disposal alternatives. In the early stages of the DMRP, it became apparent that an understanding of the actual pollution potential of dredging and discharging sediments required substantial state-of-the-art improvement in a number of fundamental biochemical areas. The basic analytical procedure specified in Public Laws 92-500 and 92-532 for use in predicting the water column pollutional impacts of the aquatic disposal of dredged material is referred to as the standard elutriate test. Particularly critical were assessments of possible biological responses to the readily mobile and bioavailable fraction of dredged material, which is evaluated chemically in the standard elutriate test, and the potential impact of this fraction to aquatic organisms. A knowledge of these effects would further support the use of the standard elutriate test as a meaningful regulatory tool.

2. While developing and initiating the several-year-long program of relevant research, it was found that existing and proposed regulatory guidelines and criteria for dredged material discharges did not include techniques that adequately reflected an effective and implementable procedure for assessing environmental impact potential. Provided an opportunity to help direct the criteria development for the recently promulgated regulatory programs, the DMRP initiated research to develop biological as well as chemical evaluative procedures to assess the bioavailability and mobility of constituents from contaminated dredged material and project their effects on the ecosystem.

3. The technical report transmitted herewith represents the results of initial and developmental laboratory biological assessments of the soluble fraction of dredged material produced through use of the standard elutriate test. This study is one of several work units included under Task 1E (Pollution Status of Dredged Material) of the DMRP; in the DMRP's management structure, it is included as part of the Environmental Impact and Criteria Development Project.

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4. This report discusses the use of selected species of algae, bacteria, and protozoans as test organisms to evaluate the possible stimulatory or inhibitory nature of the standard elutriate. Marine and freshwater species of each group were evaluated. The report evaluates the results in relation to water-quality criteria and predicted field impacts. Sediments used for this investigation originated from Bridgeport Harbor, Connecticut; Ashtabula River, Ohio; Galveston Harbor, Texas; and Arlington Channel of Mobile Bay, Alabama. The corresponding disposal sites were Eatons Neck, Long Island Sound, New York; Lake Erie, near Ashtabula, Ohio; Gulf of Mexico, near Galveston, Taxas; and an open-water disposal site adjacent to Arlington Channel in Mobile Bay, Alabama.

5. Results of the biological assessment using algae indicated both stimulatory (Bridgeport and Galveston samples) and inhibitory effects (Arlington Channel samples) when growth in the elutriate was compared with growth in the disposal site water. The results of the bacterial and protozoan bioassays were difficult to interpret because in most cases growth media had to be added to obtain a measurable response. The algal responses showed the potential utility of the standard elutriate bioassay to assess and project the pollutional nature of dredged sediments.

6. It is recommended in this report that the algal bioassay can be used in evaluating the biological effects of the chemical constituents released from sediment and their potential effect on phytoplankton at dredged material disposal sites. Bacteria and protozoa were not useful as test organisms in evaluating the ecological effect of dredged material discharges. It is further recommended that additional water-column bioassays using selected zooplankton species should be initiated and developed and that benthic bioassay development should be immediately initiated to determine the effects of the disposal of dredged material on benthic species as well as possible long-term effects of these operations.

7. The information and data published in this report are contributions to the further understanding of the complex nature of sediment, water, and chemical/biological interactions and establish a baseline from which to develop meaningful regulatory criteria. It is expected that the methodology employed in this study and the resultant interpretation of the biochemical interactions will be of significant value to those persons concerned with CE dredged material permit programs.

JOHN L. CANNON
Colonel, Corps of Engineers
Commander and Director

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This report presents some of the results obtained on the bioevaluation of the standard elutriate test developed by the U. S. Environmental Protection Agency and the Corps of Engineers.

Selected species of algae, bacteria, and protozoans were used as test organisms. Marine and freshwater species of each group were tested. Sediment

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samples were collected from four locations that are periodically dredged and water samples were collected from their corresponding disposal sites. Sediment locations were Bridgeport Harbor, Bridgeport, Connecticut; Astabula River, Astabula, Ohio; Galveston Harbor, Galveston, Texas; and Arlington Channel of Mobile Bay, Mobile, Alabama. The corresponding disposal sites were Eatons Neck, Long Island Sound, New York; Lake Erie, near Ashtabula, Ohio; Gulf of Mexico, near Galevston, Texas; and an open-water disposal site adjacent to Arlington Channel in Mobile Bay, Alabama.

The results of algal bioassays indicated both stimulatory (Bridgeport and Galveston samples) and inhibitory effects (Arlington Channel samples) when growth in the elutriate was compared to growth in disposal site water. All sediments used to prepare standard elutriates released large quantities of ammonium-nitrogen. Growth studies were conducted using the marine alga Dunaliella tertiolecta exposed to the concentrates of ammonia found in the elutriates. The concentrations used were not toxic to the test organisms.

The results of bacterial and protozoal bioassays were difficult to interpret because in most cases growth media had to be added to obtain a measureable response. The addition of media may have masked any potential effect of chemical constituents released from the sediment.

The results are discussed in relation to water quality. Algal bioassays are one method of assisting the evaluation of the suitability of a particular dredged material for disposal. Bacteria and protozoans are important in the cycling of nutrients and toxicants, but are not recommended as test organisms for water column effects. Additional research is suggested, particularly the development of bioassays using benthic organisms.

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EXECUTIVE SUMMARY

The standard elutriate test, first published in the 15 October 1973 Federal Register, was developed by members of the U. S. Environmental Protection Agency and U. S. Army Corps of Engineers as one means of assessing the potential environmental impact of the open-water disposal of dredged material. The test is initiated by 30 min of vigorous shaking of four parts of disposal site water with one part of dredged site sediment. The procedure for the standard elutriate test was later modified to specify dredged or project site water with the dredged site sediment in the 4-to-1 mixture (5 September 1975 Federal Register). The mixture is allowed to settle for 1 hr. The liquid phase is then centrifuged and filtered through 0.45-μ pore size filter. Analysis of the resultant solution, the standard elutriate, is an aid in predicting the water-soluble constituents that may be released from the sediment to the water column during disposal operations.

Early in the Dredged Material Research Program, it was decided that, in addition to the chemical constituents that were analyzed in the standard elutriate, appropriate biological testing should be conducted. This report describes the first year's preliminary developmental work (July 1974 to June 1975) in adapting or modifying existing testing methods for the biological assessment of the mixture of chemical constituents known as the standard elutriate.

Sediment was collected at three sites each from Bridgeport Harbor, Connecticut; Ashtabula River, Ohio; Galveston Harbor, Texas; and Arlington Channel of Mobile Bay, Alabama. Disposal site water was collected from the corresponding disposal sites: Eatons Neck, Long Island Sound, New York; Lake Erie, near Ashtabula, Ohio; Gulf of Mexico, near Galveston, Texas; and an open-water disposal site adjacent to Arlington Channel.

Standard elutriates were prepared from these samples, and selected species of algae, bacteria, and protozoans were used in attempting to measure biological responses such as growth, reproduction, respiration, and mortality. These organisms were selected for initial studies because

of their importance in aquatic ecosystems (such as primary production of organic matter by algae and contributions to the decomposition of organic detritus by bacteria and protozoans), as well as their overall importance in the cycling of nutrients and heavy metals.

Bridgeport Harbor Sediments

Algal bioassays were conducted using *Dunaliella tertiolecta* (no common name) as the test organism in the standard elutriates prepared with Bridgeport Harbor sediments. Three separate elutriates were prepared with sediment samples from three sites in the harbor. The results of the algal assay demonstrated that as the concentration of standard elutriate was increased, growth of algae also increased. Increased growth occurred in the three elutriates when compared to growth in the disposal site water.

Bacterial growth experiments were also conducted suing the standard elutriates prepared with the sediments from Bridgeport Harbor and a marine bacterium (MW40C). Changes in optical density were used as a measure of growth. Measurable growth did not occur in standard elutirate or disposal site water, or in any combination of the two. Growth did occur in tubes receiving nutrient additions. However, there was no difference in the amount of growth occurring in the three standard elutriates and disposal site water samples that received these nutrient additions.

The standard elutriates from Bridgeport Harbor were chemically analyzed for selected nutrients and heavy metals, and the results indicated that ammonium plus ammonia, total Kjeldahl nitrogen, total organic carbon, total inorganic carbon, manganese, and iron were released from the sediments.

Ashtabula River Sediments

Algae (*Selenastrum capricornutum*), no common name) was used as the test algae to evaluate the standard elutriates from the Ashtabula River.

There were large variations between replicate treatments during this experiment, and, because of these variations, it was impossible to determine if a significant difference in growth occurred between the disposal site water and the standard elutriates.

Bacteria (*Caulobacter bacteriodes*, no common name) was used as a test organism with the Ashtabula River samples. Growth did not occur unless growth medium was added to the test waters. In the case of nutrient additions, there were no differences in the growth that occurred regardless of the concentration of standard elutriate or disposal site water or between the elutriates from the different collection sites. A respiration study using the bacterium BLA-1 (no common name) was conducted with the elutriate prepared from sediment collection site 1 of the 3 sites sampled. The average respiration rate of BLA-1 was reduced approximately 18 percent in 100-percent elutriate when compared to the rate in 100-percent disposal site water, exhibiting a significant inhibition.

A protozoan (*Tetrahymena pyriformis*, no common name) was the test organism used to study the effect of the Ashtabula standard elutriates and disposal site water on the survival of protozoans. Survival was very variable among the treatments and the three sites; however, the trend seemed to be survival and cell division in the elutriate and a tendency to begin dying immediately after exposure to disposal site water.

Chemical analyses showed that the sediment released ammonium plus ammonia, total Kjeldahl nitrogen, and total organic carbon. Manganese was released from the three sediments and iron was released from site 1 sediments. Low-level release of cadmium, zinc, and arsenic also occurred.

Galveston Harbor Sediments

Results of algal assays using elutriates prepared with sediments collected in Galveston Harbor showed a clear and statistically significant increase in growth of *D. tertiolecta* as the elutriate concentration

was increased over the disposal site water concentration. Growth in 100-percent elutriates was 50 to 100 times greater than growth in the disposal site water exhibiting significant stimulatory effects.

Bacterial growth experiments were conducted using changes in optical density as a measure of growth. Growth was also measured by spreading bacteria on nutrient agar plates and then placing paper disks on top of the paper. The disks had been soaked in elutriate or disposal site water. The marine bacterium MB22 (no common name) showed no difference in growth among the treatments or sediment collecting sites as measured by optical density. The growth of bacteria MB22 and MW40C (no common name) was not inhibited by the elutriate or disposal site water using the paper disk method. A respiration, or oxygen consumption, study with MB22 demonstrated no change in average respiration rate between the elutriate and disposal site water treatments. Growth medium was used in all of the bacterial experiments and the presence of the medium may have masked any differential response that occurred between treatments.

A respiration study using *Uronema nigricans*, (no common name), a marine protozoan, was conducted with elutriate from site 2 of the three sites sampled in Galveston Harbor. There was no difference in the average respiration rate among any of the test conditions. Growth medium had to be added to elicit a measurable response, so the problem of interpreting the results was complicated.

Chemical analyses indicated that the Galveston sediments released orthophosphate, ammonium plus ammonia, total Kjeldahl nitrogen, and total inorganic carbon. Manganese was released in high concentrations and low-level release of nickel and arsenic occurred.

Arlington Channel Sediments

The growth of the algae *D. tertiolecta* was inhibited as the concentration of elutriates prepared with sediment from Arlington Channel sediments was increased. Growth was better in disposal site water except for site 3 elutriate where a large amount of growth occurred in the 100-percent elutriate.

A protozoan survival assay using *U. nigricans* as the test species showed no difference in survival between the elutriate and disposal site water combinations.

Chemical analyses indicated release of ammonium plus ammonia, total Kjeldahl nitrogen, total organic carbon, and manganese.

Ammonium Nitrogen Studies

Chemical analyses showed that ammonium plus ammonia had been released by all sediments tested. It was of interest to determine the effect of these components on the test organisms. Two experiments were conducted using the algae *D. tertiolecta* and concentrations of ammonium up to 49 ppm. At the pH of the tests, approximately 3 percent of the ammonium would be the un-ionized ammonia form. It is the un-ionized form that is generally considered toxic to aquatic organisms. Toxicity was not observed whether nutrient-rich conditions (algal assay medium) or nutrient-poor conditions (aged Arlington Channel disposal site water) were used as growth medium.

Summary of Test Results

Standard elutriates were prepared from collected samples of sediment and disposal site water with selected species of algae, bacteria, and protozoans were used in attempting to measure biological responses such as growth and reproduction, respiration, or mortality. The bacteria and protozoans tested did not respond to the elutriate unless nutrients were added. Interpreting the results of these studies was therefore difficult as no clear response trends were elicited. Consequently, bacteria and protozoans are not recommended as test organisms for regulatory evaluations.

Algal species tested did respond to the test solutions and showed promise as organisms for use in the testing required to make regulatory decisions. Of the standard elutriates tested, Bridgeport and Galveston demonstrated a stimulatory effect on algal growth over that in the

disposal site water, while Arlington Channel exhibited an inhibitory effect.

Chemical analyses demonstrated a consistent release of ammonium plus ammonia, total Kjeldahl nitrogen, total organic carbon, and manganese. The chemical parameters measured in elutriates of this study could not be correlated to the observed biological response.

Interpretation of Results

The algal bioassays must be interpreted as a worst-case situation since the test were conducted under static conditions where the concentrations of water-soluble components were not diluted or mixed by water currents and dispersion as would occur at a disposal site. Since the standard elutriate is a mixture of chemical constituents, an aid in evaluation the biological response would be to compare the concentration of suspected contaminants in the elutriate to those that caused toxic or stimulatory effects to the test species as well as similar species. This requires a review of relevant literature as well as published water-quality criteria and standards.

A comparison of the observed response to the elutriate with that of the published literature may help in indicating a water-quality problem. Stimulation and inhibition of algal growth are undesirable results of disposal in most cases. When no effect is seen in the bioassay, it is a good indication that the water-soluble constituents released will not produce an effect at the disposal site. When the bioassay indicates stimulation or inhibition of growth, the potential for an ecological effect exists. This potential must then be related to conditions that exist in the field such as mixing, dilution, and turnover at the discharge site and the transitory nature of water-column effects, It is most important to emphasize that the concentration of 100-percent standard elutriate is a worst-case situation and would be rapidly diluted at the disposal site.

Recommendations

Recommendations include further algal testing. The standard elutriate and a suspended particulate phase should be tested using selected zooplankton. A research effort should be initiated to assess the biological impact of the sediment that settles out of the water column using selected benthic organisms.

PREFACE

This report describes work performed during Fiscal Year 1975 by the Ecosystem Processes Research Branch (EPRB) of the Environmental Effects Laboratory (EEL), U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. The bioassays were performed for Work Unit 1E06, "Biological Assessment of Standard Elutriate Test," under Task 1E, Pollution Status of Dredged Material, of the Dredged Material Research Program.

The principal investigators and authors of the report were Dr. Peter J. Shuba, Mr. Joe H. Carroll, and Ms. Karon L. Wong, EPRB, WES.

The study was under the supervision of Dr. Robert M. Engler, Manager, Environmental Impacts and Criteria Development Project, and under the general supervision of Dr. John Harrison, Chief, EEL.

Directors of WES during the conduct of the study and preparation of the report were COL G. H. Hilt, CE, and COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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BIOLOGICAL ASSESSMENT OF SOLUBLE FRACTION OF THE DREDGED MATERIAL ELUTRIATE TEST

PART I: INTRODUCTION

Objectives and Rationale

1. One of the objectives of the Dredged Material Research Program (DMRP) is to provide more definitive information on environmental aspects of dredging and dredged material disposal and to develop technically satisfactory, environmentally compatible, and economically feasible dredging and disposal alternatives. An area of major concern is the immediate effect of chemicals released from the suspended dredged sediments on water quality and aquatic ecology during disposal operations.

2. The development of guidelines for determining the acceptability of dredged material disposal is a major area of emphasis of the DMRP. Earlier criteria developed by the Environmental Protection Agency (EPA) in 1971, based on bulk analysis of dredged material for certain chemical constituents, have received criticism for several reasons. The disadvantages of using bulk analysis are (a) little or no correlation between the total concentration of various chemical constituents within bulk sediments subject to dredging and disposal operations and consequent effects on water quality, (b) several of the variables, most notably volatile solids and chemical oxygen demand, provide little meaningful information when applied to sediments, and (c) bulk analysis does not provide any information as to the amount of total constituents that are biologically available to organisms.

3. To avoid these disadvantages while meeting the requirements set forth in the Marine Protection, Research, and Sanctuaries Act of 1972,[1] the procedures for the standard elutriate test[2] were developed by the Corps of Engineers in conjunction with EPA to determine the pollution status of dredged material prior to disposal. The elutriate test can be used to estimate water-soluble contaminants that are released from the sediment.

4. In keeping with the objectives of the DMRP, the biological assessment work unit was established to develop techniques that will be useful in interpreting the standard elutriate test. The elutriate test provides a measure of the change in concentration of possible contaminants at the disposal site. Information is lacking which relates the release of these chemicals to their effect on biota. Specific objectives of the research were to determine the biological effects of the soluble chemicals released from sediments during dredging and disposal operations. Chemical analyses of the elutriate defined the concentration of selected nutrients and heavy metals. Biological assessment documented the response of selected test organisms to the elutriate. Correlations between chemical composition and biological response could be of value in establishing criteria for the disposal of dredged material.

5. It was of interest to predict the effect of soluble chemicals released during disposal of dredged material on microbial communities. Representative species of microorganisms were selected as test organisms to serve as biological indicators in the development of analytical techniques. Microbes are abundant in most aquatic environments[3] and it is possible that an inhibitory or stimulatory effect on one or more of their biological functions would provide information useful in predicting an effect on other organisms in the ecosystems.

6. Microorganisms are important members of aquatic ecosystems. Algae are primary producers converting carbon dioxide to organic cell material that is introduced into the food chain when algae are used as food by higher trophic levels, or upon their death and decomposition. Algae produce large quantities of oxygen for use in respiration by members of the ecosystem. Problems arise in many water supplies because of the smell or taste resulting from algal blooms.[4] Procedures employing algae to assess the nutrient status of fresh and salt water are established and generally accepted.[5,6]

7. In relation to dredged material, the contaminants released from the sediments during disposal are present in the water for only short periods of time because of mixing and diffusion at the disposal

site. Microorganisms in the water column grow and reproduce at rapid rates as compared with other organisms. Therefore, the microbes may be affected to a greater degree by the short exposure and also may serve as an important entrance of nutrients and toxicants into the aquatic food web.

8. Bacteria and protozoans play important roles as decomposers and are responsible for the degradation of large amounts of organic compounds in the ecosystem. Bacteria have an important role in the cycling of elements such as carbon, nitrogen, sulfur, phosphorus, and iron.[7] Microorganisms serve as the foundation of the aquatic food chain; bacteria are food for protozoans while algae and protozoans are food for higher trophic levels.

9. Microorganisms exhibit rapid generation allowing many cells to be obtained in a short period of time. Any biological response that may occur is detected relatively quickly. Additionally, microorganisms can be handled in a small space with a minimum of equipment.

Literature Review

10. Bioassay has been defined as "any test in which organisms are used to detect or measure the presence or effect of one or more substances or conditions."[8] Alderdice stated there are three parts to a bioassay: (a) a stimulus, such as a drug, insecticide, or industrial waste; (b) a subject which may be a cell, a tissue, or a total organism; and (c) the subject's response.[9]

11. Many different organisms have been used as test species to determine the response of interest. Bioassays originated in the field of pharmacology but have been used for many different purposes including determining the nutrient status of natural bodies of water, predicting the potential pollution status of various organic and inorganic compounds, and establishing water-quality standards.

12. Few bioassay studies exist in the literature concerning dredging and dredged material disposal. Two general types of bioassays are of interest in relation to these topics. The first is concerned with

water column effects and uses plankton and nekton. The second type is concerned with the sediment phase and uses benthic epifauna and infauna.

13. The EPA has published extensive reviews of bioassay literature.[10,11] As of 1971, the volumes contain original data from over 1000 technical publications (over 2000 papers) concerning the effects of various chemicals on aquatic biota. The bioassay organisms included freshwater and saltwater species of crustaceans, fish, molluscs, and algae. Chemicals included pesticides, industrial organics, and heavy metal salts. Responses measured included stimulation or inhibition of growth, mortality, and reproduction. The concentration of the compounds that caused the response was usually determined by graphical analysis of the data.

14. The data reported in References 10 and 11 are concerned with experiments in which the chemicals were added to water (usually some form of "natural water") at various concentrations with observations being made of the effect on the organism. The levels causing the effect were usually based on the response of a single biological species to a soluble chemical in an aquatic environment. Little or no data were given concerning sediment toxicity and bioassays with benthic organisms.

15. The EPA has published "Proposed Criteria for Water Quality," Volumes I and II.[12,13] Volume I lists the maximum acceptable level of various chemicals in fresh water, marine water, recreational water, and other aquatic environments. Volume II lists the concentration of heavy metals found in selected U. S. waters and suggested physical, chemical, and biological methods for use in water-quality determinations. The majority of bioassays used to establish these water-quality criteria were performed using fish as test organisms and were conducted using soluble chemicals in an aqueous environment.

16. There are some published bioassays pertinent to dredging operations. Emerson[14] used two species of benthic polychaetes, Ophryotrocha labronica and Capitella capitata, for biological assessment of the standard elutriate. Four sediment sampling sites were selected in Los Angeles Harbor and standard elutriates were prepared from each sediment. In addition, a series of sediment extracts were prepared

using various ratios of sediment to water from each site. Particulate matter remained in suspension during the tests. Mortality of C. capitata was less than 50 percent in all 96-hr exposures. Long-term (28 day) experiments were unsuccessful with C. capitata because of technical difficulties. O. labronica had no mortalities during 96-hr exposures. Twenty-eight-day exposures decreased reproductivity at high concentrations of resuspended sediment. Lower concentrations of resuspended sediment produced a stimulatory effect on reproduction. It was suggested that dredged material may have a role as a "sea fertilizer." Chemical data were given for the sediments but not for the elutriates.

17. Lee et al.[15] conducted bioassays using the freshwater cladoceran Daphnia magna and the saltwater shrimp Palaemonetes pugio. Elutriate preparation was modified by using different percentages of sediment (5, 10, 15, and 20 percent) and by sparging with compressed air rather than shaking. In some cases growth media were used instead of dredge site water to prepare the elutriates. The 10-percent sediment elutriate prepared from Bridgeport sediments had a toxic effect on P. pugio in 96-hr tests while elutriates from Ashtabula and Corpus Christi Harbors had little or no toxicity. Manganese was released from all sediments tested and its effect was determined using acute lethal 96-hr bioassays on D. magna and P. pugio. No effects were observed at the concentrations used with either organism. Lee recommended the abandonment of bulk analysis in favor of the elutriate test for water column effects and benthic organism bioassays for long-term effects.

18. Hoss et al.[16] used sediment extracts made from seawater and marine sediments to determine the effects of soluble compounds released from the sediments on larval fish. They found that responses varied among the seven species when any one particular site was considered, and variations occurred for the same species when the response to different sediment sites was considered. They also found that the sediment-to-water ratio used in preparing the extract was an important variable in determining survival of the larvae.

19. During dredging and disposal operations, DeCoursey and

Vernberg[17] collected water samples from a disposal area, from 183 m downstream of a dredge site, and from a dredge site and used the water to conduct bioassays on larval and juvenile zooplankton (Daphnia, Paleomonetes, and Polydora). They noted that, generally, dredge site water was least toxic to the test species; the water from 182.9 m downstream of the dredge site was intermediate in toxicity; and disposal site water was the most toxic.

20. Hendricks[18] used 0.3-M phosphate buffer at pH 7.0 to elute loosely associated chemical nutrients from river bottom sediments. The buffer eluates contained protein, ammonium-nitrogen, and hexoses in concentrations 4 to 6 times greater than those found in the river water. Attempts to elute these nutrients from the sediment with river water produced no measureable increase in eluate concentrations. Respiration rate studies demonstrated that the pathogenic and nonpathogenic enteric bacteria used as test organisms had increased respiration rates in the phosphate eluate when compared with the rates in river water.

21. Gannon and Beeton[19] conducted bioassays on dredged material from Great Lakes harbors. They used benthic fauna (Pontoporeia, Gammarus, and Chironomus larvae), Daphnia, native zooplankton, native phytoplankton, and Cladophora. Sediment selectivity, benthos viability, and algal uptake of carbon-14 labeled carbon dioxide were among the assay methods used. Algal assays using direct counts and light scattering were unsuccessful because the algal cells clumped with the sediment. Cladophora experiments failed because the algae did not grow without the addition of soil extract.

22. For the bioassays using carbon-14 uptake, sediment "extracts" were used by Gannon and Beeton[19] rather than suspended sediments. Cell numbers were not determined. An increased incorporation of carbon-14 into algal cells was observed during a 4-day period. This was interpreted as a stimulation of algal growth. If this were true, the population would increase, resulting in an increase in the rate of incorporation of carbon-14. Lee and Plumb[20] point out that when the carbon-14 data are corrected for time of contact, the algal photosynthetic activity decreased. However, the data did indicate in many cases that as

the percentage of sediment extract was increased, the amount of total carbon-14 taken up also increased.

23. Gannon and Beeton[21] have recommended the use of the sediment selectivity and benthos viability tests they have devised. While their data demonstrate that the test organisms did prefer certain sediments over others, there were no clear-cut correlations between chemical or physical characteristics of the sediment and selection by the organisms. It is interesting to note that Pontoporeia affinis selected sediments from open-water areas containing high proportions of sand. The organisms used for this study were collected from an open-water area where the sediments had a high percentage of sand. It is possible that the organisms simply chose sediments to which they were accustomed and, under other conditions, could easily adapt to different sediment types. It is possible that certain sediments were not selected because these sediments did not contain suitable nutrients for P. affinis including a native bacterial flora which these organisms prefer. The benthos viability studies suffered from a lack of dissolved oxygen measurements. As stated by Gannon and Beeton, the possibility exists that the high oxygen demand of some sediments may have caused the death of test organisms, rather than any toxic materials that may have been present in the sediments.

24. Bryan and Hummerstone[22] used estuarine sediments that contained high concentrations of heavy metals to determine toxicity levels for the polychaete Nereis diversicolor. The data for copper indicated that the concentrations in the worms were, in general, related to the concentrations in the sediments. The sediments containing high mean concentrations of copper had polychaetes that also contained high mean concentrations. The concentrations of zinc, lead, manganese, and iron in the worms were relatively constant regardless of the concentrations in the sediments. They suggested that the organisms may have regulatory mechanisms for zinc, lead, manganese, and iron, but not for copper. These mechanisms would exclude the accumulation of zinc, lead, manganese, and iron by the organisms, but copper could accumulate in concentrations that would be related to sediment concentrations.

PART II: MATERIALS AND METHODS

Sampling Locations

25. Four harbors and four disposal sites were sampled for sediments and water to prepare elutriates for use in the biological assessment studies. Sediment samples were collected from Bridgeport Harbor, Bridgeport, Connecticut (Figure 1); Ashtabula River, Ashtabula, Ohio (Figure 2); Galveston Harbor, Galveston, Texas (Figure 3); and Arlington Channel of Mobile Bay, Mobile, Alabama (Figure 4). Sediments were collected from three different sites within each location, and a minimum of three core samples were taken at each site. Cores were collected using a modified Wildco-Ballcheck Oceanographic Core Sampler (Wildlife Supply Co., Saginaw, Michigan) containing a 61 cm long plastic core liner with an 8.1-cm internal diameter. The liner was removed from the core sampler immediately after the sample was taken, capped to prevent any sediment loss, and then placed in an insulated barrel containing ice. The sediment samples were kept on ice until they were returned to the laboratory.

26. Water samples were collected from the corresponding disposal sites. These were Eatons Neck, Long Island Sound, New York; Lake Erie, near Ashtabula, Ohio; Gulf of Mexico, near Galveston, Texas; and an open-water disposal site adjacent to Arlington Channel in Mobile Bay, Alabama. A Van Dorn sampler was used and a composite water column sample was obtained by mixing water collected a few centimetres from the bottom, midway in the water column and just below the water surface. The samples were transported in plastic 18.9-ℓ bottles, which were refrigerated until they arrived at the laboratory. All sampling equipment that came in contact with sediment or water had been washed in a 10-percent hydrochloric acid bath and thoroughly rinsed with deionized water prior to use.

27. Core samples from an individual site within a location were mixed thoroughly in the lab by means of stirring before being used to prepare an elutriate. Each location had three sites; therefore, three separate elutriates were prepared for each location.

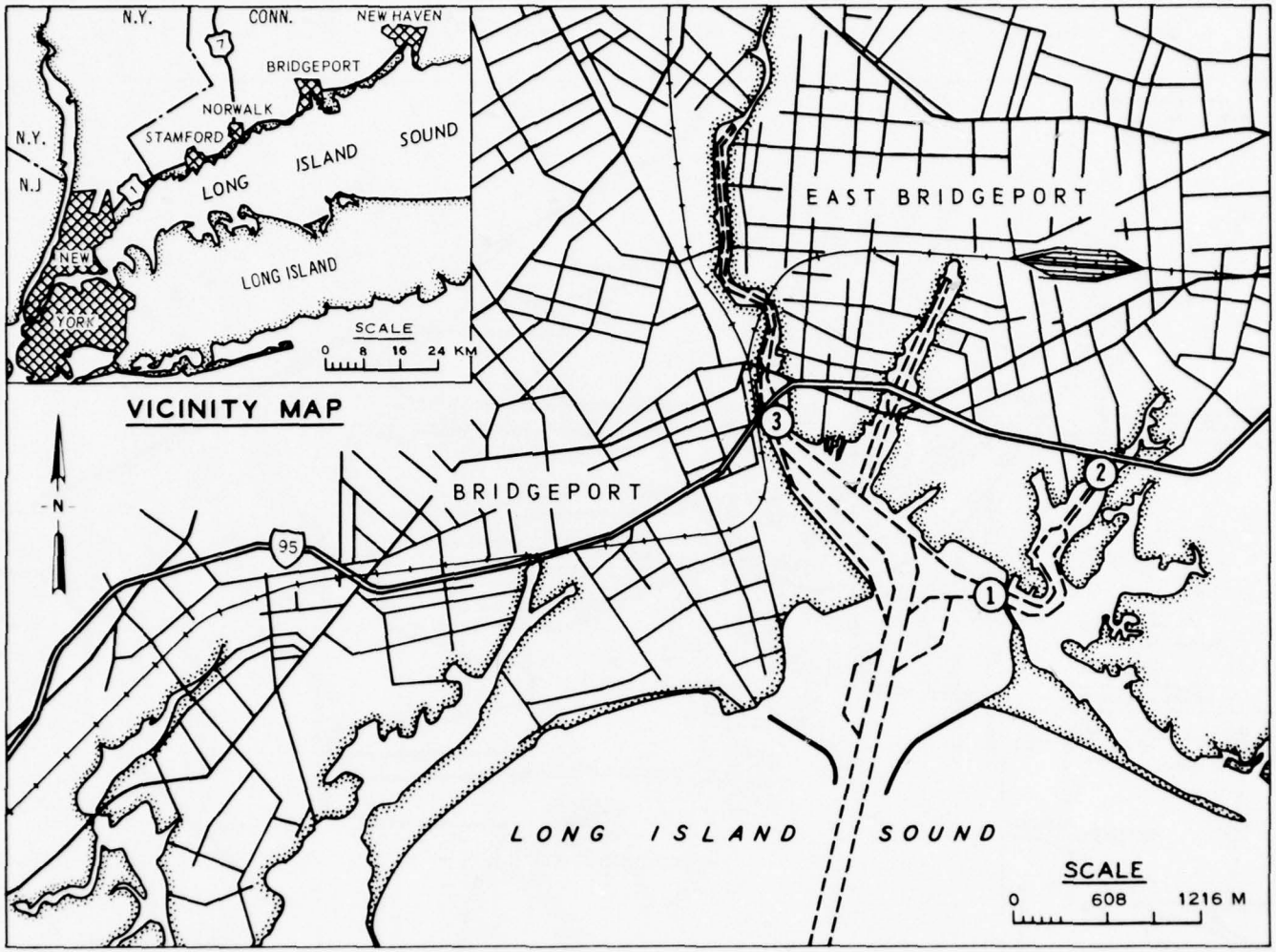


Figure 1. Sediment sampling sites (1,2,3) in Bridgeport Harbor, Connecticut

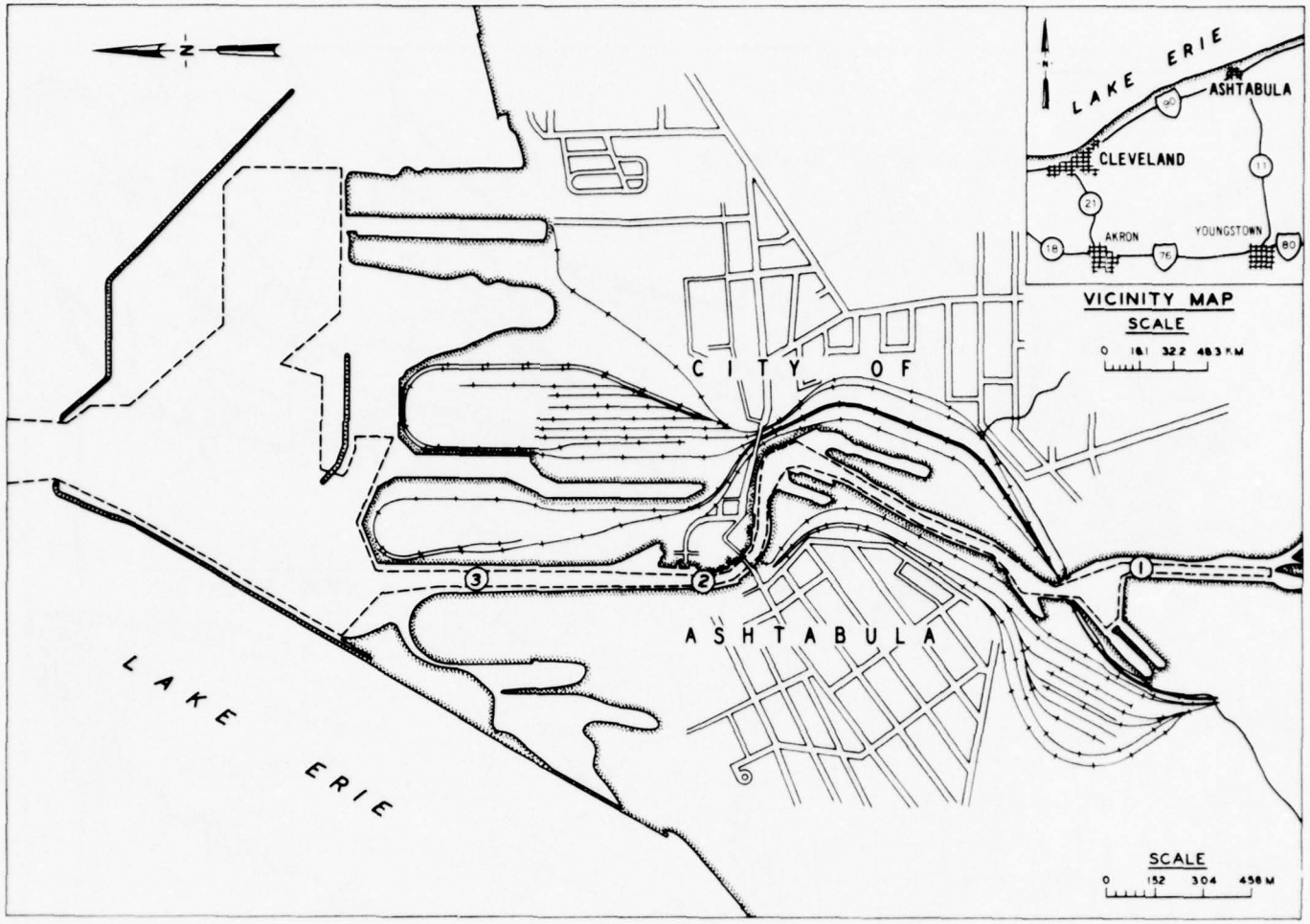


Figure 2. Sediment sampling sites (1,2,3) in the Ashtabula River Harbor, Ohio

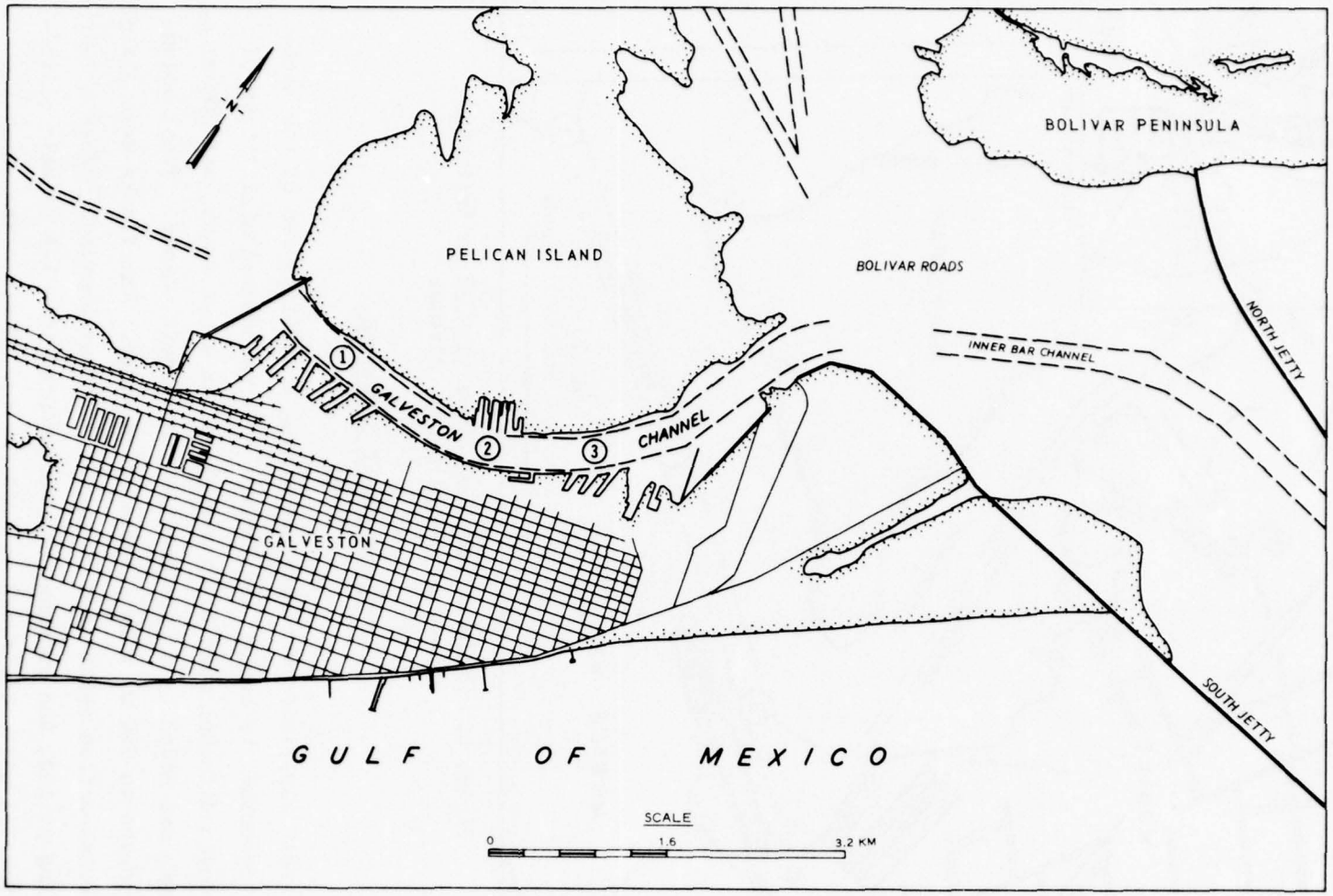


Figure 3. Sediment sampling sites (1,2,3) in the Galveston Ship Channel, Texas

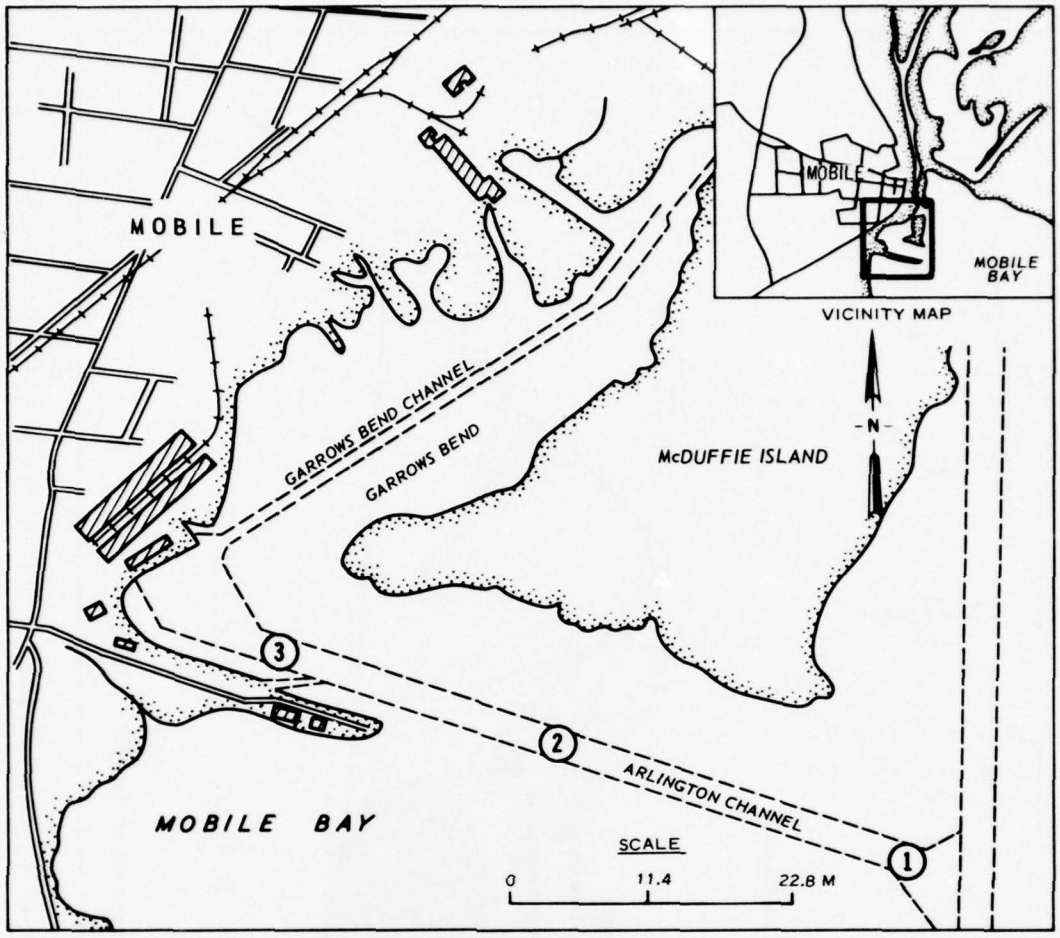


Figure 4. Sediment sampling sites (1,2,3) in Arlington Channel of Mobile Bay, Alabama

Preparation of Elutriate

28. Elutriates were prepared using a modification of the technique described by Keeley and Engler.[2] Three hundred millilitres of unfiltered disposal site water was placed in a 1-ℓ flask, and 100 ml of sediment was added by displacement of the liquid volume. Final volume was brought to 500 ml with disposal site water. The flasks were placed on a wrist-action shaker for 30 min of vigorous shaking. After a 1-hr settling period, the supernatants were poured into 1-ℓ plastic centrifuge bottles and centrifuged at 6000 rpm for 10 min. The resulting

supernatant was filtered twice. The first filtration was through 0.45-μm pore-size millipore filters; the second filtration was through sterile 0.22-μm pore-size millipore filters. The second filtrate was collected in sterile flasks to obtain an elutriate free of microorganisms. The disposal site water used to dilute the elutriates was filtered in the same manner.

Chemical Analyses

29. Disposal site water and elutriates from the three sites at each location were analyzed by the Analytical Laboratory Group (ALG) of the Environmental Effects Laboratory (EEL) at the Waterways Experiment Station (WES). Procedures and methods for chemical analyses were those described in "Methods for Chemical Analysis of Water and Waste"[23] and Standard Methods for the Examination of Water and Wastewater.[24] Nutrient analyses included ammonia plus ammonium-nitrogen (NH_3-N),* nitrate-nitrogen (NO_3-N), and nitrite-nitrogen (NO_2-N), orthophosphate-phosphorus (OPO_4-P), total organic carbon (TOC), total inorganic carbon (TIC), and total Kjeldahl nitrogen (TKN). The values listed in the tables accompanying the analyses are the concentrations for the elements of the nutrients. Heavy metal concentrations were determined for cadmium (Cd), nickel (Ni), zinc (Zn), manganese (Mn), lead (Pb), copper (Cu), iron (Fe), and arsenic (As).

Algal Assay Procedures

30. The algal assays consisted of establishing a series of treatments and controls using elutriate and filtered disposal site water. These experimental units were then inoculated with a test organism taken from a stock culture and held under a specified set of test conditions while carrying out a sampling program for evaluating effects. The algal assays for freshwater dredging and disposal sites were based on the procedures described in "Algal Assay Procedures: Bottle Test."[5] The

* For convenience, symbols and unusual abbreviations are listed and defined in the Notation (Appendix C).

assays for marine or estuarine dredging and disposal sites followed the procedures described in "Marine Algal Assay Procedure: Bottle Test."[6]

31. Selenastrum capricornutum was selected as the test algae for freshwater biological assessment studies; Dunaliella tertiolecta was used as a representative marine algae. Stock cultures of both organisms were obtained from the EPA's National Environmental Research Center, Corvallis, Oregon. Selenastrum capricornutum is a unicellular green algae, Class Chlorophyceae, Order Chlorococcales. Individual Selenastrum cells are curved and range in size from 20 to 48 μm in length and from 3 to 9 μm in width. Dunaliella tertiolecta is a green unicellular flagellate, Class Chlorophyceae, Order Volvocales. Cells are ovoid and attain a size of 5 to 8 by 10 to 12 μm with two long flagella at the anterior end.

32. Stock algal cultures were grown in synthetic nutrient media (Appendix A). Fresh cultures were started once a week by transferring 0.1 ml of a 1-week-old culture to 100 ml of fresh media using aseptic techniques. Stock cultures were grown at laboratory temperature (approximately 23°C) under continuous cool-white fluorescent lighting at an intensity of approximately 1500 $\mu W/cm^2$ while being shaken continuously at 110 rpm.

33. Culture vessels were 500-ml Pyrex Erlenmeyer flasks stoppered with polyurethane foam plugs. All glassware was washed with detergent, rinsed with tap water, placed in a 10-percent hydrochloric acid bath for a few hours, and rinsed five times with tap water and five times with distilled water.

34. Treatment levels were established using dredged material elutriate, disposal site water, and an inoculum of the test organism in 500-ml Erlenmeyer flasks with a total liquid volume of 100 ml. The following treatment levels were used:

Percent Elutriate	Percent Disposal Site Water
0	100
25	75
50	50
75	25
100	0

Controls included 100- and 10-percent synthetic algal nutrient media. Also, 100-percent disposal site water, 100-percent elutriate, and a combination of 50-percent disposal site water and 50-percent elutriate received an addition of growth media equivalent to 10 percent of the stock medium concentration. The elutriate and the elutriate to disposal site water mixtures were repeated for the three sediment sampling sites of each location.

35. Three replicates of each treatment level and control were established. The flasks were randomly distributed in two Psychrotherm incubators (New Brunswick Scientific Co., Inc.). Temperature was 18°C for marine algal assays and 24°C for freshwater assays (±2°C). Cool-white fluorescent bulbs were used to obtain constant illumination of approximately 1100 to 1300 $\mu W/cm^2$. The shaking rate was 110 rpm throughout the assays. The assays lasted from 8 to 18 days.

36. The inoculum was prepared by centrifuging and washing stock culture cells with sterile water containing 15 mg of sodium bicarbonate per litre for the freshwater algae or with sterile artificial seawater minus nutrients for the marine algae. The inoculum cell concentration was adjusted by dilution then pipetted into the test water to give an initial concentration in the test waters of 1000 cells/ml for S. capricornutum and 100 cells/ml for D. tertiolecta.

37. Growth of the test organisms, as measured by total cell numbers, was used to indicate the effect of the elutriate on the organisms. Cell numbers were monitored by periodic removal of aliquots from the test units, fixing the algal cells in Lugol's Iodine solution, and counting the cells microscopically in a Sedgwick-Rafter counting chamber using the method described in Standard Methods for the Examination of Water and Wastewater.[24] Three separate counts were averaged for each cell count measurement reported.

38. Optical density and dry weight determinations were attempted in the early phase of the project. Both methods were found to be variable at low cell numbers; these methods were not used in the later phase of the project. During the last month of the program, a Coulter Electronic Particle Size Analyzer Model TA II was obtained. This

instrument was found to be very suitable for counting cells. Cells were counted with the Coulter Counter during the experiment on the effect of added ammonium-nitrogen (NH_4-N) to Mobile Bay disposal site water.

39. Two different growth parameters were used to describe the growth of a test algae in the Bottle Test: maximum specific growth rate and maximum standing crop. Maximum standing crop, defined as the maximum algal biomass achieved during incubation, proved to be the best parameter for comparative purposes in these studies.

40. Statistical analyses of the data included analysis of variance and Duncan's new multiple-range test for comparison of the effects resulting from the combination of elutriate and disposal site water.[25]

Protozoan Assays

41. A number of protozoan assay approaches were attempted throughout the period of research. These included the drop culture method,[26] measurements of respiration rates as a function of the ratio of elutriate and disposal site water, and assays in which the survival of protozoans was determined as a function of elutriate and disposal site water concentration.

42. Tetrahymena pyriformis was the freshwater test protozoan used in the drop culture assays. T. pyriformis is a large, motile ciliated protozoan easy to observe and count under a microscope. It is easily grown, has a generation time of 3 hr, is widely distributed in nature, and has been in culture for 30 yr. The original stock culture used in this study was obtained from Dr. Ivan L. Cameron, Department of Anatomy, University of Texas Medical School, San Antonio, Texas. The stock culture of T. pyriformis was grown at approximately 24°C, in a 2 percent (w/v) proteose peptone medium containing 0.1-percent (w/v) liver extract. Stock cultures were transferred weekly.

43. The saltwater protozoan used in biological assessments of dredged material elutriates, Uronema nigricans, was supplied by Dr. A. T. Soldo, Veterans Administration Hospital, Miami, Florida. U. nigricans is a filter feeding, ciliated protozoan about 12 by 25 μm in size. This

organism has been axenically cultured and studied in detail and considerable information is available on its nutritional requirements. The growth media for maintaining stock cultures are given in Appendix A. Fresh cultures were started weekly.

44. The drop culture assay method consisted of placing washed cells of T. pyriformis in solutions of elutriate and disposal site water. After thorough mixing, a few drops of each sample were removed, placed in a plastic petri dish, and covered with a drop of mineral oil. The cells were then counted microscopically at 1, 2, 4, 8, 24, 48, 72, and 96 hr. The following treatment solutions were tested:

Percent Elutriate	Percent Disposal Site Water
0	100
25	75
50	50
100	0

Controls were 100-percent growth medium and distilled water inoculated with cells. The test units were incubated at 25°C for the test period.

45. Respirometry studies were conducted using a Gilson Differential Respirometer to determine if the standard elutriate had an effect on oxygen uptake by U. nigricans. Each test unit was inoculated with 2.5 ml of a washed 2-day-old stock culture for a final concentration or 2.5×10^5 cells/ml. Uninoculated controls were run on each of the treatment solutions to provide a background determination of oxygen uptake. Temperature during the test was 24°C (±1°C).

46. Flask assays were used to determine protozoan survival as a function of elutriate and disposal site water concentrations. Each test unit consisted of a 500-ml Erlenmeyer flask containing 100 ml of test solution inoculated with a sufficient number of organisms to provide an initial concentration of approximately 3×10^4 cells/ml. The inoculum was prepared by centrifuging, washing, and resuspending the cells in artificial seawater of the appropriate salinity. The cell concentrations were measured by counting, using a Sedgwick-Rafter counting chamber and a microscope. The treatment levels used in the protozoan

flask assays were the same as those for the algal flask assays. Controls were 100-percent artificial seawater and 100-percent growth medium. The test units were incubated at lab temperature (22 to 24°C) for a period of 6 days. Samples were initially removed 1 hr after inoculation, then at daily intervals for the duration of the test. Additional details on methods will be given when particular experiments are discussed.

Bacterial Assay Procedures

47. Various experiments were conducted to determine the effect of elutriate and disposal site water on the growth of selected bacterial species. Respirometry studies were conducted using a Gilson Differential Respirometer.

48. The marine bacteria used were designated as MW40C and MB22. Both organisms are gram-negative vibrios isolated from the Atlantic Ocean near Chesapeake Bay. These organisms were provided by Dr. Max Tyler of the University of Florida. The test species used for freshwater studies were isolated from Brown's Lake located near the WES. They were designated BL-1 and BL-2. Both were gram-positive rods. Caulobacter bacteriodes was also used as a freshwater organism. Growth media used to culture the bacteria are given in Appendix A.

49. Initial attempts to grow the bacteria in elutriate or disposal site water were unsuccessful, probably because of a lack of nutrients. Later, growth medium was added to the test units. Growth of the test organisms was then monitored by measuring the changes in optical density using a Spectronic 20 Spectrophotometer or a Klett-Summerson Photocolorimeter. Since each experiment was conducted using a different approach, additional details will be given when individual experiments are discussed.

PART III: RESULTS

Bridgeport Harbor Sediments and Eatons Neck Disposal Site Water

Physical characteristics of the samples

50. Sediment and water samples were collected on 19 October 1974. Water depth at sediment site 1 was 12 m. At the water-sediment interface the salinity was 27 ppt, temperature 15°C, and the dissolved oxygen concentration was 7.8 ppm. The sediment in the core samples had a pH of 7.1 and a temperature of 12.5°C immediately after collection.

51. For sediment site 2, water depth was 4 m, bottom salinity 26.6 ppt, temperature 14.1°C, and dissolved oxygen 8.0 ppm. Core sample temperature was 15°C and the pH was 7. At sediment site 3, the water depth was 10 m, salinity at the water-sediment interface was 27 ppt, temperature 15.5°C, and dissolved oxygen 7.8 ppm. Core sample temperature was 15°C and the pH was 6.8.

52. The composite disposal site water samples collected from Eatons Neck had a salinity of 30 ppt, temperature of 11°C, dissolved oxygen concentration of 10 ppm, and pH of 8.3.

Chemical analyses

53. Table 1 lists the concentrations of nutrients and heavy metals found in the samples from Eatons Neck disposal site water and in the elutriates prepared from the three sediment sampling sites of Bridgeport Harbor. Disposal site water was filtered through 0.45-μ millipore filters prior to chemical analyses. Elutriates were filtered as previously described.

54. The increase in nitrate concentrations in the elutriates probably represents oxidation of some of the ammonium-nitrogen that was in the elutriates since it is unlikely that the sediments released nitrates. The samples used for nutrient analyses were not acidified and remained in storage about 48 hr before chemical analyses were performed. Nitrite concentrations were essentially the same in the disposal site water and the elutriates. A slight amount of orthophosphate

Table 1

Chemical Analyses of Eatons Neck Disposal Site Water and Bridgeport Harbor Elutriates Before Biological Assessment

Constituents	Disposal Site Water	Elutriate Site 1	Elutriate Site 2	Elutriate Site 3
Nutrients (ppb):				
NO_2-N	35	25	30	32
NO_3-N	99	152	126	124
OPO_4-P	58	42	38	40
Nutrients (ppm):				
NH_3-N	<0.8	27	14	18
TKN-N	<1.0	33	17	23
TOC-C	1.0	9	6	8
TIC-C	25.0	63	26	44
Heavy metals (ppb):				
Cd	2	4	2	18
Ni	300	400	400	400
Zn	14	15	21	7
Mn	12	113	27	62
Pb	2	5	5	3
Cu	18	18	18	18
Fe	30	20	79	27
As	7	2	2	1

was adsorbed by the sediments as indicated by the decreased concentrations found in the elutriates.

55. The sediments from the three sites released large quantities of ammonium-nitrogen. Total organic carbon was also released from the three sediments and total inorganic carbon was released from sites 1 and 3.

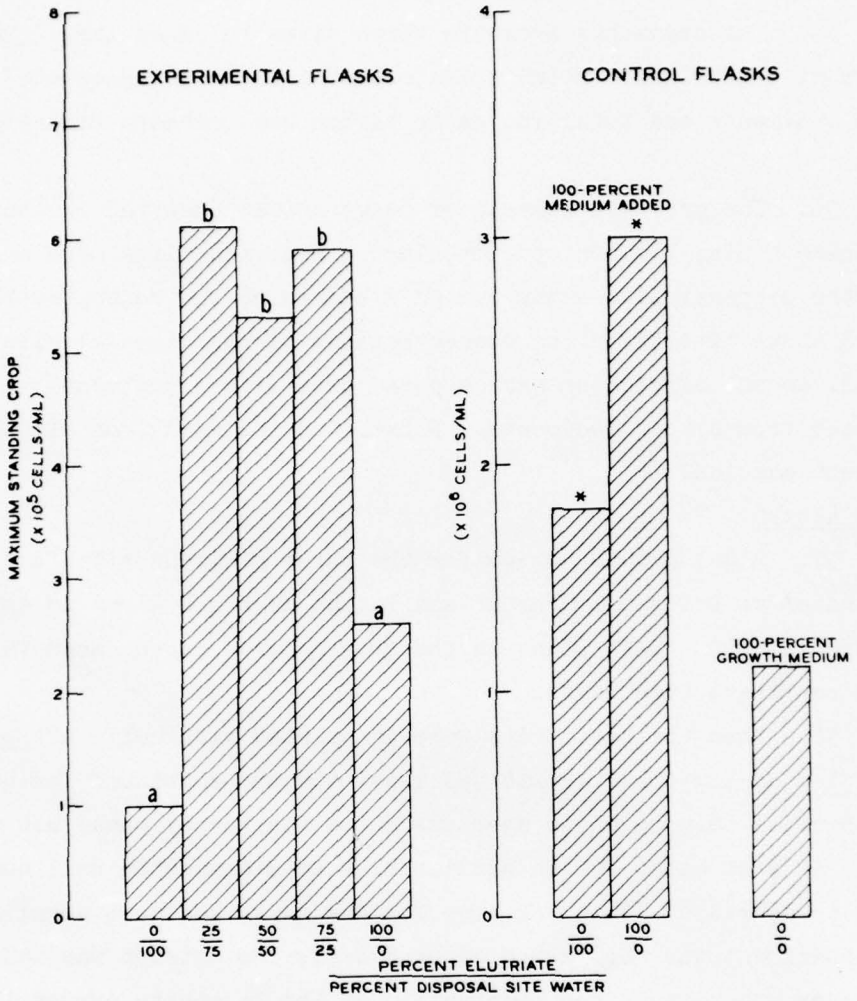
56. The greatest release of heavy metals occurred in the case of manganese. Site 1 elutriate contained almost ten times more manganese than the disposal site water. Site 2 had twice the concentrations, and site 3 about five times the concentrations of the disposal site water. A small amount of cadmium was released from site 3 sediments; iron was released from site 2 sediments. Nickel was released from all three sediment samples.

Algal assays

57. Algal growth curves for the three sediment sites and disposal site water of Bridgeport Harbor and Eatons Neck are given in Appendix B (Figures B1-B3). Each point on the growth curve is the mean value of three replicate treatments.

58. When the test units were inoculated with cells of Dunaliella tertiolecta, log-phase growth was initiated and continued for 4 days. Growth rates then began to decrease and a stationary phase was maintained in most cases for an additional 2 to 3 days when cell numbers began to decrease. This was true for the three sediment sampling sites. The experiment was terminated after 8 days. No attempt was made to calculate growth rates since observation of the log-phase curves indicated the rates were very similar in most cases.

59. Figures 5, 6, and 7 show the maximum standing crop (cells/ml) obtained for D. tertiolecta in disposal site water and elutriates prepared from sites 1, 2, and 3, respectively. Maximum growth obtained in control flasks is also illustrated. The scale given for the experimental units is 10^5 cells/ml, while the scale for the controls is 10^6 cells/ml. The data presented are the mean for three replicates of experimentals and controls. The maximum standing crop did not always occur on the same day for different treatments. This method of data



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE $P < 0.05$ LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT ($P < 0.05$) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 5. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 1 of Bridgeport Harbor and disposal site water collected from Eatons Neck

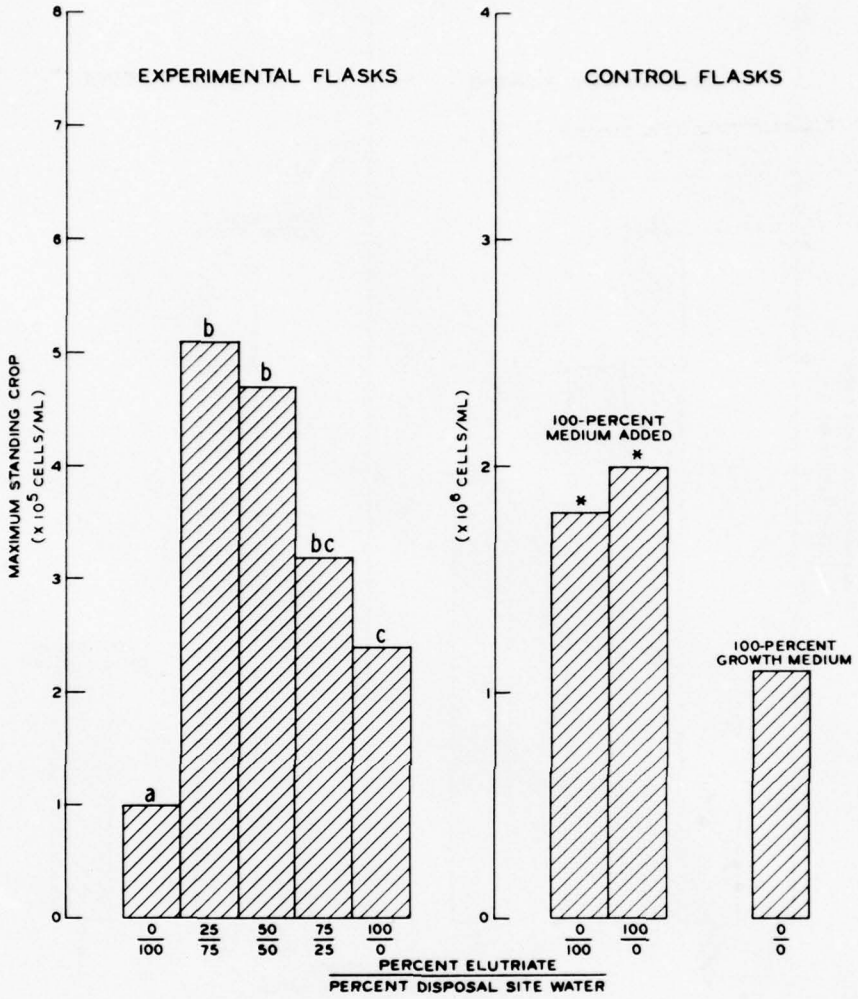


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Figure 6. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 2 of Bridgeport Harbor and disposal site water collected from Eatons Neck

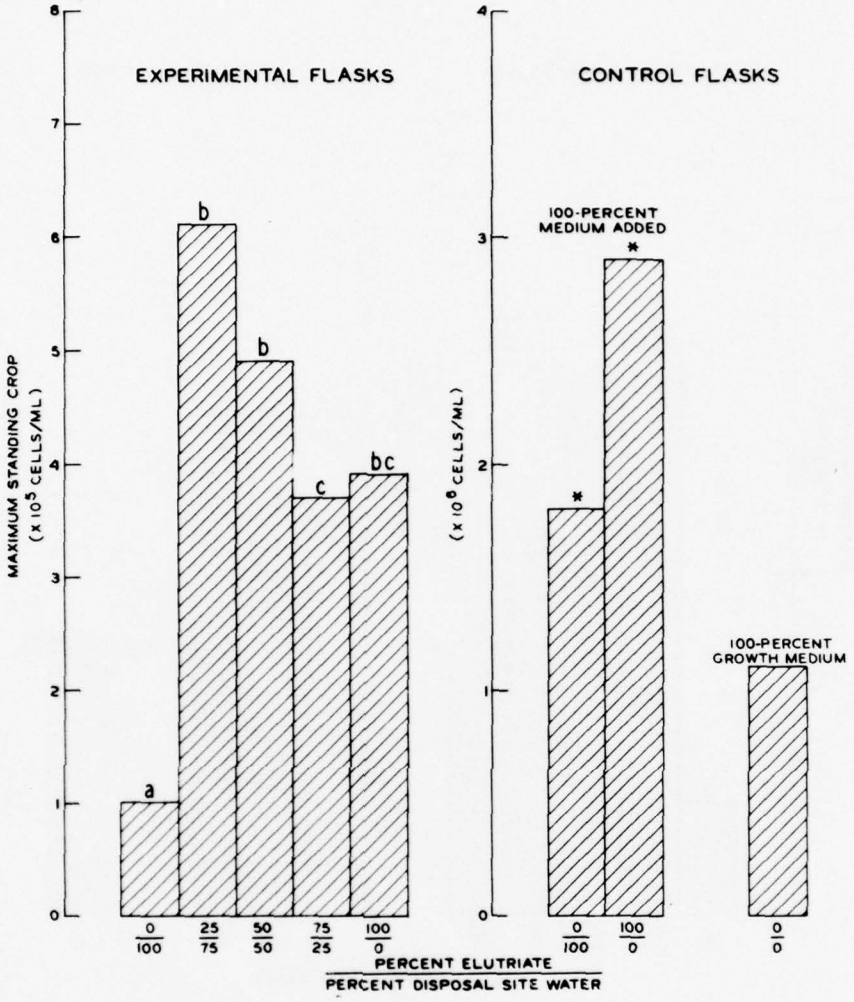

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Figure 7. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 3 of Bridgeport Harbor and disposal site water collected from Eatons Neck

presentation was used for all locations discussed in this report.

60. D. tertiolecta grew better in all combinations of elutriate and disposal site water than in 100-percent disposal site water or in 100-percent elutriate. However, as the concentration of elutriate was increased the amount of growth generally decreased. For site 1 (Figure 5), growth was better in 25-percent elutriate to 75-percent disposal site water (6.1×10^5 cells/ml) than in 100-percent disposal site water (1×10^5 cells/ml) or 100-percent elutriate (2.6×10^5 cells/ml). Variations between replicate flasks were larger than the mean values for some of the treatments making interpretation difficult. Adding an amount of medium equivalent to 100-percent of the nutrients used to culture the organisms increased growth significantly in the disposal site water and the elutriate.

61. Growth in the elutriate prepared from site 2 (Figure 6) shows the trend more clearly. Growth was stimulated by the elutriate and as the elutriate concentration was increased, the maximum cell numbers decreased. Growth in 25-percent elutriate was 5.1×10^5 cells/ml; while in 100-percent elutriate, it was 2.4×10^5 cells/ml. Nutrient addition to the elutriate stimulated growth (2.0×10^6 cells/ml) when compared with unspiked elutriate (2.4×10^5 cells/ml).

62. Growth yields for site 3 (Figure 7) show that growth was stimulated at all concentrations of elutriate, but was lower in the higher percentages of elutriate.

63. The data indicated that chemical constituents released from the sediments produced a dual effect on the growth of D. tertiolecta. At low concentrations of elutriate, growth was stimulated over that which occurred in the disposal site water. As more elutriate was added to the disposal site water, the maximum growth decreased. Nutrients that were released from the sediments stimulated growth (e.g., NH_3-nitrogen, TOC, TIC, Table 1). The elutriates may have contained one or more toxic substances that increased in concentration as more elutriate was added. However, spiking 100-percent elutriate and 100-percent disposal site water increased growth significantly, arguing against the presence of a toxic substance.

64. Only trends can be observed when discussing the three sample sites for Bridgeport Harbor and Eatons Neck disposal site. In some cases, variations of growth between replicate treatments were larger than the difference observed for growth between different treatments. However, it is clear that (a) elutriates from the three sample sites stimulated growth when compared with growth in disposal site water, (b) maximum cell numbers generally decreased as the concentration of elutriate was increased, and (c) adding nutrient spikes to 100-percent elutriate from the three sites and to 100-percent disposal site water increased growth over that obtained in the corresponding unspiked samples.

Bacterial assays

65. Growth experiments were conducted using MW40C as the test organism in combinations of diposal site water and elutriate as shown in Table 2. One-hundred-millilitre spectrophotometer tubes were used as growth vessels. The change in optical density at 620 μm was measured with a Spectronic-20 Spectrophotometer. Liquid volume was 20 ml/tube. Three replicates of each treatment were used.

66. Readings prior to 24 hr were difficult to obtain. The experimental tubes reached a maximum optical density of 0.03 to 0.04 in 29 hr (Table 2). There were no apparent differences between sites or dilution of the elutriates with disposal site water. The controls that received growth medium grew for about 71 hr, then remained at an optical density of 0.15 to 0.18. No growth occurred in the uninoculated controls (not shown in Table 2).

67. The optical density of 0.03 to 0.04 in the experimental tubes indicates a very small amount of growth. Growth occurred in the control tubes receiving nutrients, indicating that the elutriates and disposal site water did not have sufficient nutrients for growth of the test bacterium. The slight amount of growth that did occur in the experimental tubes may have been caused by nutrient carryover because the bacteria were grown in a complex growth medium prior to inoculation of the test units.

68. The bacterial growth experiment demonstrated that the

Table 2

Optical Density Readings of Marine Bacteria MW40C in Elutriates and Disposal Site Water from Ashtabula Harbor

Sample	Percent Disposal Site Water	Percent Elutriate	Millilitres of Medium	Optical Density at Elapsed Time of 24 hr	29 hr	46 hr	54 hr	71 hr
Site 1	100	0	0	0.033	0.037	0.030	0.018	0.011
	100	0	1	0.120	0.150	0.170	0.165	0.175
	75	25	0	0.031	0.033	0.028	0.018	0.011
	50	50	0	0.037	0.041	0.031	0.020	0.014
	50	50	1	0.104	0.130	0.154	0.146	0.156
	25	75	0	0.027	0.033	0.025	0.016	0.008
	0	100	0	0.029	0.035	0.029	0.016	0.010
	0	100	1	0.092	0.120	0.146	0.136	0.148
Site 2	75	25	0	0.029	0.034	0.028	0.014	0.010
	50	50	0	0.029	0.035	0.031	0.017	0.012
	50	50	1	0.095	0.125	0.147	0.145	0.150
	25	75	0	0.021	0.032	0.025	0.013	0.009
	0	100	0	0.021	0.027	0.027	0.014	0.010
	0	100	1	0.083	0.110	0.131	0.129	0.138
Site 3	75	25	0	0.029	0.029	0.029	0.019	0.007
	50	50	0	0.029	0.027	0.027	0.011	0.007
	50	50	1	0.110	0.151	0.151	0.149	0.154
	25	75	0	0.032	0.037	0.029	0.018	0.012
	0	100	0	0.028	0.033	0.024	0.012	0.006
	0	100	1	0.091	0.121	0.141	0.140	0.140
Medium	0	0	20	0.104	0.132	0.247	0.273	0.330
Controls	0	0	0	--	--	--	--	--

chemical constituents released from the sediments would not stimulate bacterial growth beyond that which would occur in the disposal site water. Growth of the test organisms was not inhibited by the elutriates when nutrients were added, indicating that no toxic compounds were present.

Ashtabula River Sediments and Lake Erie Disposal Site Water

Physical characteristics of the samples

69. Water and sediment samples were collected on 6 November 1974. Water depth at sediment site 1 was 3 m, bottom temperature was 10°C, and dissolved oxygen concentration was 10.5 ppm. Sediment site 2 had a water depth of 5 m, temperature was 10°C, and dissolved oxygen concentration was 9.8 ppm. Site 3 sediments were collected in 7 m of water, temperature was 10.5°C, and dissolved oxygen concentration was 9.5 ppm. Surface temperature of the water at the disposal site was 10°C and dissolved oxygen concentration was 11.5 ppm. Since high winds prevented the collection of a composite water column sample, only surface water was obtained at the disposal site.

Chemical analyses

70. Table 3 lists the chemical analyses for Ashtabula Harbor elutriates and Lake Erie disposal site water. The nitrate-nitrogen found in the elutriates was probably a result of microbial oxidation of the ammonium-nitrogen in the elutriates because the unacidified samples were stored at 4°C for 3 days prior to nutrient analyses. Ammonium-nitrogen increased when compared with disposal site water concentrations. Total organic and inorganic carbon compounds were released from the sediments. Manganese was released from the sediments of the three sites as were small amounts of zinc and arsenic. Iron was released from site 1 sediments. Conversely, iron was removed from disposal site water by sediments from sites 2 and 3.

Algal assays

71. Growth curves for Selenastrum capricornutum in elutriates prepared with sediments from Ashtabula Harbor are given in Appendix B (Figures B4-B6). Growth in the disposal site water is also shown.

Table 3

Chemical Analyses of Lake Erie Disposal Site Water and Ashtabula Harbor Elutriates Before Biological Assessment

Constituents	Disposal Site Water	Elutriate Site 1	Elutriate Site 2	Elutriate Site 3
Nutrients (ppb):				
NO_2-N	10	18	18	18
NO_3-N	60	830	980	830
OPO_4-P	3	5	6	7
Nutrients (ppm):				
NH_3-N	0.2	19	13	14
TOC-C	<1	11	11	7
TIC-C	16	22	21	26
TKN-N	0.3	25	16	20
Heavy metals (ppb):				
Cd	0.3	1	2	1
Ni	21	13	32	21
Zn	13	19	44	25
Mn	3	87	83	81
Pb	8	6	5	2
Cu	2	1	6	1
Fe	48	80	<5	6
As	<1	10	6	10

72. Figure 8 shows the maximum standing crop obtained for elutriate prepared from sediment site 1. The data, as presented, would indicate an initial stimulation of growth and then an inhibitory effect as the percentage of elutriate was increased. However, statistical analysis of the data showed that these differences were not significant at the $P < 0.05$ level because there were large variations between replicate flasks and the trend could not be proven statistically. Nutrient additions to 100-percent elutriate and 100-percent disposal site water stimulated growth; the stimulation was greater in 100-percent disposal site water (1.8×10^6 cells/ml) than in 100-percent elutriate (0.9×10^6 cells/ml).

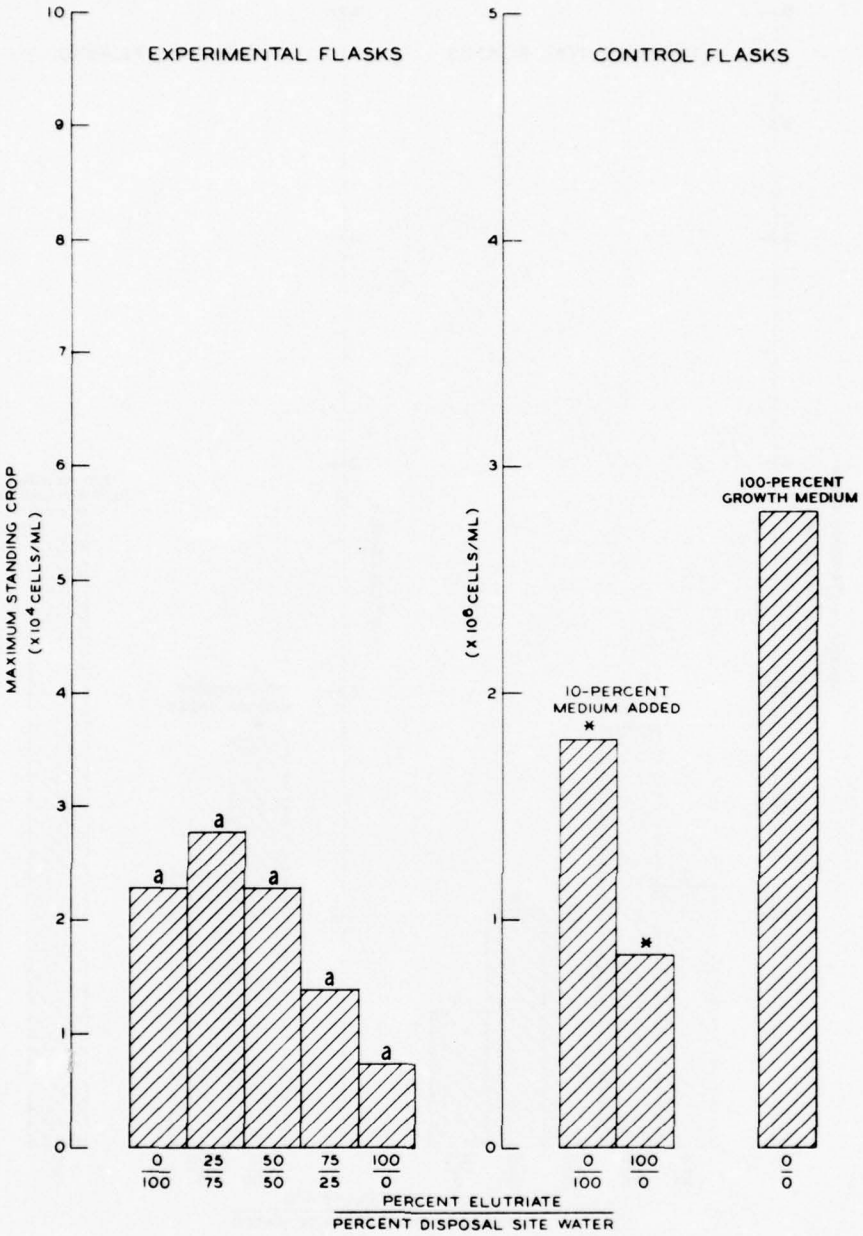
73. The maximum standing crop for each treatment of site 2 did not indicate any trend (Figure 9). Analysis of the data for site 3 (not shown) yielded the same problems as similar analyses of sites 1 and 2 (high variability among replicate treatments).

Bacterial assays

74. Bacterial growth study. Washed cells of Caulobacter bacteroides were used to determine the effect of elutriate and disposal site water on the growth of bacteria. Spectrophotometer test tubes were used as growth vessels, and growth was determined by measuring optical density in the tubes with a Spectronic-20 Spectrophotometer at a wavelength of 620 nanometres. Liquid volume was 20 ml/tube. Elutriates from the three sediment sampling sites were tested; three replicates of each experimental or control unit were used. The tubes were incubated at 24°C in the dark.

75. Experimental and control units were established as follows:

Experimental Units	
Percent Disposal Site Water	Percent Elutriate
100	0
75	25
50	50
25	75
0	100



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE $P < 0.05$ LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT ($P < 0.05$) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 8. Maximum standing crop of S. capricornutum in elutriate prepared from sediment site 1 of Ashtabula Harbor and disposal site water collected from Lake Erie

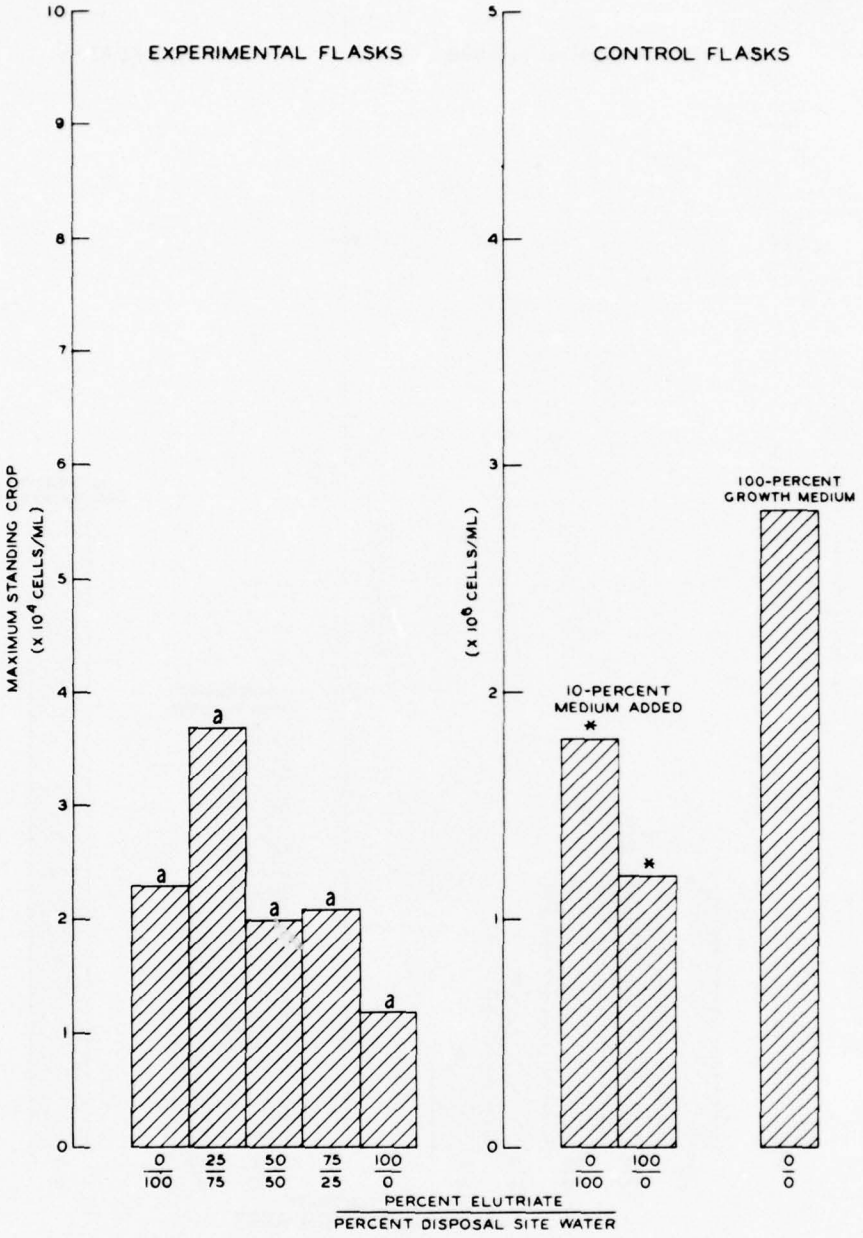


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Figure 9. Maximum standing crop of S. capricornutum in elutriate prepared from sediment site 2 of Ashtabula Harbor and disposal site water collected from Lake Erie

Control Units Inoculated with Bacteria		
Percent Disposal Site Water	Percent Elutriate	Millilitres of Growth Medium
100	0	1.0
50	50	1.0
0	100	1.0
0	0	20.0

Control Units Not Inoculated with Bacteria		
Percent Disposal Site Water	Percent Elutriate	Millilitres of Growth Medium
100	0	1.0
50	50	1.0
0	100	1.0
0	0	20.0
100	0	0.0
50	50	0.0
0	100	0.0

76. Growth did not occur in any of the experimental tubes during a 240-hr incubation period. Controls receiving 1.0 ml of growth medium had measurable growth after 20 hr (approximate optical density 0.1) and reached an optical density of 0.2 at 240 hr. There were no apparent differences among these controls regardless of the concentration of elutriate or the sediment-sample site used for elutriate preparation. One-hundred-percent growth medium controls grew well throughout the experiment and reached an optical density greater than 0.1 after 240 hr.

77. Bacterial respiration study. The effect of elutriate and disposal site water on respiration rate was determined using washed cells of the bacterium designated as BLA-1. Elutriate combinations from Ashtabula sediment site 1 and Lake Erie disposal site water were established as shown in Table 4. Flasks 1 through 9 were controls and 10 through 17 were experimentals. Flasks 15, 16, and 17 were replicates for flasks 11, 13, and 14. The respiration rate of BLA-1 was reduced approximately 18 percent when 1 ml of elutriate was present as compared with the rate in the presence of 1-ml disposal site water (flasks 10

Table 4

Respirometry Experiment Using BLA-1 Bacteria in Elutriate Prepared from Ashtabula Harbor Sediments and Disposal Site Water from Lake Erie

Flask No.	Flask Contents, ml					Average Respiration Rate-Oxygen Uptake µl/min
	Cells	Disposal Site Water	Elutriate	Medium	Distilled Water	
1	3.0	0.0	0.0	0.0	1.0	6.5
2	3.0	0.0	0.0	0.0	1.0	6.7
3	3.0	0.0	0.0	0.0	1.0	6.6
4	3.0	0.0	0.0	0.0	1.0	6.6
5	3.0	0.0	0.0	1.0	0.0	9.8
6	3.0	0.0	0.0	1.0	0.0	9.8
7	0.0	1.0	0.0	3.0	0.0	0.0
8	0.0	0.5	0.5	3.0	0.0	0.0
9	0.0	0.0	1.0	3.0	0.0	0.0
10	3.0	1.0	0.0	0.0	0.0	6.9
11	3.0	0.75	0.25	0.0	0.0	7.0
12	3.0	0.5	0.5	0.0	0.0	6.4
13	3.0	0.25	0.75	0.0	0.0	6.3
14	3.0	0.0	1.0	0.0	0.0	5.7
15	3.0	0.75	0.25	0.0	0.0	7.2
16	3.0	0.25	0.75	0.0	0.0	6.7
17	3.0	0.0	1.0	0.0	0.0	5.9

through 14). The reduction in respiration rate was the same for both sets of experimental flasks when 0.25-ml elutriate (flasks 11 and 15) was compared with 1.0-ml elutriate (flasks 14 and 17). The reduction was 1.3 µl/min (7.0 - 5.7 and 7.2 - 5.9).

Protozoan assays

78. Tetrahymena pyriformis was the test organism used to study the effect of elutriate and disposal site water on the survival of protozoans. The drop culture protozoan assay, and the combinations of elutriate and disposal site water used were described in Part II: Materials and Methods.

79. Figure 10 shows the survival of T. pyriformis in elutriate prepared from site 1 and in the disposal site water. The organisms survived best in 100-percent elutriate and seemed to begin dying in 100-percent disposal site water immediately after exposure. Cells divided in 100-percent elutriate, but were unable to do so in the disposal site water.

80. The same trend occurred in site 2 elutriate (Figure 11). Increasing the amount of disposal site water produced an inhibitory effect on cell division. The results for site 3 elutriate (Figure 12) show a different pattern from the first two sites. The percent survival in 25-percent elutriate to 75-percent disposal site water was approximately the same as it was for 100-percent elutriate, except for the 96-hr values.

Galveston Harbor Sediment and Gulf of Mexico Disposal Site Water

Physical characteristics of the samples

81. Sediment and water samples were collected on 16 January 1975. Water depth at all sediment sites was 9 m. Salinity directly above the sediment was 28 ppt, dissolved oxygen concentration was 7.9 ppm, and the temperature was 15°C at the three sediment collecting sites.

82. The composite water column samples collected from the Gulf of Mexico disposal site had a salinity of 30 ppt, and the temperature was 15°C immediately after collection.

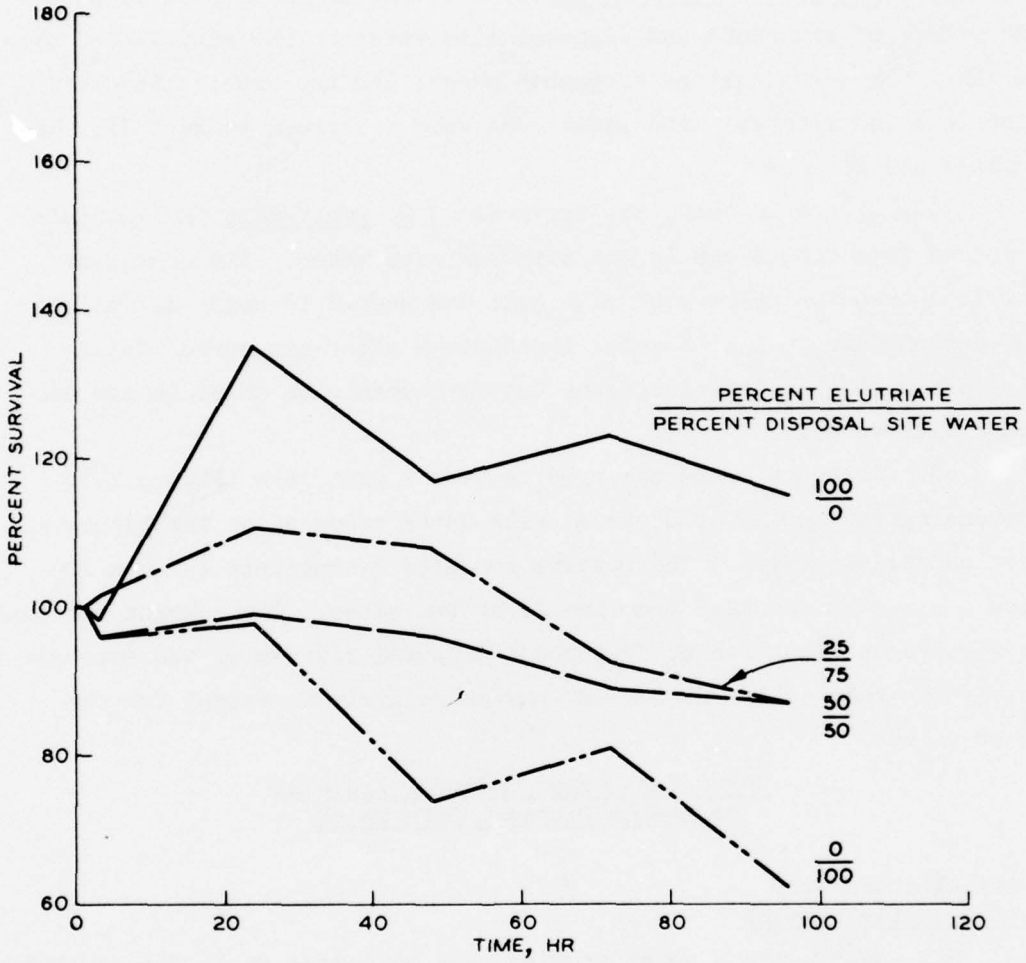


Figure 10. Percent survival of T. pyriformis in elutriate prepared from sediment site 1 of Ashtabula Harbor and disposal site water collected from Lake Erie

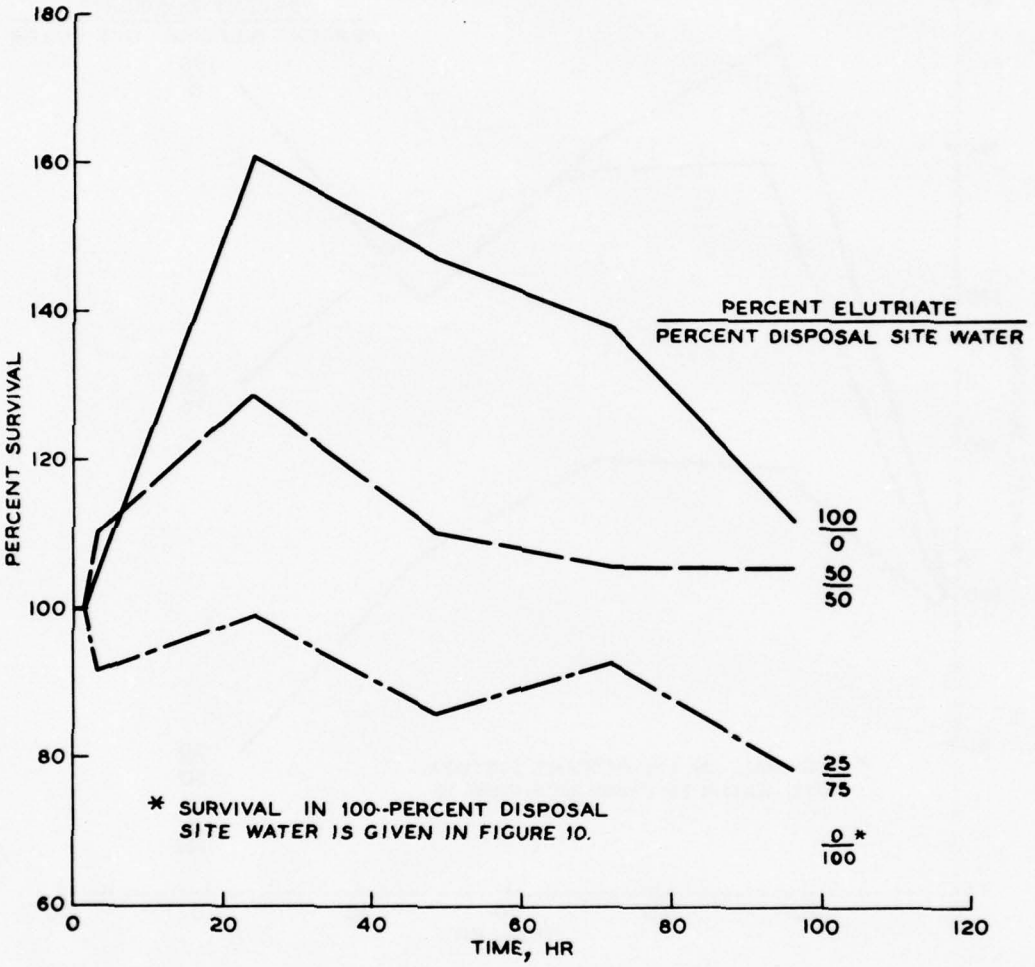


Figure 11. Percent survival of T. pyriformis in elutriate prepared from sediment site 2 of Ashtabula Harbor and disposal site water collected from Lake Erie

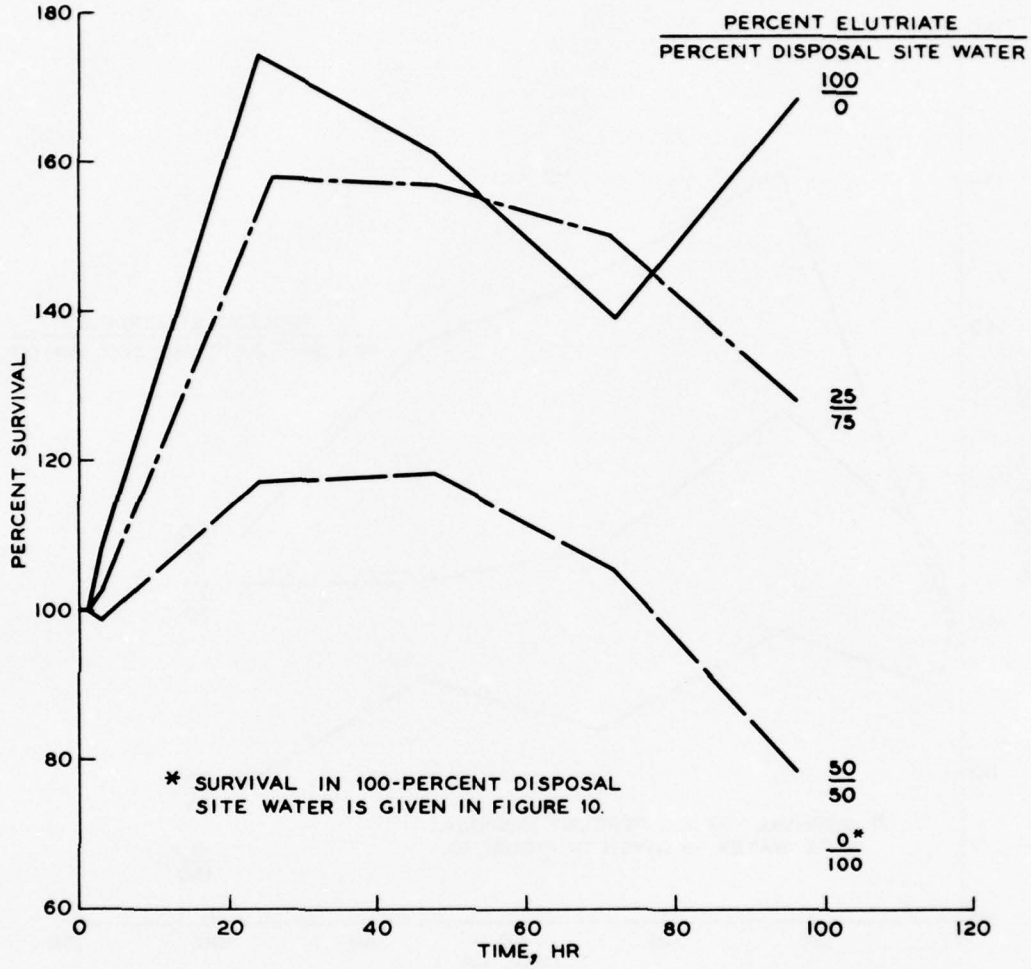


Figure 12. Percent survival of T. pyriformis in elutriate prepared from sediment site 3 of Ashtabula Harbor and disposal site water collected from Lake Erie

Chemical analyses

83. Table 5 lists the chemical analyses of the Gulf of Mexico disposal site water and Galveston Harbor elutriates before algal bioassessment studies were conducted. The slight increase in the nitrate concentrations of the elutriates as compared with disposal site water probably resulted from microbial oxidation of the ammonium nitrogen present. Orthophosphate was released from the sediments of each site. Ammonium-nitrogen was released in large quantities, and total inorganic carbon also increased. Of the heavy metals analyzed, manganese concentrations in the elutriates increased in large quantities as compared with the disposal site water concentrations.

Algal assays

84. Growth curves for Dunaliella tertiolecta are shown in Appendix B (Figures B7-B9) for the elutriates and disposal site water collected from the Galveston area. Growth rates were very similar for the various treatment levels during the exponential phase of growth.

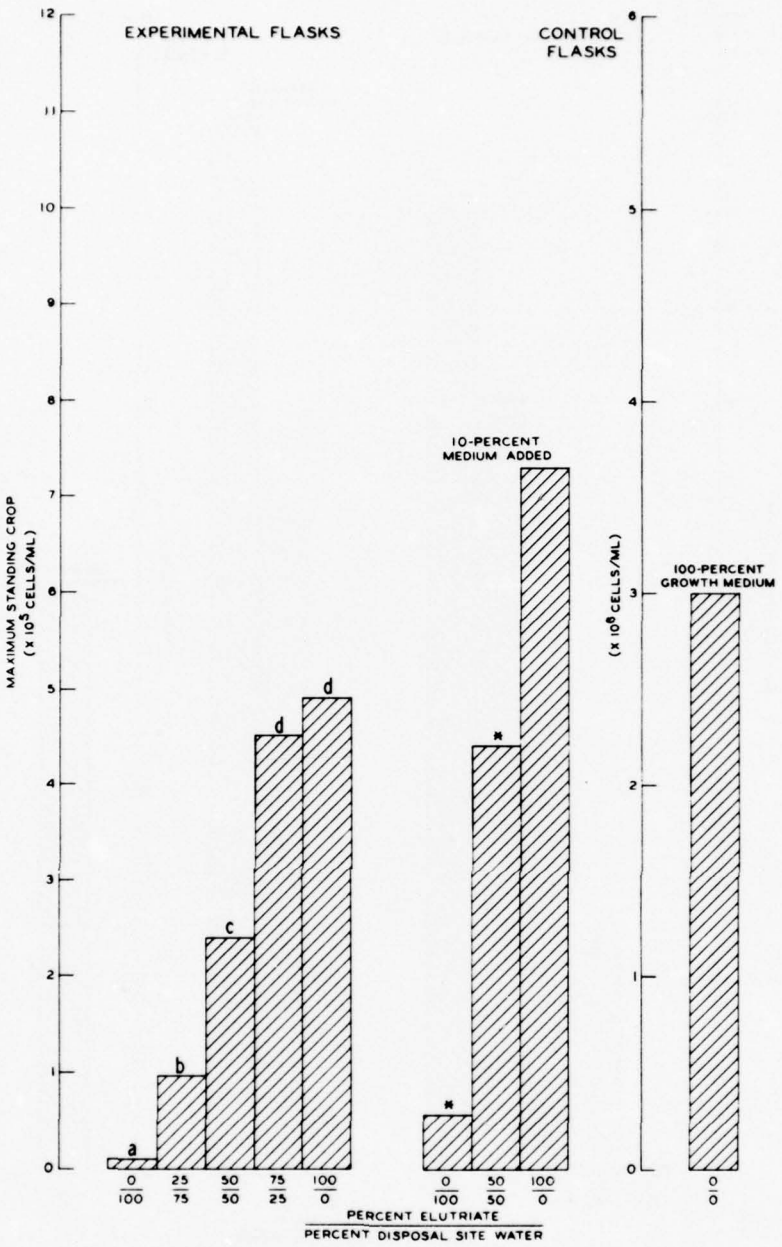
85. When maximum cell numbers were considered, D. tertiolecta responded in a similar way to the elutriates prepared from the three sediment sites (Figures 13-15). As the concentration of elutriate increased, the maximum cell number also increased. The maximum growth in 100-percent disposal site water was 1×10^4 cells/ml. Elutriate prepared from site 1 sediments (Figure 13) produced a maximum cell number of approximately 4.9×10^5 cells/ml in 100-percent elutriate. The results from sediment sites 2 and 3 (Figures 14 and 15) are similar to those of site 1. However, maximum standing crop for 100-percent elutriate was 1.0×10^6 cells/ml for site 2 and 7.3×10^5 cells/ml for site 3. Statistical analysis of growth in the treatment flasks indicated that there was a significant difference ($P < 0.05$) between treatments in all cases except when the 75-percent elutriate to 25-percent disposal site water and 100-percent elutriate of site 1 were compared (Figure 13).

86. Adding a 10-percent growth-medium spike to the disposal site water increased the maximum growth to approximately 5×10^4 cells/ml. Nutrient spikes also increased growth in the 50-percent disposal site water to 50-percent elutriate combinations for the three sediment

Table 5

Chemical Analyses of Gulf of Mexico Disposal Site Water and Galveston Harbor Elutriates Before Algal Biological Assessment

Constituents	Disposal Site Water	Elutriate Site 1	Elutriate Site 2	Elutriate Site 3
Nutrients (ppb):				
NO_2-N	6	6	11	9
NO_3-N	6	31	40	26
OPO_4-P	3	45	64	32
Nutrients (ppm):				
NH_3-N	0.02	21	16	16
TOC-C	4	9	9	11
TIC-C	15	98	92	85
TKN-N	0.03	26	21	21
Heavy metals (ppb):				
Cd	9	8	12	21
Ni	6	30	15	37
Zn	4	7	1	3
Mn	33	116	114	116
Pb	8	3	5	5
Cu	4	20	5	10
Fe	26	28	24	23
As	1	5	6	4



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE P <005 LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT (P <005) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 13. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 1 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

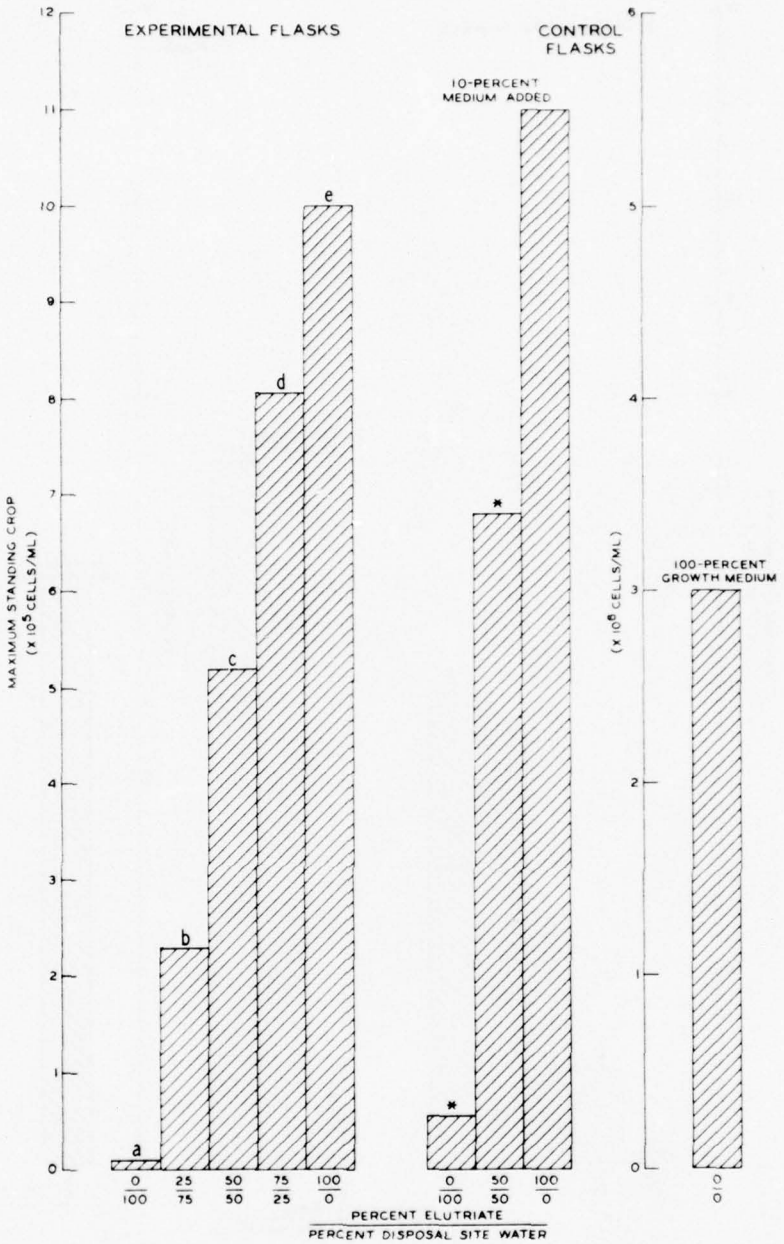


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Figure 14. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 2 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

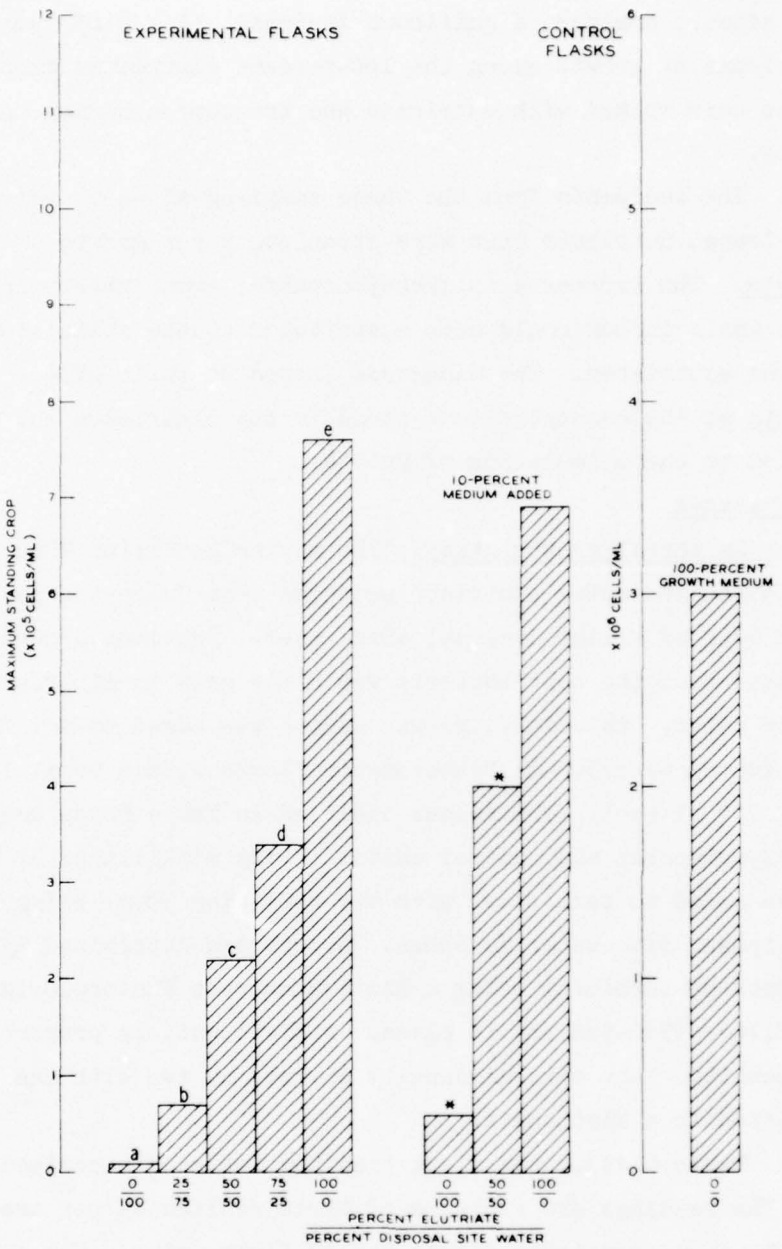

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Figure 15. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 3 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

sampling sites. However, significant increases ($P < 0.05$) were not found in comparisons of growth among the 100-percent elutriates from the three sites that were spiked with nutrients and the corresponding unspiked treatments.

87. The sediments from the three sampling sites of Galveston Harbor released nutrients that were stimulatory for growth of D. tertiolecta. The increases in orthophosphate, ammonium-nitrogen, and total inorganic carbon could have contributed to the stimulatory effect of the elutriates. The manganese showed no toxic effect on D. tertiolecta at the concentrations found in the elutriates and may have contributed to the stimulation of growth.

Bacterial assays

88. Bacterial growth study. The marine bacterium MB22 was used for growth studies using elutriate prepared from Galveston Harbor sediments and Gulf of Mexico disposal site water. Previous growth studies demonstrated that the test bacteria would not grow in elutriate or disposal site water. Therefore, growth medium was added to all flasks. Culture vessels were 500-ml Nephelometer flasks with a total liquid volume of 100 ml each. The scheme outlined in Table 6 was used to establish experimental and control units. Fifty millilitres of growth medium was added to each flask with the remaining 50 ml being elutriate and/or disposal site water as shown. Growth was determined by periodic measurements of turbidity using a Klett-Summerson Photocolorimeter with a green filter (550-580 μm) in place. A Klett unit is proportional to optical density: the optical density divided by two with the decimal place omitted is a Klett unit.

89. Table 6 lists the Klett readings for each experimental condition. The readings are averages of three replicates per treatment. Variability among replicate flasks was ±3 Klett units. The cultures reached maximum turbidity in 7.5 hr and remained stationary for an additional 40.5 hr. No difference in growth was observed for any of the treatment levels within a site, or among sites when compared with growth in disposal site water.

90. Bacterial respiration study. Table 7 lists the flask

Table 6

Klett Readings of Marine Bacteria MB22 in Elutriates and Disposal Site Water from Galveston Area

Sample	Percent Disposal Site Water	Percent Elutriate	Klett Reading at Elapsed Time of 2.5 hr	7.5 hr	14 hr	21.5 hr	27.5 hr	48 hr
Site 1	75	25	18	54	58	54	53	50
	50	50	18	56	57	51	52	49
	25	75	17	54	57	53	50	47
	0	100	17	54	55	50	49	46
Site 2	75	25	19	54	58	56	56	51
	50	50	23	58	63	57	54	54
	25	75	22	58	63	55	54	--
	0	100	20	57	62	54	55	50
Site 3	75	25	22	55	61	57	56	56
	50	50	21	56	62	57	57	56
	25	75	21	57	60	57	56	53
	0	100	24	58	62	58	57	50
Controls	100	0	20	56	59	57	56	53
100 ml growth medium			22	91	100	96	96	92
50 ml growth medium plus 50 ml artificial seawater			8	18	35	38	38	40

Table 7

Respirometry Study Using Marine Bacteria MB22 with Galveston Elutriate and Disposal Site Water

Flask No.	Flask Contents, ml					Average Respiration Rate Oxygen Uptake µl/min
	Elutriate*	Disposal Site Water	Cells	ASW**	Growth Medium	
1	0.0	0.0	2.0	0.0	2.0	1.8
2	0.0	0.0	2.0	0.0	2.0	2.2
3	0.0	0.0	2.0	0.0	2.0	1.9
4	0.0	0.0	2.0	2.0	0.0	1.0
5	0.0	0.0	2.0	2.0	0.0	1.1
6	0.0	0.0	2.0	2.0	0.0	1.1
7	0.0	0.0	2.0	2.0	0.0	1.0
8	2.0	0.0	0.0	0.0	2.0	0.0
9	1.0	1.0	0.0	0.0	2.0	0.0
10	0.0	2.0	0.0	0.0	2.0	0.0
11	2.0	0.0	2.0	0.0	0.0	1.0
12	2.0	0.0	2.0	0.0	0.0	1.2
13	1.5	0.5	2.0	0.0	0.0	1.2
14	1.5	0.5	2.0	0.0	0.0	1.2
15	1.0	1.0	2.0	0.0	0.0	1.1
16	1.0	1.0	2.0	0.0	0.0	1.2
17	0.5	1.5	2.0	0.0	0.0	1.0
18	0.5	1.5	2.0	0.0	0.0	1.3
19	0.0	2.0	2.0	0.0	0.0	1.2
20	0.0	2.0	2.0	0.0	0.0	1.2

* Elutriate was prepared with sediment from site 2.
** Artificial seawater without organic nutrients.

contents for a respirometry experiment that measured oxygen uptake by the marine bacterium MB22 in Galveston Harbor elutriate prepared from sediment site 2 and disposal site water. Flasks 1 through 10 were controls, and flasks 11 through 20 were experimentals.

91. Table 7 also lists the average respiration rate (µl oxygen uptake/min) for a 130-min period for the treatments and controls. Treatment flasks were done in duplicate. Respiration rates were essentially the same for all combinations of elutriate and disposal site water (flasks 11 through 20), indicating that chemicals released from the sediments had no effect on the respiration of the organisms during the experimental period. The rates of the experimentals were similar to those of the controls diluted with artificial seawater (flasks 4, 5, 6, and 7); while controls in growth medium had a higher rate of respiration (flasks 1, 2, and 3).

92. Bacterial sensitivity test using filter paper disks. This test method consisted of spreading bacteria, washed free of growth medium, evenly over the surface of an agar plate. Immediately after spreading the bacteria, Whatman No. 3 filter-paper disks (1.5 cm diam), which had been soaked in 100-percent elutriate or 100-percent disposal site water, were placed on top of the agar. As controls, dry disks and disks soaked in artificial seawater medium were also placed on the agar surface. After incubation for 24 hr, the plates were examined for zones of inhibition or stimulation of growth around the disks as compared with other areas on the agar surface.

93. The marine bacteria MB22 and MW40C were used in the sensitivity test with elutriate from site 2 and disposal site water. Neither inhibition nor stimulation of growth was observed with these organisms in two separate experiments.

Protozoan assays

94. Uronema nigricans was used as the test organism for respiration rate studies using elutriate prepared from sediment site 2 of Galveston Harbor. Table 8 lists the contents of the experiment and control flasks as well as the average respiration rate produced in each flask for a 235-min period. The average oxygen uptake (µl/min) was

Table 8

Respirometry Study Using Protozoa *Uronema Nigricans* with Galveston Elutriate and Disposal Site Water

Flask No.	Flask Contents, ml					Average Respiration Rate Oxygen Uptake µl/min
	Elutriate	Disposal Site Water	Cells*	ASW**	Growth Medium†	
1	0.0	0.0	2.5	2.0	0.5	0.6
2	0.0	0.0	2.5	2.0	0.5	0.6
3	0.0	0.0	2.5	2.0	0.5	0.5
4	2.0	0.0	0.0	0.0	3.0	0.0
5	2.0	0.0	0.0	0.0	3.0	0.0
6	1.0	1.0	0.0	0.0	3.0	0.0
7	1.0	1.0	0.0	0.0	3.0	0.0
8	0.0	2.0	0.0	0.0	3.0	0.0
9	0.0	2.0	0.0	0.0	3.0	0.0
10	0.0	0.0	0.0	2.0	3.0	0.0
11	0.0	0.0	0.0	2.0	3.0	0.0
12	2.0	0.0	2.5	0.0	0.5	0.5
13	2.0	0.0	2.5	0.0	0.5	0.6
14	2.0	0.0	2.5	0.0	0.5	0.5
15	1.0	1.0	2.5	0.0	0.5	0.7
16	1.0	1.0	2.5	0.0	0.5	0.5
17	1.0	1.0	2.5	0.0	0.5	0.5
18	0.0	2.0	2.5	0.0	0.5	0.5
19	0.0	2.0	2.5	0.0	0.5	0.7
20	0.0	2.0	2.5	0.0	0.5	0.5

* Three-day axenic culture of Uronema nigricans with 2.5×10^5 cells µl/min.

** Artificial seawater without organic nutrients.

† Sterile protozoan growth medium.

approximately the same for 100-percent elutriate, 100-percent disposal site water, and 50-percent elutriate to 50-percent disposal site water (flasks 12 through 20). The rates for these experimental units were approximately the same as the controls (flasks 1, 2, and 3) that were not exposed to elutriate or disposal site water.

Summary of Galveston bioassays

95. For the Galveston samples, the algal bioassays demonstrated that nutrients were released from the sediments, stimulating the growth of the test algal species. Toxic compounds were not present in the elutriates, or they were not present in sufficient concentrations to have an inhibitory effect on algal growth. The bacterial bioassays measuring growth and respiration rate and those using filter paper disks indicated that the elutriate had no effect on the test organisms. However, to evoke a response with bacteria, growth medium had to be added to the test units. These additions could have masked any potential response caused by chemicals released from the sediments. The protozoan respiration study also required the addition of growth medium to obtain a measurable rate of oxygen uptake. No apparent differences were observed between any of the treatments, but any potential effect in rate may have been obscured by the addition of growth medium.

Arlington Channel Sediments and Disposal Site Water

Physical characteristics of the samples and elutriates

96. Sediment and water samples were collected from Arlington Channel of Mobile Bay on 18 March 1975. Water depth was 7 to 8 m at the three sediment sampling sites. Bottom temperature was 18°C at the three sites. Dissolved oxygen concentrations directly above the sediment surfaces were 7.2 ppm at site 1 and 5.2 ppm at sites 2 and 3. The composite water column sample used to prepare the elutriates had a pH of 8.3 and a salinity of 10.5 ppt. Elutriate from sediment site 1 had a pH of 8.1 and a salinity of 11.5 ppt. The pH of site 2 elutriate was 7.8 and salinity was 12.5 ppt. For site 3 the pH was 7.9 and salinity was 10.5 ppt.

Chemical analyses

97. Table 9 lists the results of chemical analyses of Arlington Channel disposal site water and elutriates before algal biological assessment studies. Ammonia-nitrogen was released from all sediments, as was TOC and TIC. Some orthophosphate was removed by all sediments. Heavy metal analyses indicated that manganese was released from all sites in large quantities. Lead and nickel were released from all sites. Iron was removed from the disposal site water by the three sediments used in preparing the elutriates.

98. Table 10 lists the chemical analyses of filtered disposal site water and elutriates after algal growth. Nitrate-nitrogen decreased in all of the test units. Orthophosphate concentrations decreased in the disposal site water and site 2 elutriates, was unchanged in site 1, and increased in site 3 elutriates. Eighty percent of the ammonia-nitrogen was gone in the three elutriates, but the concentration in the disposal site water remained unchanged. Total inorganic carbon was used under all conditions listed; approximately 50 percent was taken up in site 1 elutriate while the organisms in sites 2 and 3 elutriates used almost all of the inorganic carbon.

99. The manganese concentration decreased to very low levels in all experimental units. Iron decreased in the disposal site water but was approximately the same concentration in the elutriates as it was before algal growth.

Algal assays

100. Growth curves for the elutriates prepared from the three sediment sampling sites of Arlington Channel and the disposal site water of Mobile Bay are shown in Appendix B (Figures B10-B12). Exponential growth was similar for all treatments for the first 2 days. Growth rates for some treatments began to decline after the second day, while others continued at an exponential rate. Maximum cell yields were obtained on day six for most of the treatments.

101. D. tertiolecta grew better in 100-percent disposal site water than in any of the elutriate-disposal site water combinations of site 1 (Figure 16). Maximum standing crop for disposal site water was

Table 9

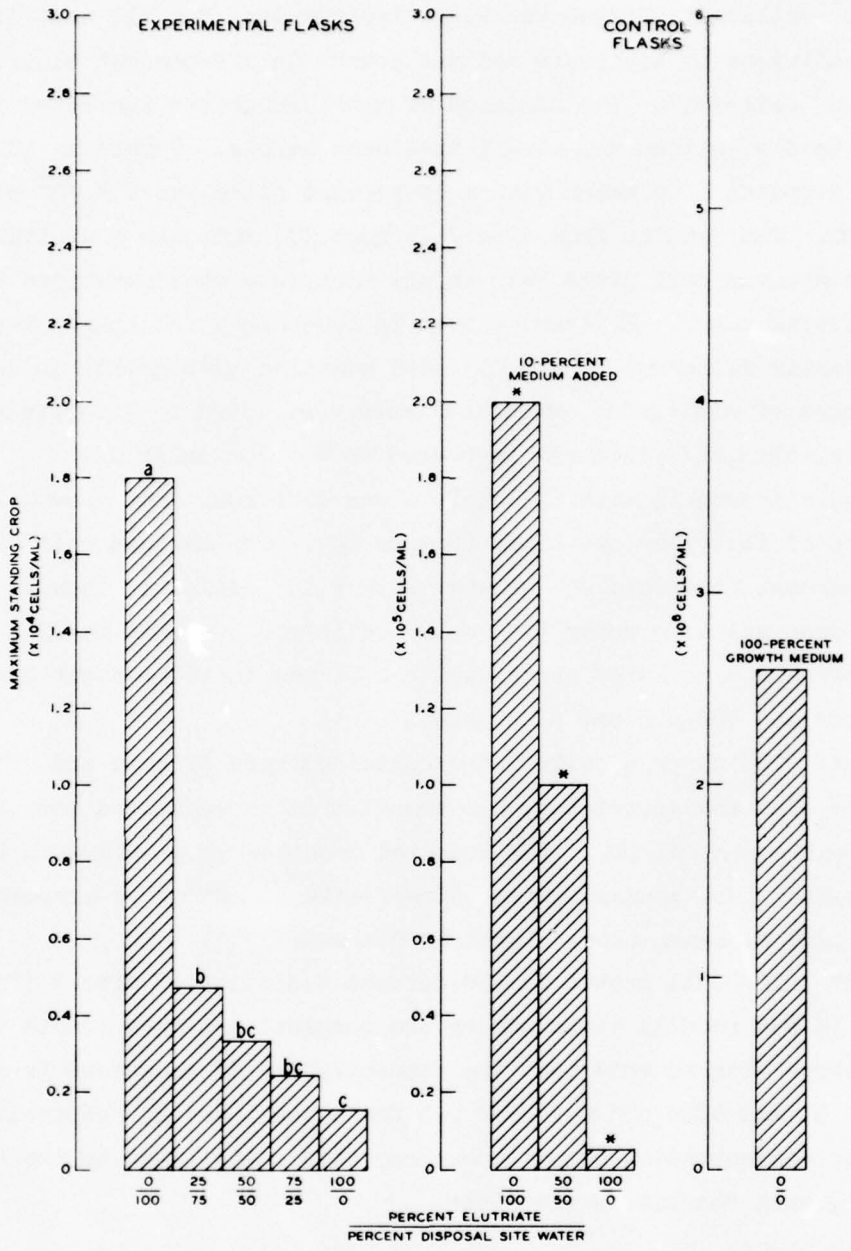
Chemical Analyses of Arlington Channel Disposal Site Water and Mobile Bay Elutriates Before Algal Bioassessment

Constituents	Disposal Site Water	Elutriate Site 1	Elutriate Site 2	Elutriate Site 3
Nutrients (ppb):				
NO_2-N	10	20	30	30
NO_3-N	190	80	70	70
OPO_4-P	128	96	96	96
Nutrients (ppm):				
NH_3-N	0.3	29	15	11
TOC-C	9	26	14	15
TIC-C	11	22	42	31
TKN-N	0.4	35	20	15
Heavy metals (ppb):				
Cd	1	3	3	5
Ni	16	22	29	19
Zn	9	9	5	6
Mn	70	4800	2900	700
Pb	18	44	69	32
Cu	10	15	5	14
Fe	117	13	16	14
As	<1	3	1	1

Table 10

Chemical Analyses of Arlington Channel Disposal Site Water and Mobile Bay Elutriates After Algal Bioassessment

Constituents	Disposal Site Water	Elutriate Site 1	Elutriate Site 2	Elutriate Site 3
Nutrients (ppb):				
NO_2-N	<15	<15	<15	<15
NO_3-N	<10	30	30	30
OPO_4-P	64	96	64	128
Nutrients (ppm):				
NH_3-N	0.1	5	3	2
TOC-C	10	29	20	16
TIC-C	7	12	<1	4
TKN-N	0.4	18	11	6
Heavy metals (ppb):				
Cd	3	4	1	1
Ni	18	24	24	20
Zn	8	3	14	4
Mn	3	1	1	<1
Pb	13	60	15	30
Cu	7	10	5	6
Fe	11	10	10	10
As	5	3	2	2



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE $P < 0.05$ LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT ($P < 0.05$) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 16. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 1 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

1.8×10^4 cells/ml. Growth was significantly less for all test flasks having additions of elutriate and was lowest in 100-percent elutriate (1.8×10^3 cells/ml). The addition of nutrient spikes increased the growth yield significantly at all treatment levels. Growth in 100-percent disposal site water with a 10-percent spike was 2×10^5 cells/ml.

102. The results from site 2 (Figure 17) indicate a similar trend with the maximum cell yield less in all elutriate concentrations than in disposal site water. However, growth in 100-percent elutriate was not significantly different ($P < 0.05$) when compared with growth in lower percentages of elutriate. When nutrients were added to 100-percent elutriate, the cell yield was increased to 4×10^4 cells/ml.

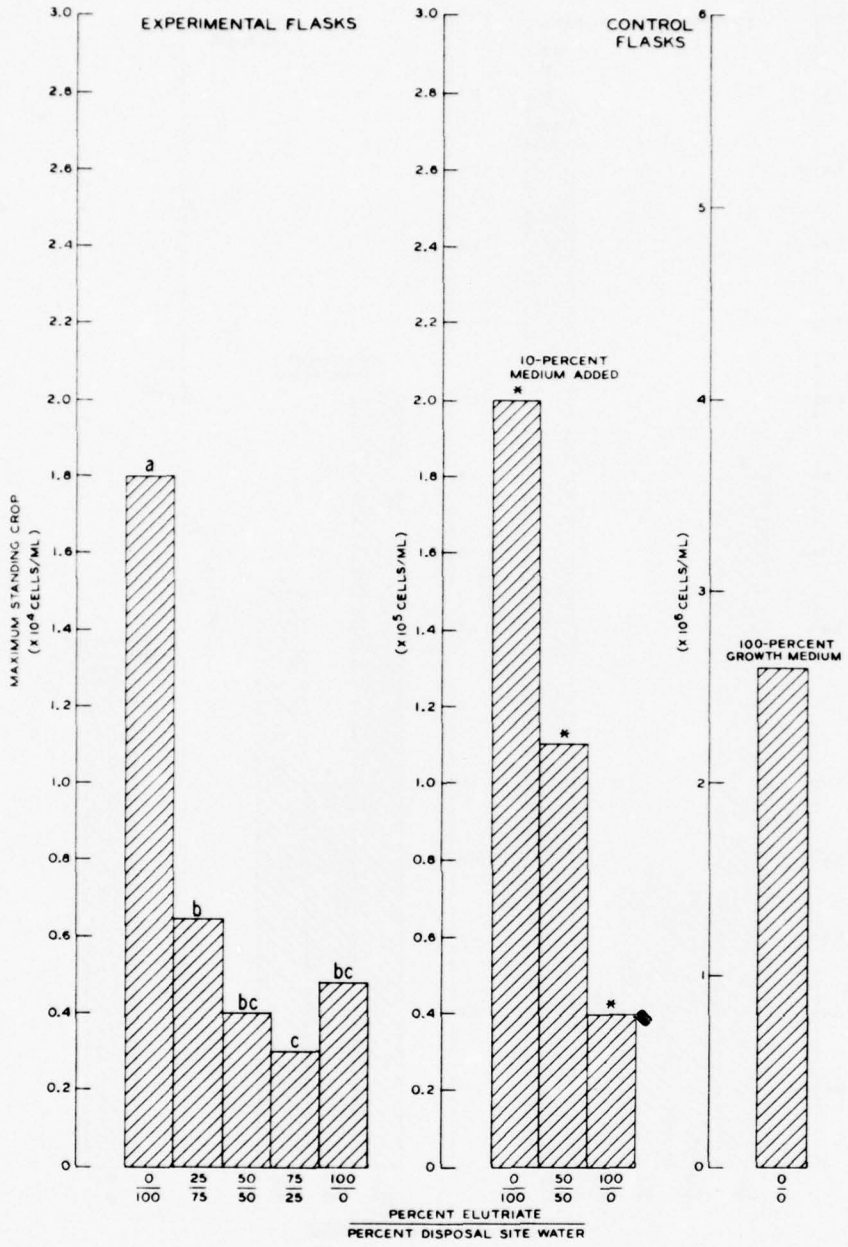
103. Growth in site 3 elutriate was different from growth in elutriate of the other two sites (Figure 18). The maximum cell yield in 100-percent elutriate was greater (6.2×10^4 cells/ml) than in 100-percent disposal site water (1.8×10^4 cells/ml). Growth in 25-, 50-, and 75-percent elutriates was lower than it was in 100-percent disposal site water and 100-percent elutriate.

104. The disposal site water contained more nitrate and orthophosphate than the elutriates, but the elutriates contained more ammonium-nitrogen and TIC. The observed decrease in growth with increasing elutriate concentration (except site 3) cannot be explained by the nutrient concentrations listed in Table 9.

105. The cell growth in 100-percent elutriate of site 3 (Figure 18) is not readily explained by the chemical analyses (Table 9). It is interesting to note that the concentration of manganese is 700 ppb for site 3, and 4800 ppb and 2900 ppb for sites 1 and 2, respectively. The lower concentration of manganese may have contributed to the increased growth for site 3 elutriate.

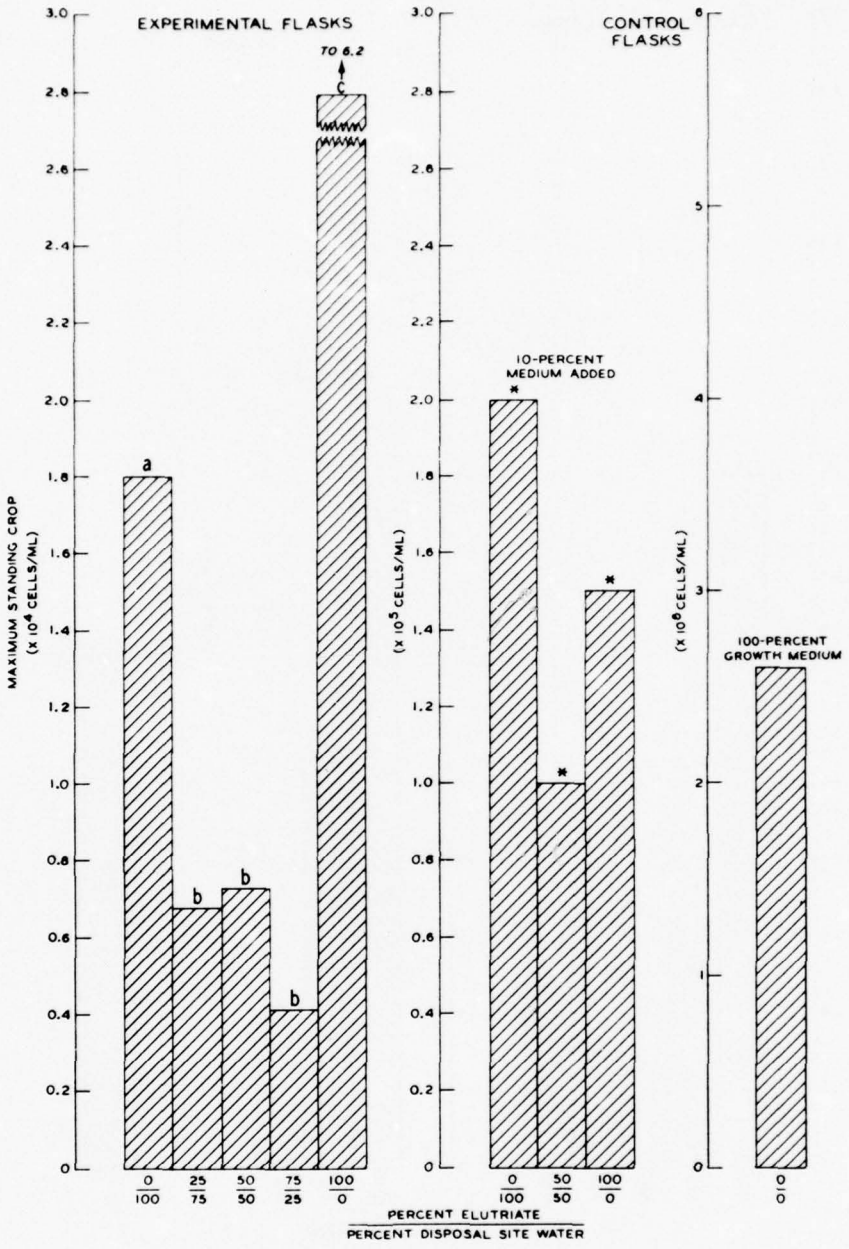
Protozoan assay

106. Survival of U. nigricans was determined in various concentrations of elutriate from site 2 and disposal site water of Arlington Channel. The results are shown in Figure 19. Cells divided in all of the treatment levels. Because of a lack of nutrients, the populations started dying after about 72 hr of incubation in all cases.



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE P <0.05 LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT (P <0.05) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 17. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 2 of Arlington Channel and disposal site water collected adjacent to Arlington Channel



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE P <0.05 LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS. ASTERISKS ABOVE THE BARS FOR THE CONTROL FLASKS INDICATE THAT GROWTH WAS SIGNIFICANTLY DIFFERENT (P <0.05) FROM THAT OBTAINED IN THE CORRESPONDING EXPERIMENTAL FLASKS.

Figure 18. Maximum standing crop of D. tertiolecta in elutriate prepared with sediment from site 3 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

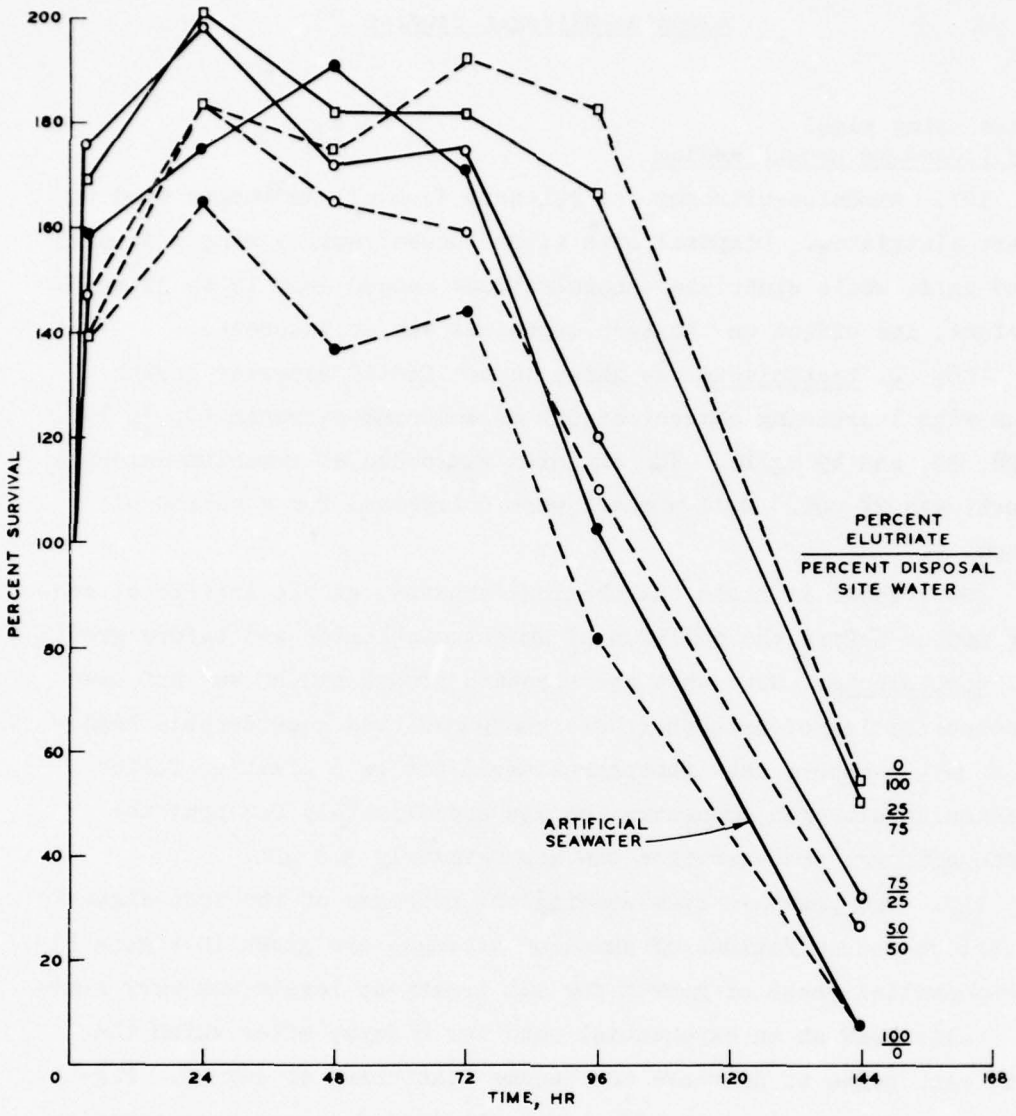


Figure 19. Percent survival of U. nigricans in elutriate prepared with sediment from site 2 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

No trend was observed as a function of elutriate concentration. The slight difference in die-off rate between the various treatment levels could be attributed to experimental errors in counting the cells microscopically.

Ammonium-Nitrogen Studies

Results using algal
assay procedure growth medium

107. Ammonium-nitrogen was released from all sediments used to prepare elutriates. Disposal site water concentrations ranged from 23 to 240 µg/ℓ, while elutriate concentrations ranged from 12 to 35 mg/ℓ. Therefore, its effect on the test organisms was of interest.

108. D. tertiolecta was grown in artificial seawater growth medium with increasing concentrations of ammonium-nitrogen (0, 7, 14, 21, 28, 35, and 49 mg/ℓ). The ammonium was added as ammonium-chloride. Salinity was 24 ppt. Cell numbers were determined for a period of 18 days.

109. Table 11 lists the chemical analyses of the artificial seawater medium before the addition of ammonium-chloride and before growth of D. tertiolecta. Note that the standard growth medium was not used. The concentration of orthophosphate phosphorus was considerably higher than usual to ensure that phosphorus would not be a limiting factor. The ammonium-nitrogen concentration was approximately 0.3 ppm; the nitrate-nitrogen concentration was approximately 3.8 ppm.

110. The growth curves showing the response of the test algae to the various concentrations of ammonium-nitrogen are given in Figure B13. The exponential phase of growth for all treatment levels was very similar. Cells grew at an exponential rate for 8 days, after which the growth rate began to decrease and became stationary at day 13. Figure 20 shows the maximum standing crop obtained for each concentration of ammonium-nitrogen. There were no significant differences ($P < 0.05$) in the number of cells obtained at the different ammonium-nitrogen concentrations. The pH of the medium used was 7.5, and the bioassay was conducted at 18°C. Therefore, the un-ionized ammonium-nitrogen in solution was approximately 1 percent of the ammonium-nitrogen added.[27] It is the ammonia form that is toxic to many aquatic organisms.

111. Table 12 lists the nutrients remaining in filtered samples after the growth of D. tertiolecta at various ammonium-nitrogen

Table 11

Nutrient Analyses of Artificial Seawater Growth Medium Before the Addition of Ammonium-Chloride and Inoculation with *Dunaliella tertiolecta*

Nutrient	Concentration, ppm
NO_2-N	<0.05
NO_3-N	3.8
OPO_4-P	2.2
NH_3-N	0.3
TKN-N	0.3
TOC-C	4.0
TIC-C	15.0

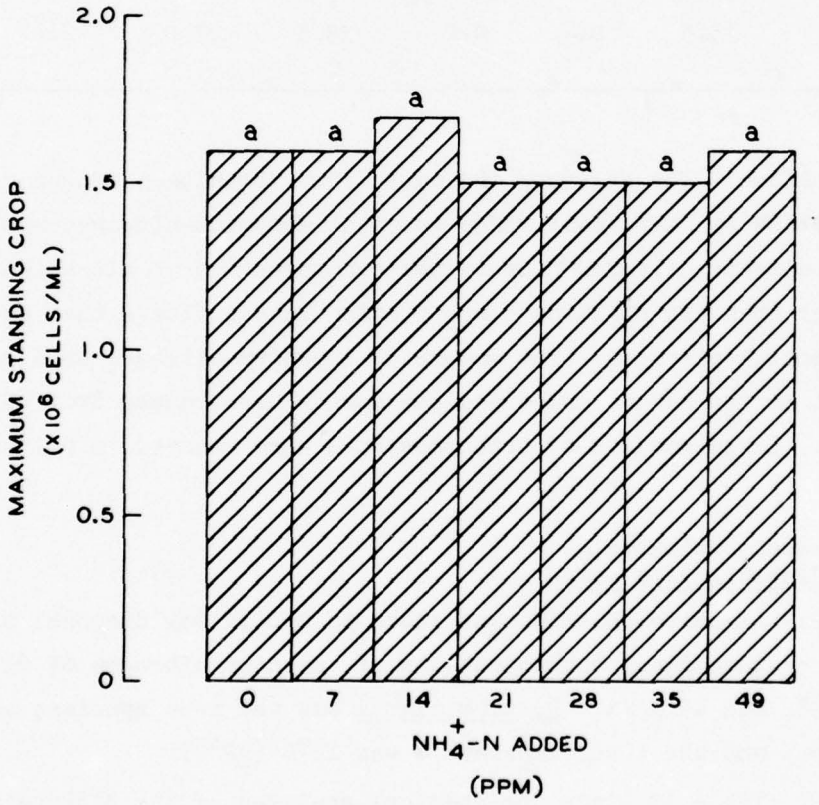


Figure 20. Maximum standing crop of D. tertiolecta in artificial seawater growth medium with various additions of ammonium-nitrogen added. The a's above the columns indicate that statistically growth was not significantly different between treatments at the $P < 0.05$ level

Table 12

Chemical Analysis of Nutrients Remaining After the Growth of *Dunaliella tertiolecta* at Various Concentrations of Added Ammonium-Chloride

Calculated Addition of NH_4-N, ppm	Final Nutrient Concentration Determined by Chemical Analysis, ppm						
	NO_3-N	NO_2-N	OPO_4-P	NH_3-N	TOC-C	TIC-C	TKN-N
0.0	0.8	0.4	0.5	0.2	20.0	2.2	4.0
7.0	9.0	0.6	0.7	0.3	24.0	6.0	3.0
14.0	8.1	2.2	0.7	2.2	25.0	3.0	7.0
21.0	14.1	0.1	0.5	10.0	22.0	4.0	22.0
28.0	16.3	0.3	0.6	13.3	25.0	4.0	28.0
35.0	14.1	0.1	0.4	21.7	24.0	3.0	46.0
49.0	15.5	0.1	0.6	33.5	27.0	2.0	73.0

concentrations. The analyses indicated that ammonium-nitrogen was used in preference to nitrate-nitrogen and that ammonium-nitrogen was oxidized to nitrate-nitrogen because the concentration of nitrate-nitrogen was higher than the starting concentration in all flasks that received added ammonium-nitrogen. The amount of ammonium-nitrogen used was not dependent on the amount added and the amount used ranged from 6.7 to 15.5 ppm. Large amounts of orthophosphate were removed in all treatment flasks.

Results using Arlington Channel disposal site water

112. Ammonium-chloride was added to Mobile Bay disposal site water to produce final concentrations of ammonium-nitrogen of 0, 7, 14, 21, 28, 35, and 49 mg/ℓ. D. tertiolecta was the test species; salinity was 10 ppt; and the test temperature was 18°C (±2°C).

113. Table 13 lists the chemical analyses of the disposal site water before the addition of ammonium-chloride and inoculation with D. tertiolecta. The disposal site water used in the study was collected

Table 13

Chemical Analyses of Arlington Channel Disposal Site Water Before the Addition of Ammonium-Chloride and Inoculation with *Dunaliella tertiolecta*

Nutrient	Concentration, ppb
NO_3-N	<10
NO_2-N	<10
OPO_4-P	<10
NH_3-N	131
TOC-C	17,000
TKN-N	200
Heavy Metal	**Concentration, ppb**
Cd	2.0
Ni	30.0
Zn	16.0
Mn	11.0
Pb	23.0
Cu	20.0
Fe	18.0
As	0.6

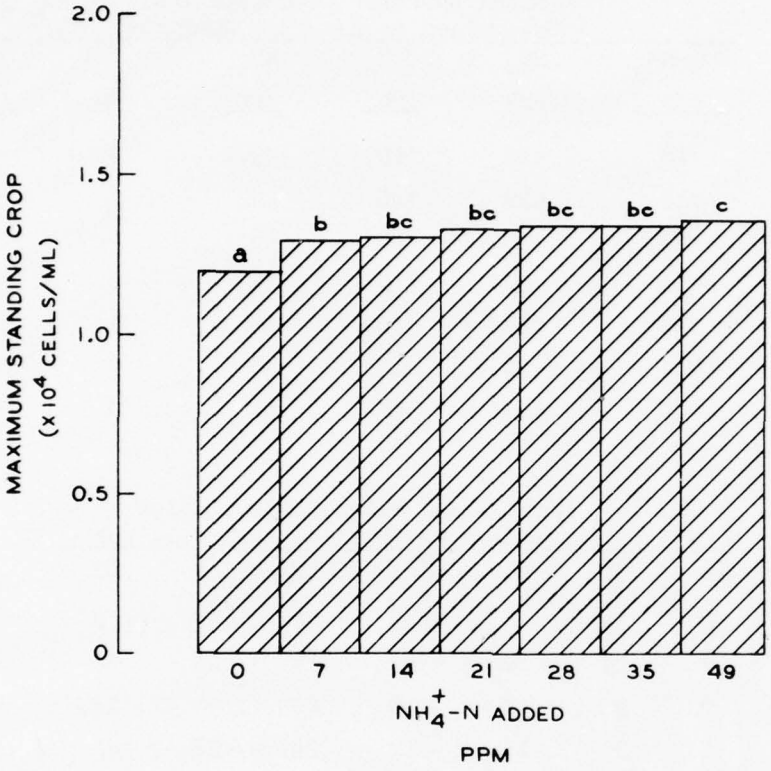
at the same time as the water used for the algal bioassay of Mobile Bay elutriates and disposal site water. However, the portion used for the ammonium-nitrogen study was stored at 4°C for 6 weeks prior to use. When chemical analyses at the two different times were compared, nitrate- and nitrite-nitrogen concentrations had decreased to below detectable levels as did orthophosphate concentrations. Ammonium-nitrogen concentrations decreased, but total organic carbon increased. The only heavy metals that showed any significant change were manganese and iron, both of which showed decreases (Table 9).

114. Figure B14 shows the growth curves for D. tertiolecta at each of the ammonium-nitrogen concentrations. Exponential growth rates

were similar for all treatment levels for the first 3 days, after which the growth rates began to level off.

115. Figure 21 shows the maximum growth obtained at each concentration of ammonium-nitrogen added. Maximum growth in 100-percent disposal site water was approximately 1.4×10^4 cells/ml for 49 mg of ammonium-nitrogen/ℓ, compared with 1.2×10^4 cells/ml for flasks receiving no added ammonium-nitrogen. The results indicate that ammonium-nitrogen would not be toxic to D. tertiolecta in water that is low in nutrients.

116. Table 14 lists the nutrients and heavy metals remaining in filtered samples after growth of the algae. Most of the nutrients were below the detectable limits before and after growth making comparisons impossible. Total organic carbon decreased, and the measured levels of ammonium-nitrogen remaining after growth were higher than the calculated amount added. Of the heavy metals, only the manganese concentration changed, being higher after growth.



NOTE: THE LETTERS ABOVE THE BARS FOR THE EXPERIMENTAL FLASKS INDICATE STATISTICAL SIGNIFICANCE. DIFFERENT LETTERS INDICATE A STATISTICAL DIFFERENCE AT THE $P < 0.05$ LEVEL, WHILE THE SAME LETTER INDICATES THAT GROWTH WAS NOT SIGNIFICANTLY DIFFERENT BETWEEN TREATMENTS.

Figure 21. Maximum standing crop of D. tertiolecta in Arlington Channel disposal site water with various concentrations of ammonium-nitrogen added

Table 14

Chemical Analyses of Nutrients and Heavy Metals Remaining After the Growth of *Dunaliella tertiolecta* at Various Concentrations of Added Ammonium-Chloride

Calculated Addition of NH_4-N ppm	Final Nutrient Concentration Determined by Chemical Analyses					
	NO_3-NO_2 ppb	NO_2 ppb	OPO_4 ppb	NH_4 ppm	TOC ppm	TKN ppm
0	<10	<10	<10	0.2	9	0.2
7	<10	<10	<10	8	7	10
14	<10	<10	<10	16	6	20
21	<10	<10	<10	24	6	29
28	<10	<10	<10	30	7	35
35	<10	<10	13	38	9	41
49	<10	<10	16	66	9	70

	Final Heavy Metal Concentration Determined by Chemical Analyses, ppb							
	Cd	Ni	Zn	Mn	Pb	Cu	Fe	As
0	1	29	9	113	21	9	8	<0.5
7	2	29	9	113	14	29	32	<0.5
14	2	28	7	116	20	54	13	1.0
21	1	39	17	113	24	13	25	2.0
28	2	27	12	102	20	12	8	<0.5
35	2	33	15	116	21	12	11	<0.5
49	1	49	9	113	20	15	11	<0.5

PART IV: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion of the First Year's Work

117. The results described in this report represent the first year (FY 1975) of in-house bioassay work. During that period, the bioassay laboratory was established and experiments were conducted, some of which are described in this report.

118. A number of improvements were made during FY 1975. This may be seen in the results, which were presented in chronological order. Variation of cell numbers among replicate treatments decreased considerably in the Galveston Harbor tests and ammonium-nitrogen studies compared with earlier studies. The variations among replicates for algal bioassays conducted during FY 1976 have been less than ±10 percent. This can be attributed to better techniques among workers as well as the use of a Coulter Electronic Particle Counter (Model TA-II) at the end of FY 1975.

Application to Water Quality and Criteria

119. There are two approaches that can be used in applying bioassay data to determine the acceptability of a particular dredged material for disposal. The first method involves comparing growth in 100-percent elutriate with growth in 100-percent disposal site water. The effect of diluting the elutriate with disposal site water should be considered. The second approach involves growth of the test organism in elutriates that have been characterized for major chemical constituents and attempting to compare the biological responses of the test organisms with the concentrations of various nutrients and heavy metals found in the elutriates. State and Federal water-quality standards, as well as published literature, can be used in evaluating the data. This method could help establish criteria or standards for disposal of dredged material, but the method suffers from the fact that a chemical constituent not included in the chemical analyses may have caused an observed effect.

Also, there is a lack of knowledge as to the biological response caused by a mixture of chemicals (e.g., heavy metals).

120. In relation to the first approach, algal bioassays of the elutriate would indicate the bioavailability of dissolved constituents released from dredged material and the possible effect on phytoplankton productivity at the disposal site. If observed growth in the elutriate were equivalent to observed growth in disposal site water, no adverse effect on the phytoplankton at the disposal site would be indicated. If exposure to the elutriate produced some effect in the test population, it must be remembered that the static bioassay described represents the worst-case situation because the tests are performed on the elutriate without dilution of the nutrients and contaminants released from the sediment at a disposal site. If a stimulatory or inhibitory response was observed in the elutriate cultures, mixing and diffusion at the disposal site would have to be considered in evaluating the bioassay results. The procedure described in this report involved use of various ratios of elutriate and disposal site water in an attempt to simulate dilution. Duration of exposure to a particular elutriate concentration was not considered, and each dilution was a worst-case situation. The various dilutions used were considered arbitrary; more appropriate dilutions could be substituted as needed.

121. The EPA has proposed water-quality criteria for marine aquatic life.[12] They suggested that concentrations greater than 0.4 mg of ammonia per litre are unacceptable. The Bridgeport Harbor elutriates had a pH of 8.0. The test was conducted at 18°C. The concentrations listed in Table 1 include ammonia plus ammonium-nitrogen. At the pH and temperature used for the bioassay, about 4 percent of the reported values were in the un-ionized form.[25] Therefore, in site 1 elutriate, the concentration of ammonia was 1.3 ppm; site 2 had 0.7 ppm; and site 3 had 0.9 ppm--all exceeding the suggested level. Two heavy metals also exceeded the recommended levels for marine aquatic life. The concentration of nickel in the disposal site water and the three elutriates exceeded the suggested 10 ppb. Manganese in site 1 elutriate was slightly higher than the 100 ppb recommended in the criteria. The EPA

proposed criteria are usually based on exposure of organisms to a toxicant for 96 hr. The concentration of the toxicant in the test chamber is constant except for any uptake that may occur by the organism.

122. Algal growth was greater in the three elutriates prepared from Bridgeport sediments than it was in Eatons Neck disposal site water. Increasing the elutriate decreased the maximum cell growth in each case. The heavy metals may have been exerting a toxic effect, singularly or in combination, as their concentration was increased.

123. Statistical analysis of the Ashtabula Harbor data indicated no significant difference ($P < 0.05$) between the various treatments. It is therefore impossible to discuss the chemical constituents in relation to observed growth. However, ammonium-nitrogen and manganese were released from the sediments of the three sampling sites. The suggested EPA maximum level of ammonia for freshwater aquatic life is 0.02 mg/ℓ, which was exceeded in the three elutriates. There are no suggested limits for manganese in fresh water for aquatic life.

124. The Galveston Harbor elutriates demonstrated a very clear stimulation of algal growth beyond that exhibited in disposal site water; increasing the elutriate concentration increased the maximum cell growth.

125. Ammonia and manganese exceeded the suggested levels in the elutriates prepared from the three sediment sampling sites of Galveston Harbor, but a toxic effect was not observed.

126. Chemical analyses of Arlington Channel elutriates showed the maximum acceptable level of ammonia to be exceeded in the three cases (1.4, 0.7, and 0.5 ppm for sites 1, 2, and 3, respectively). The algal growth data demonstrated a definite toxic effect of the elutriates from sites 1 and 2. Site 3 elutriate was also toxic, but had unexplainably high growth in 100-percent elutriate.

127. The ammonium studies demonstrated that the concentrations of ammonium plus ammonia found in the elutriates were not toxic to the test alga, Dunaliella tertiolecta. Under nutrient-poor conditions (Arlington disposal site water), the ammonium was slightly stimulatory to algal growth.

128. Erickson et al.[28] have shown that a concentration of 450 ppb copper inhibited the growth of D. tertiolecta by 50 percent of that observed in the controls. Overnell[29] inhibited the photosynthetic oxygen evolution of D. tertiolecta by 50 percent in the presence of 640 ppb copper. The toxic level of copper for eight species of green algae was reported by Kemp et al.[30] to be 2.0 ppm. The maximum concentration of copper found in the elutriates was 20 ppb, far less than any of the reported values that caused toxic effects.

129. Rachlin and Farran[31] found that the growth of the green alga Chlorella vulgaris was reduced approximately 50 percent in the presence of 2.0 ppm zinc. Payne[32] reported that in waters not containing chelating agents, the toxic level of zinc was 45 ppb for Selenastrum capricornutum. The highest concentration of zinc found in any elutriate was 44 ppb (Table 3). It is interesting to note that the algal assay procedure growth medium contains 15 ppb zinc.

Recommendations for Additional Research

130. Algal bioassays are useful in evaluating the biological effects of the chemical constituents released from sediments and their potential effect on phytoplankton at dredged material disposal sites. Stimulation, as well as toxicity, of algal growth has been demonstrated in the initial bioassays. Algal bioassays should be developed further as an aid in predicting the biological impact of the disposal of dredged material.

131. Bacteria have not shown promise as test organisms in evaluating the ecological effect of dredged material and have been particularly ineffective in criteria development. While they are important organisms in aquatic ecosystems, it is doubtful that bacterial bioassays will aid in the development of criteria for disposal of dredged material. Bacteria bioassay development for this purpose should be discontinued.

132. The use of protozoa as test organisms is questionable. A small effort should be made to determine the mortality of the organisms as a function of various conditions. If a simple, rapid bioassay can

be developed, it may be useful. On the other hand, if after a few additional attempts, protozoans do not show more promise than they have, they should be discarded as possible test organisms for the development of criteria. Protozoans and bacteria are very important in the cycling of nutrients and toxicants in sediments. The short-term effect of the disposal of dredged material on bacteria and protozoans may not be significant and will be difficult to use in establishing disposal criteria. These organisms will be extremely important in the long-term release of certain heavy metals and contaminants such as pesticides from deposited sediments.

133. Additional water column bioassays using selected zooplankton species are needed. Tests should be conducted using standard elutriates and unfiltered elutriates with particulate matter remaining in suspension.

134. Benthic bioassays need to be developed as aids in determining the effects of the disposal of dredged material on benthic species as well as possible long-term effects of these operations.

135. The biological laboratory data should be compared with field data. Comparisons are planned for the future when field data are compiled from ongoing DMRP field studies. Also, field data are needed in order to develop suitable test organism exposure times for various concentrations of test materials.

136. Ammonium-nitrogen and manganese were released from all sediments tested. Therefore, it is important to conduct bioassays using the concentrations of these chemicals measured in the elutriates under proper disposal site conditions. The effect of ammonium-nitrogen has been partially tested using D. tertiolecta and should be extended to Selenastrum capricornutum as well as any other organism used as a test specie. The effect of manganese on test species should also be determined. Since the elutriate and dredged material are really mixtures of chemicals, experiments should be conducted to determine the synergistic and antagonistic effects of various chemicals found in the test material.

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APPENDIX A: MICROBIAL GROWTH MEDIA

Algal Assay Procedure Growth Medium for *Selenastrum capricornutum*

1. For macronutrients, the following salts are Biological or Reagent Grade in milligrams per litre of distilled water.

Compound	Concentration mg/ℓ	Element	Concentration mg/ℓ
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
$MgCl_2$	5.700	Mg	2.904
$MgSO_4 \cdot 7H_2O$	14.700	S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	C	2.143
$NaHCO_3$	15.000	Ca	1.202
		Na	11.001
		K	0.469

2. For micronutrients, the following salts are Biological or Reagent Grade in micrograms per litre of distilled water.

Compound	Concentration µg/ℓ	Element	Concentration µg/ℓ
H_3BO_3	185.520	B	32.460
$MnCl_2$	264.264	Mn	115.374
$ZnCl_2$	32.709	Zn	15.691
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$FeCl_3$	96.000	Fe	33.051
$Na_2EDTA \cdot 2H_2O$	300.000		

3. Concentrated solutions of macronutrients and micronutrients can be made to suit individual requirements. The $FeCl_3$ and $Na_2EDTA \cdot 2H_2O$ should be made up as a mixture separately from the other micronutrients and added after filtration of the medium through 0.45-µm membrane filters.

Artificial Seawater Growth Medium for *Dunaliella tertiolecta*

4. For basal medium, use Analytical Reagent or Reagent Grade chemicals.

Compound	g/ℓ	g/4 ℓ
NaCl	23.48	93.92
Na_2SO_4	3.92	15.68
$NaHCO_3$	0.19	0.76
KCl	0.66	2.64
KBr	0.10	0.38
H_3BO_3	0.03	0.10
$MgCl_2 \cdot 6H_2O$	10.61	42.44
$SrCl_2 \cdot 6H_2O$	0.04	0.16
$CaCl_2 \cdot 2H_2O$	1.47	5.88
H_2O to	1,000 ml	4,000 ml

5. For dilution to various salinities:

Salinity, %	Artificial Seawater Stock, ℓ	H_2O (glass distilled), ℓ
35	4.000	0.000
30	3.43	0.57
24	2.74	1.26
20	2.29	1.71
16	1.83	2.17
12	1.37	2.63
8	0.91	3.09
5	0.57	3.43

6. For any given final salinity, mix well, adding the following levels of nutrients:

$NaNo_3$	102 mg/4-ℓ batch (4.2 mg N/ℓ)
K_2HPO_4	4.18 mg/4-ℓ batch (0.186 mg P/ℓ)
Na_2EDTA	1200 µg/4-ℓ batch (300 µg/ℓ)

Filter through 0.45 µ membrane filter, add <u>after</u> filtration, sterilized $FeCl_3$, 384 µg/4-ℓ batch (33.05 µg Fe/ℓ).

7. For Nutrient Algal Assay Medium (NAAM) trace metal solution, add the following per 500 ml of distilled water:

0.0928 g H_3BO_3	0.208 g $MnCl_2$ $4H_2O$
0.016 g $ZnCl_2$	0.714 mg $CoCl_2 \cdot 6H_2O$
0.0107 mg $CuCl_2 \cdot 2 H_2O$	3.63 mg $Na_2MoO_4 \cdot 2H_2O$

Add 1 ml of this concentrate to each litre of medium. Adjust to pH of 8.0 (±0.1), if necessary.

8. Additional information on the algal growth media can be obtained in the EPA's "Algal Assay Procedure: Bottle Test"[5*] and "Marine Algal Assay Procedure: Bottle Test."[6]

Growth Medium for *Caulobacter bacteroides*

9. <u>Caulobacter</u> was grown at 30°C in the following medium (g/ℓ of distilled water): 2.0 peptone, 1.0 yeast extract, and 0.2 mg $SO_4 \cdot 7H_2O$.

Growth Medium for BLA-1 and BLA-2

10. The freshwater bacteria from Brown's Lake were isolated and maintained in culture using the following medium:

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* Raised numbers refer to similarly numbered items in the References at the end of the main text.

Compound	g/ℓ
$CaCl_2 \cdot 2H_2O$	0.02
$MgCl_2 \cdot 6H_2O$	0.02
KNO_3	0.01
$CoCl_2 \cdot 6H_2O$	0.01
NH_4Cl	0.20
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.01
$NaHCO_3$	2.00
KH_2PO_4	0.02
$FeSO_4$	0.01
Yeast extract	0.10

Plus Glacial Acetic Acid at 1.0 ml/ℓ.

11. The KH_2PO_4 and $FeSO_4$ were made as solutions, autoclaved separately and added when all solutions were at room temperature. The $FeSO_4$ solution contained 0.05-percent cysteine-HCl. Two-percent agar was used when solid medium was required.

Growth Medium for MW40C and MB 22

12. The marine bacteria were grown on the following medium:

Compound	g/ℓ
NaCl	24.0
KCl	0.7
$MgCl_2 \cdot 6H_2O$	5.3
$MgSO_4 \cdot 7H_2O$	7.0
Phytone	1.0
Nutrient gelatin	10.0
Yeast extract	0.1

AD-A040 087 ARMY ENGINEER WATERWAYS EXPERIMENT STATION VICKSBURG MISS F/G 13/2
BIOLOGICAL ASSESSMENT OF THE SOLUBLE FRACTION OF THE STANDARD E--ETC(U)
MAR 77 P J SHUBA, J H CARROLL, K L WONG

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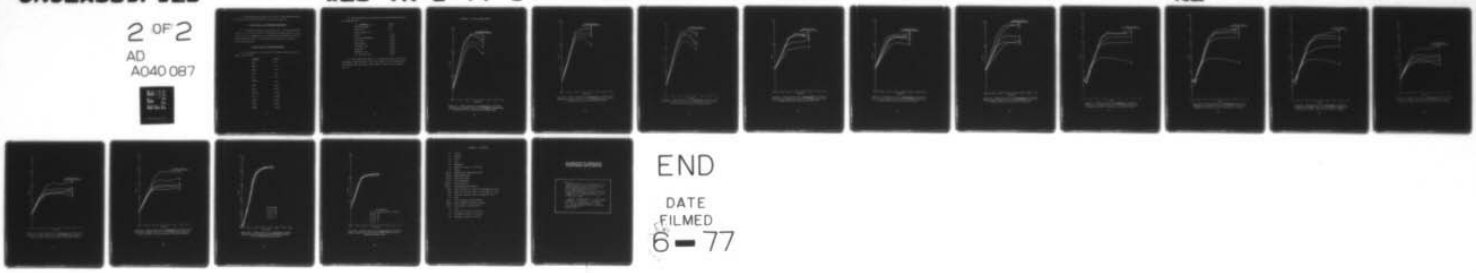

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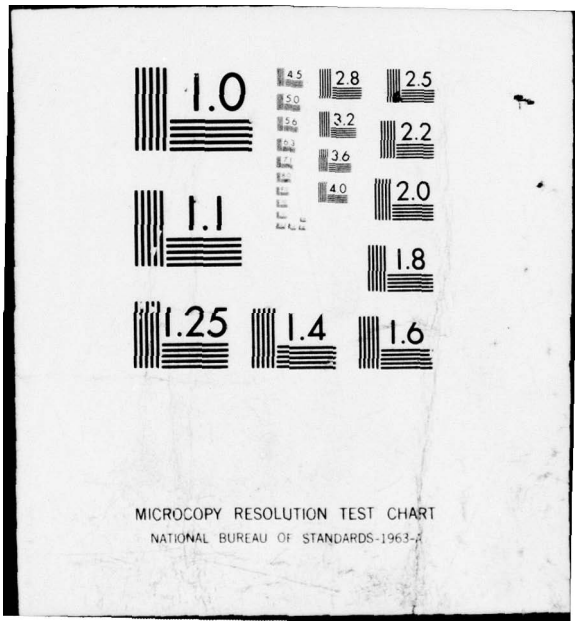
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MICROCOPY RESOLUTION TEST CHART
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13. The medium was dispensed into tubes or flasks and autoclaved. Two-percent agar was used when solid medium was required.

Growth Medium for *Tetrahymena pyriformis*

14. Ten grams of proteose peptone and 1.0 g of liver extract were added to 1 ℓ of distilled water. The mixture was heated gently to dissolve most of the components, then centrifuged at 2000 rpm for 10 min to remove particulate matter. The medium was dispensed into tubes of flasks and autoclaved.

Growth Medium for *Uronema nigricans*

15. The composition of the artificial seawater used to grow the marine protozoan was:

Compound	g/ℓ
NaCl	28.3
$MgSO_4$	3.43
$MgCl_2$	2.40
$CaCl_2$	1.22
KCl	0.76
$NaHCO_3$	0.21
NaBr	0.082
H_3BO_4	0.062
$NaSi_4O_9$	0.0098
Al_2Cl_3	0.0066
H_3PO_4	0.0049
$LiNO_2$	0.0035
NH_4OH	0.0018

16. The concentration of nutrients and vitamins added to artificial seawater were:

Compound	g/ℓ
Proteose peptone	10.0
Tryptricase	10.0
Yeast nucleic acid	1.0
Biotin	0.0001
Calcium panthothenate	0.0010
Folic acid	0.0005
Nicotinamide	0.0005
Pyridoxal · HCl	0.0005
Riboflavin	0.0005
Thiamine · HCl	0.0150
DL - thioctic acid	0.0001

17. The vitamins were made as a concentrated mixed solution and dispensed in small portions. These were gassed with nitrogen, sealed in airtight vials and frozen until needed. Final pH of the medium was 7.2.

APPENDIX B: ALGAL GROWTH CURVES

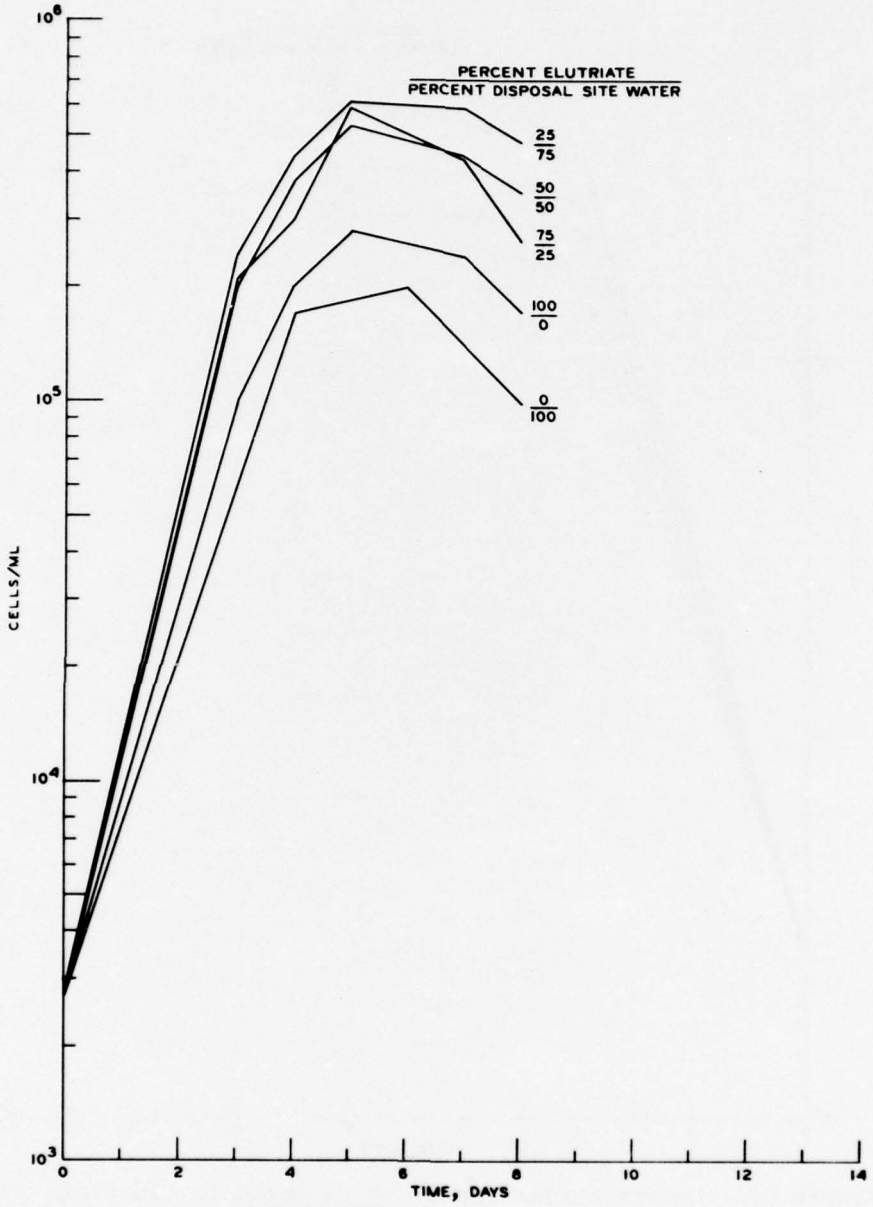

Figure B1. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 1 of Bridgeport Harbor and disposal site water collected from Eatons Neck

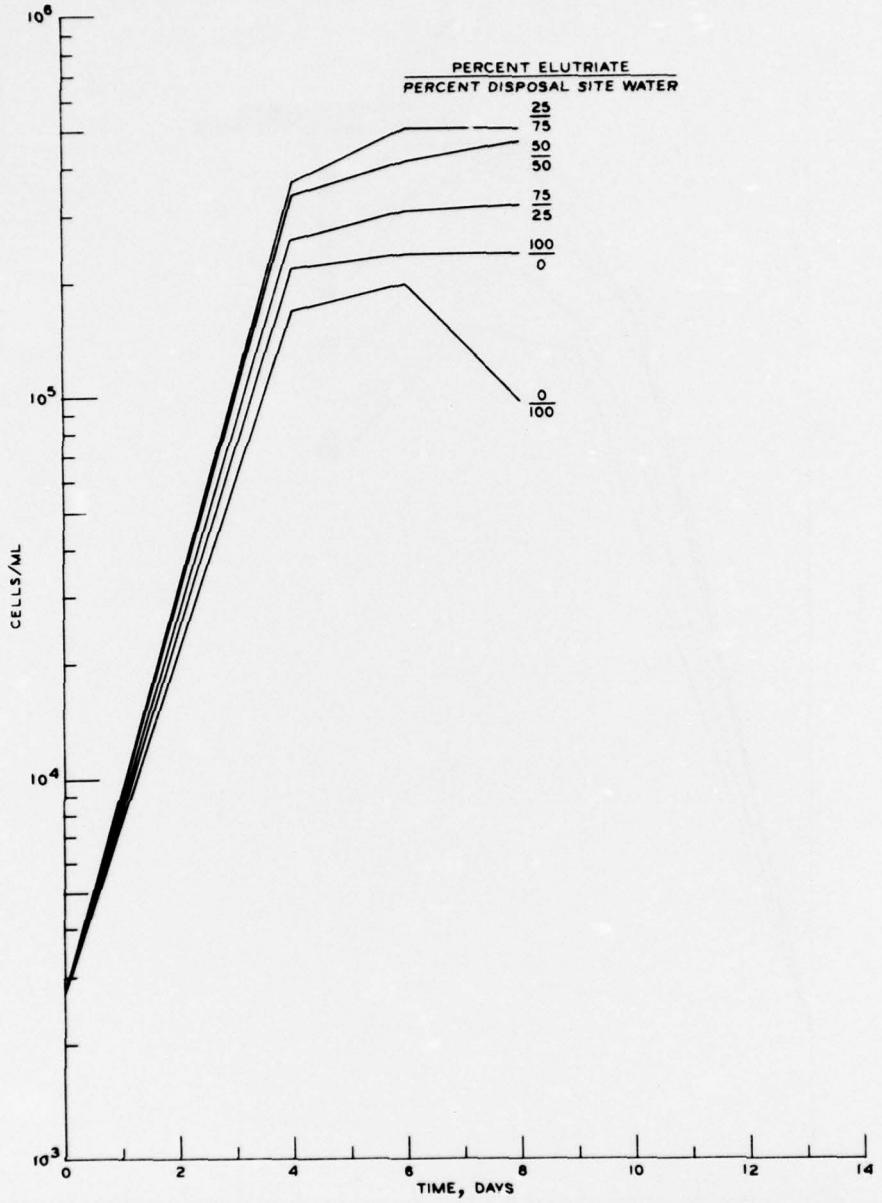

Figure B2. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 2 of Bridgeport Harbor and disposal site water collected from Eatons Neck

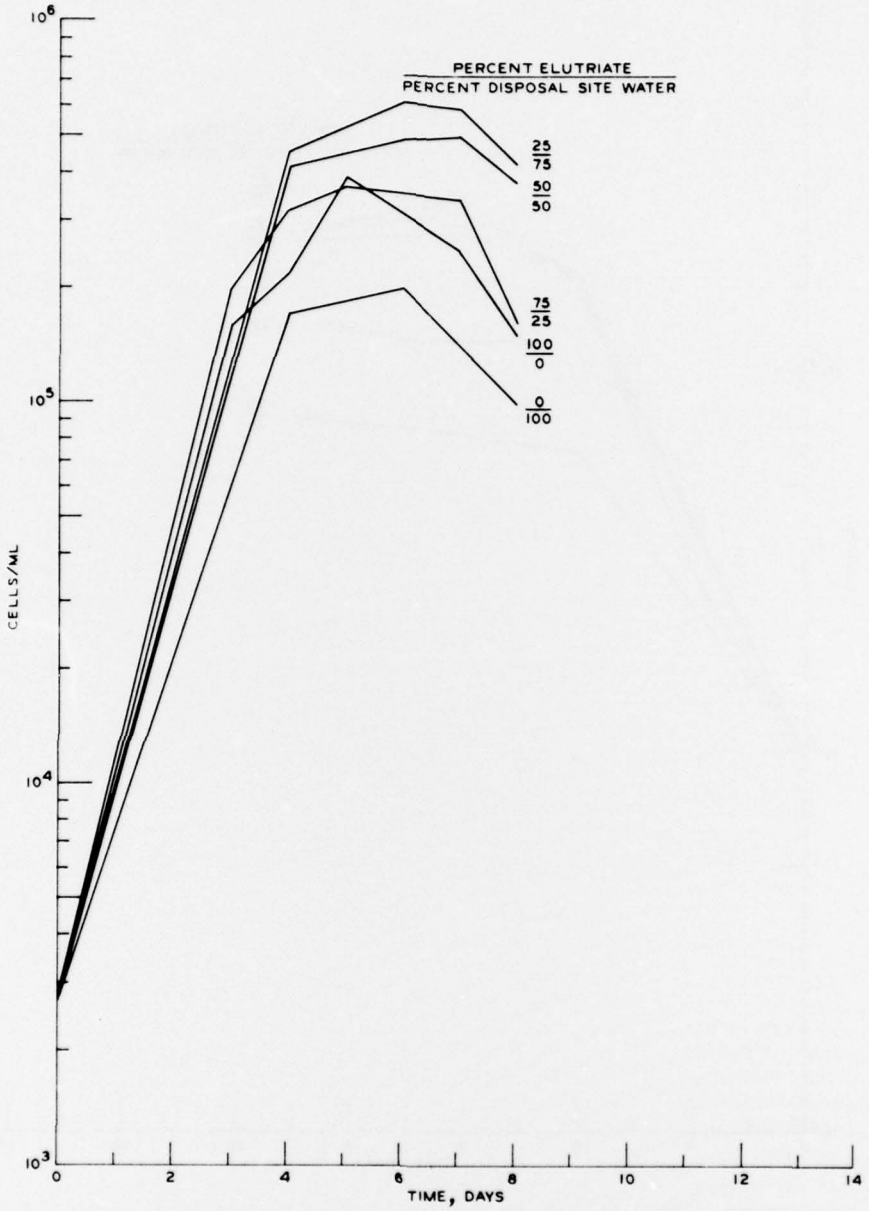

Figure B3. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 3 of Bridgeport Harbor and disposal site water collected from Eatons Neck

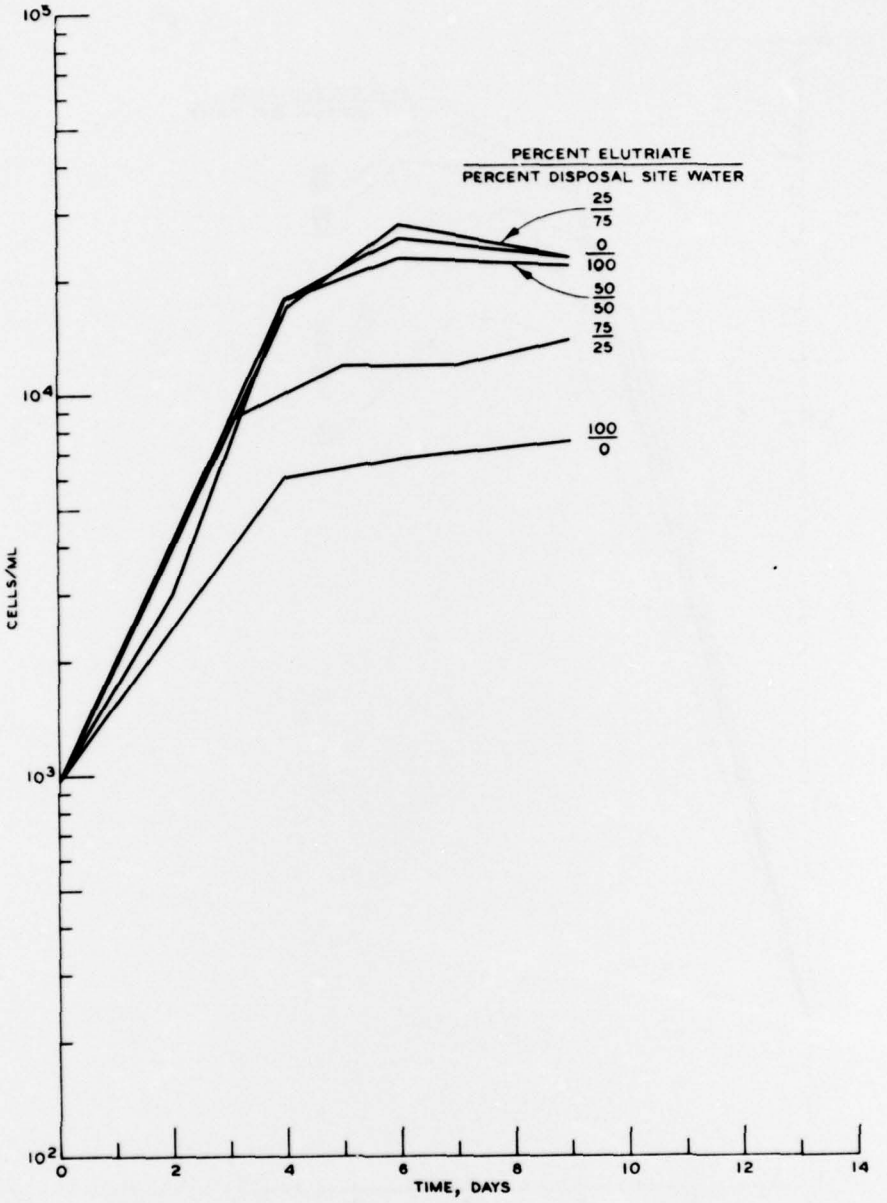

Figure B4. Growth curves for S. capricornutum in elutriate prepared with sediment from site 1 of Ashtabula Harbor and disposal site water collected from Lake Erie

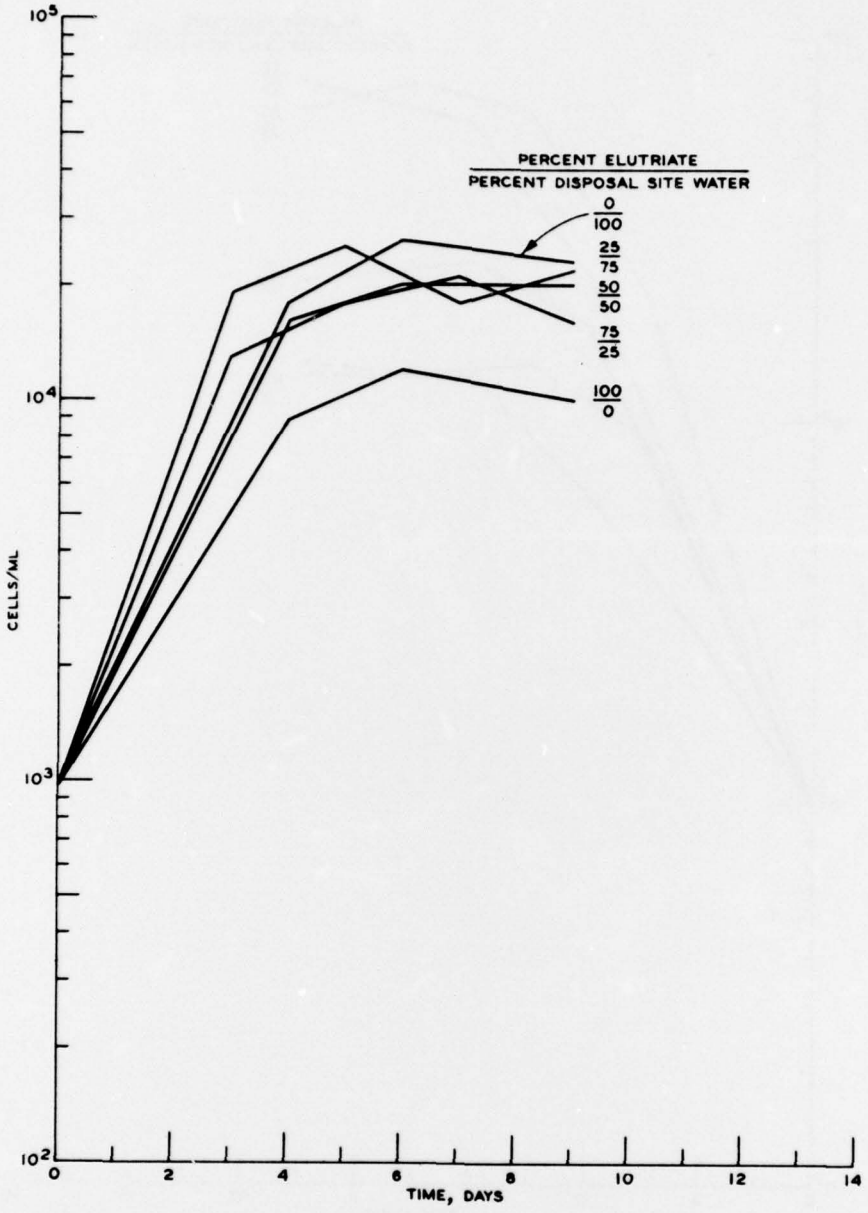

Figure B5. Growth curves for S. capricornutum in elutriate prepared with sediment from site 2 of Ashtabula Harbor and disposal site water collected from Lake Erie

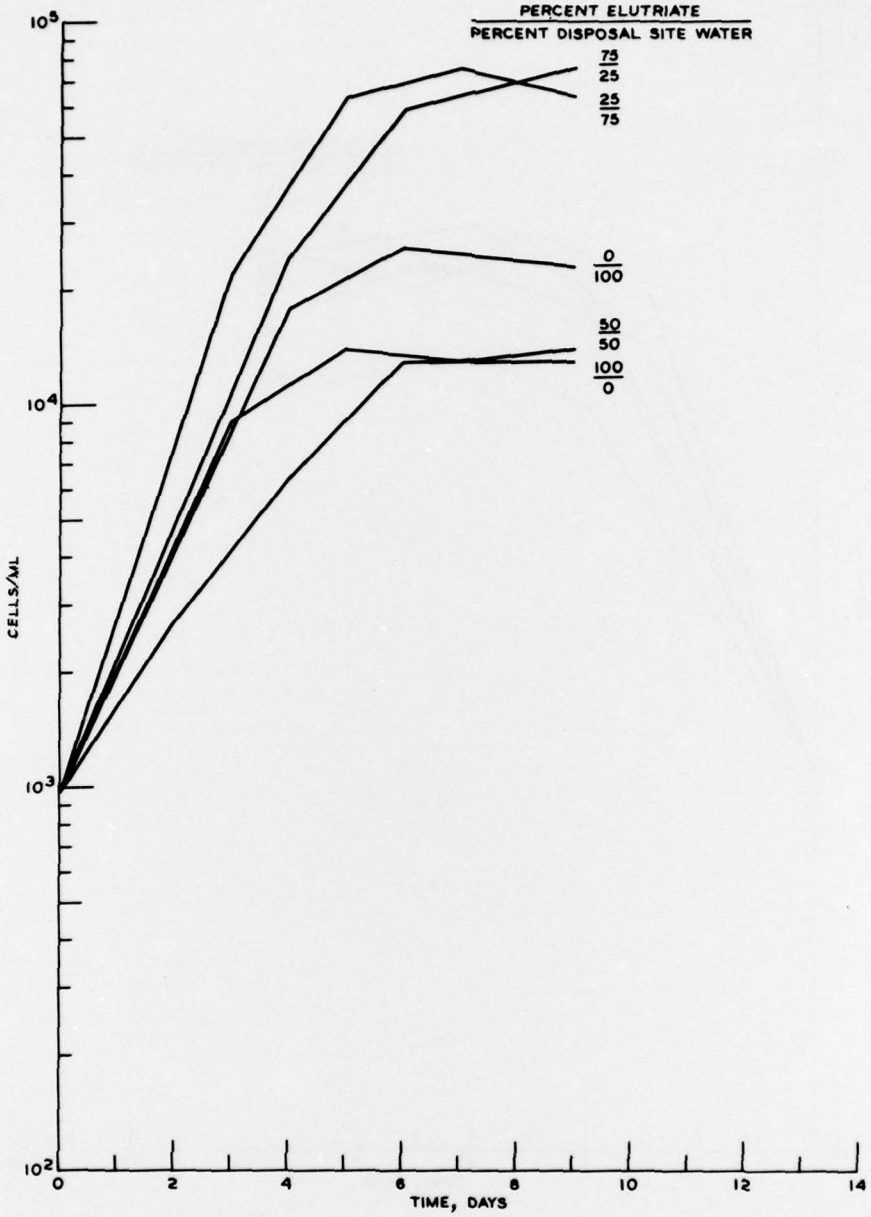

Figure B6. Growth curves for S. capricornutum in elutriate prepared with sediment from site 3 of Ashtabula Harbor and disposal site water collected from Lake Erie

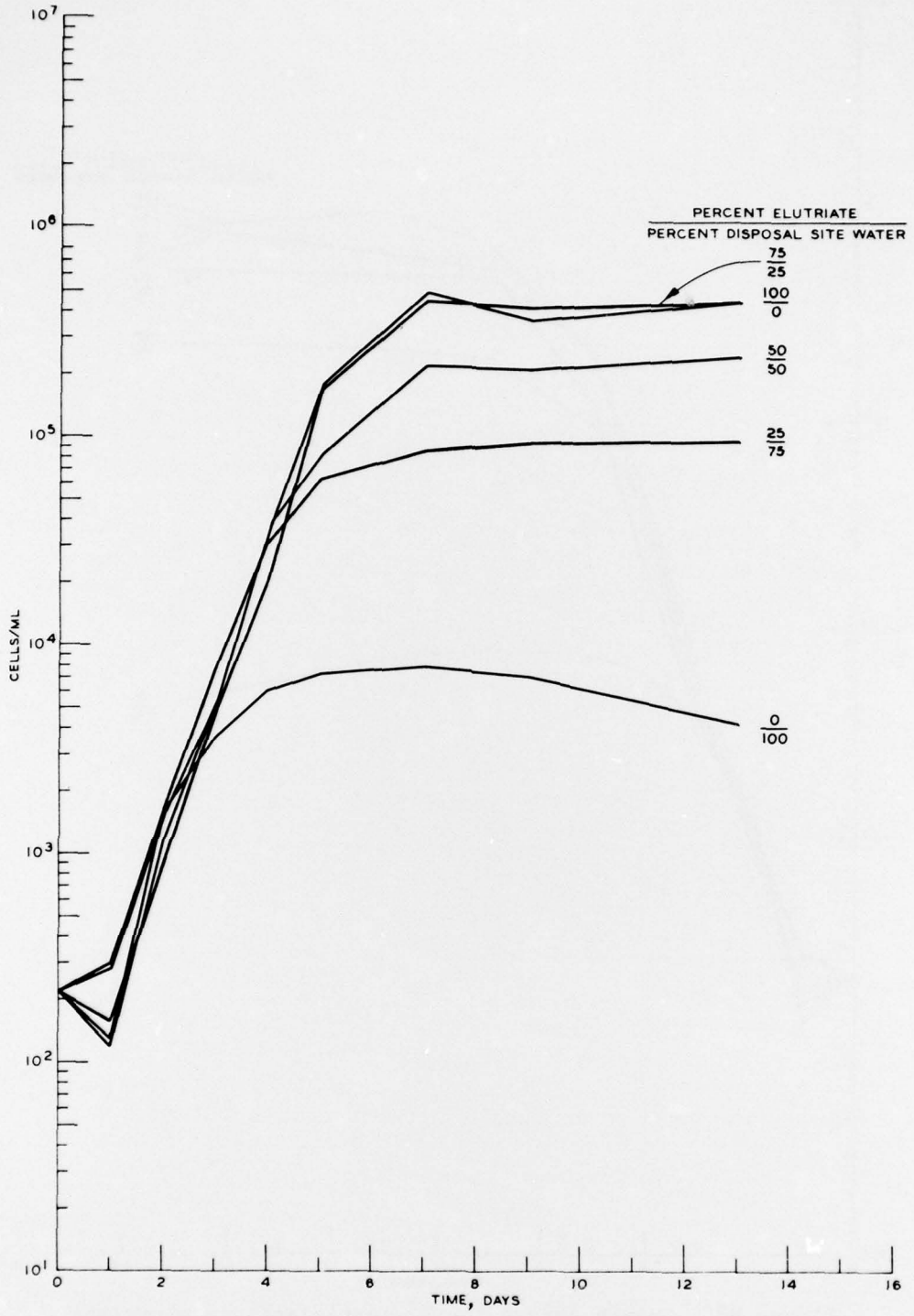

Figure B7. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 1 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

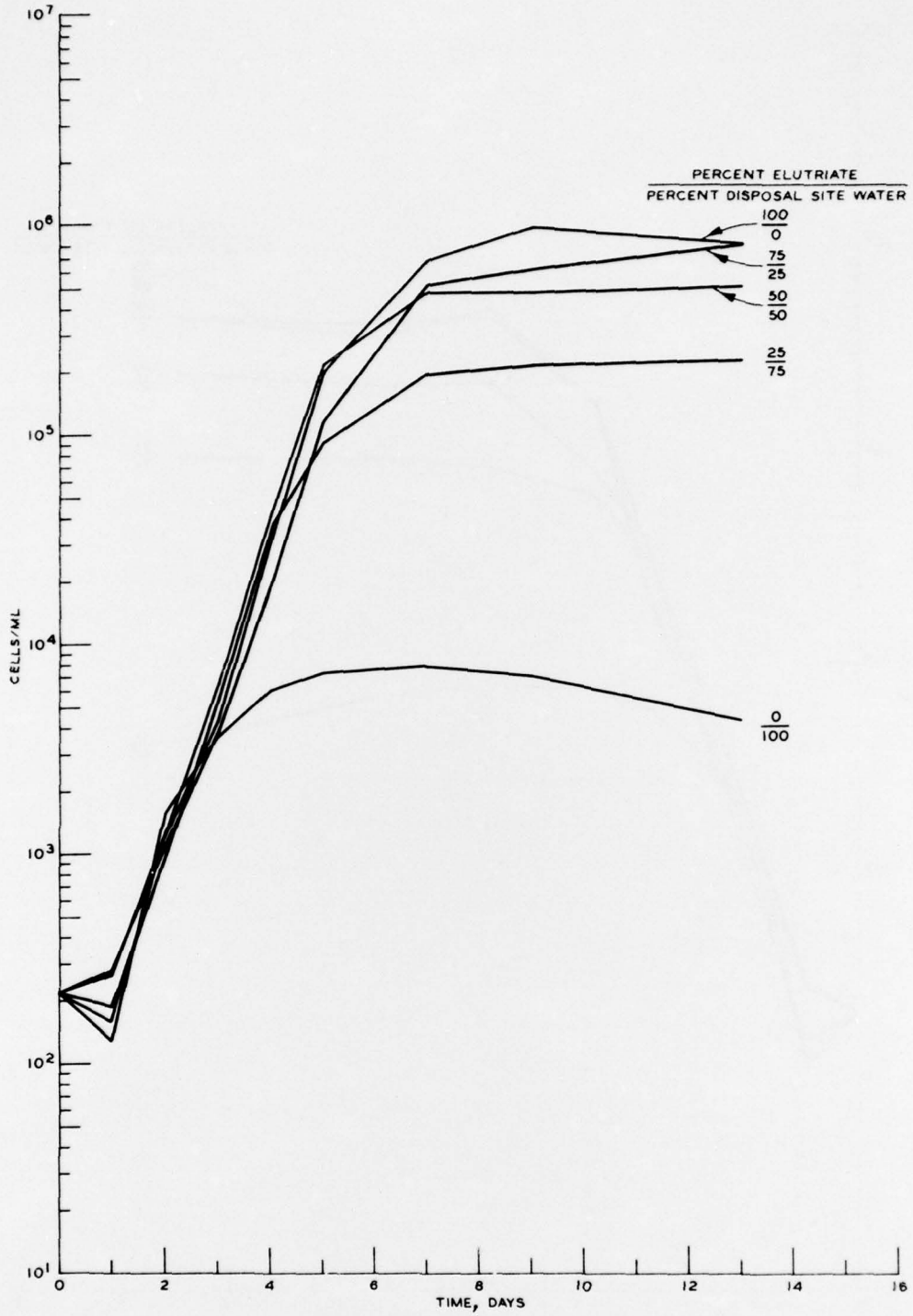

Figure B8. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 2 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

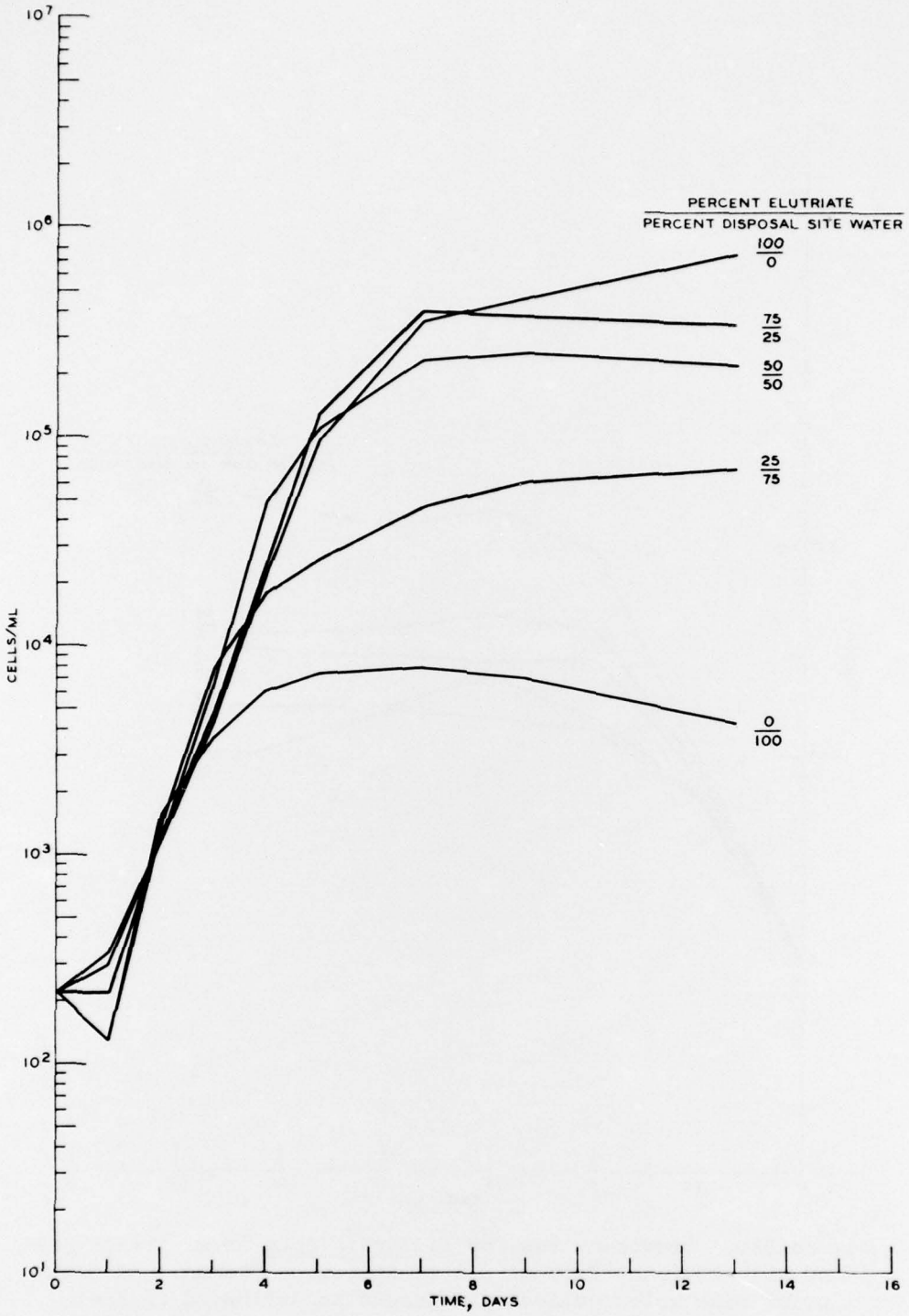

Figure B9. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 3 of Galveston Harbor and disposal site water collected from the Gulf of Mexico

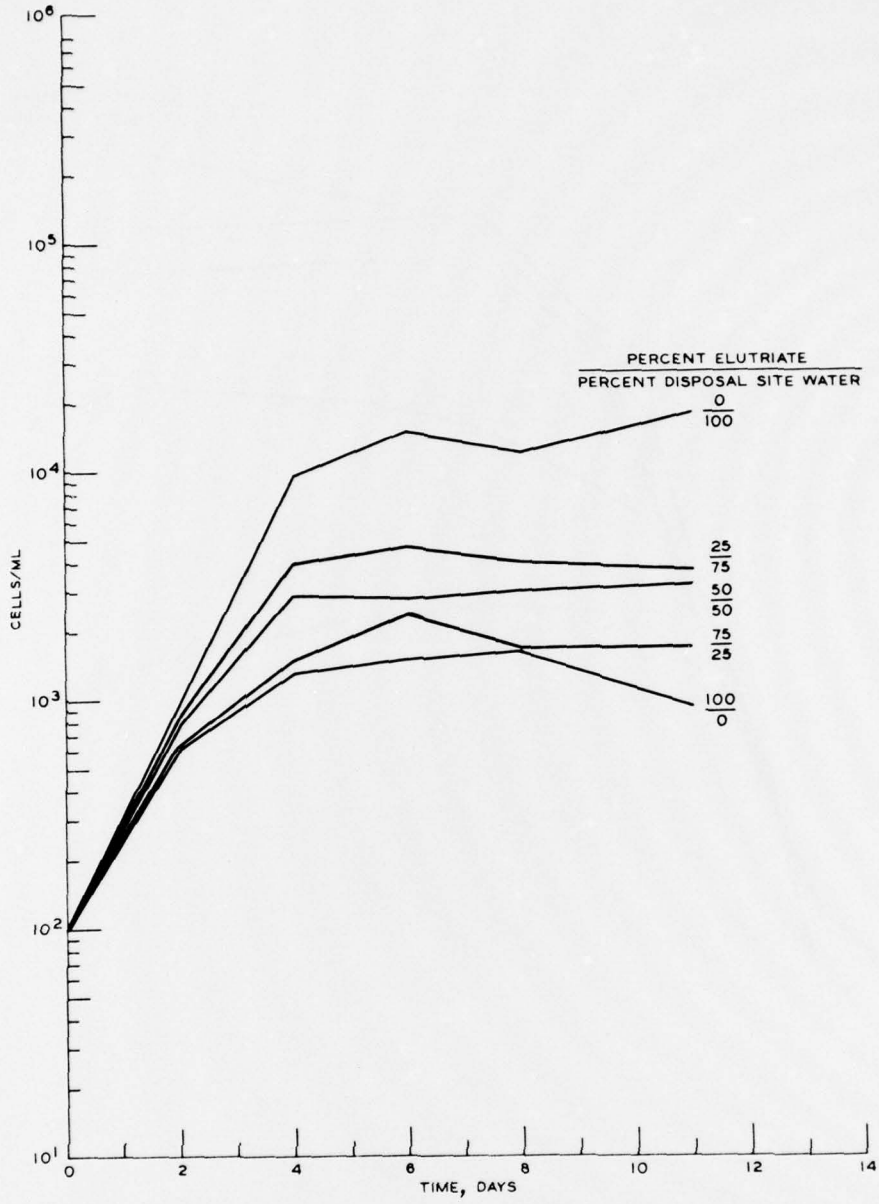

Figure B10. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 1 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

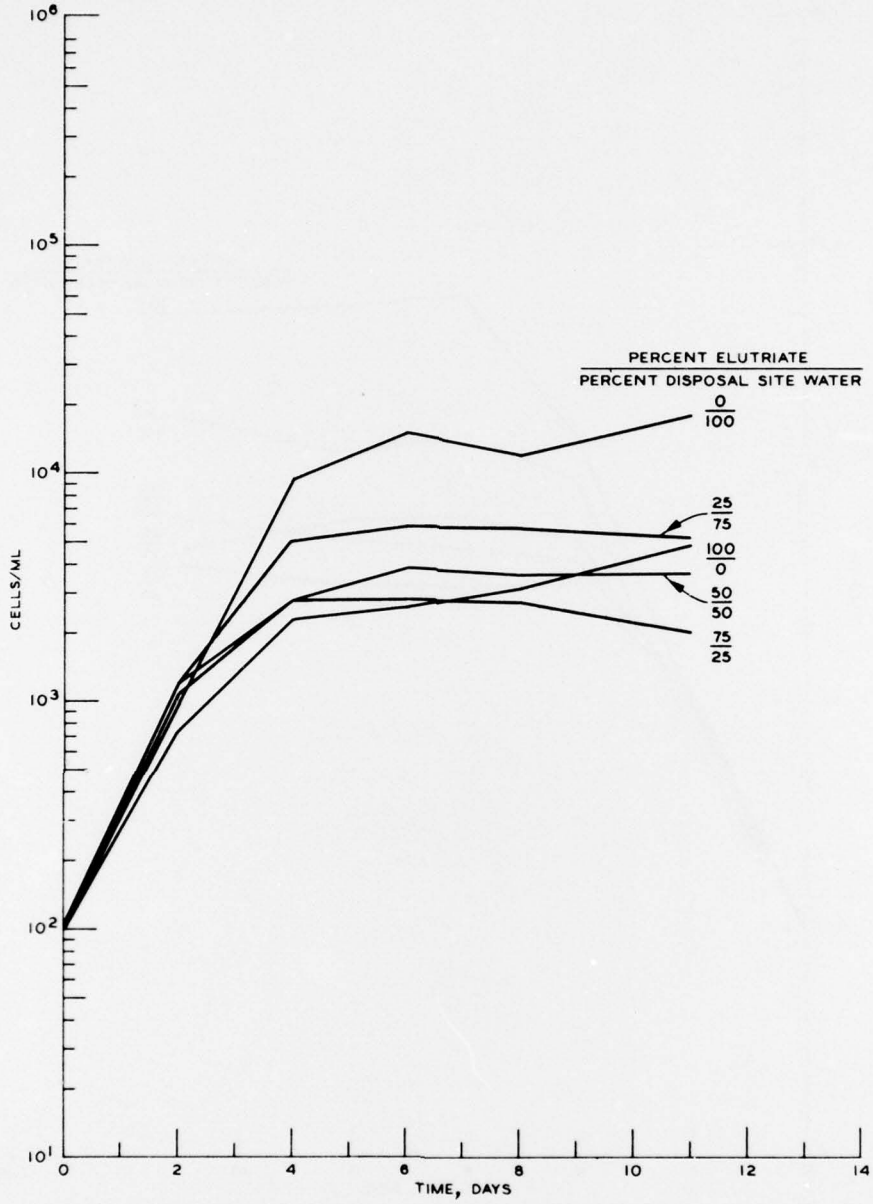

Figure B11. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 2 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

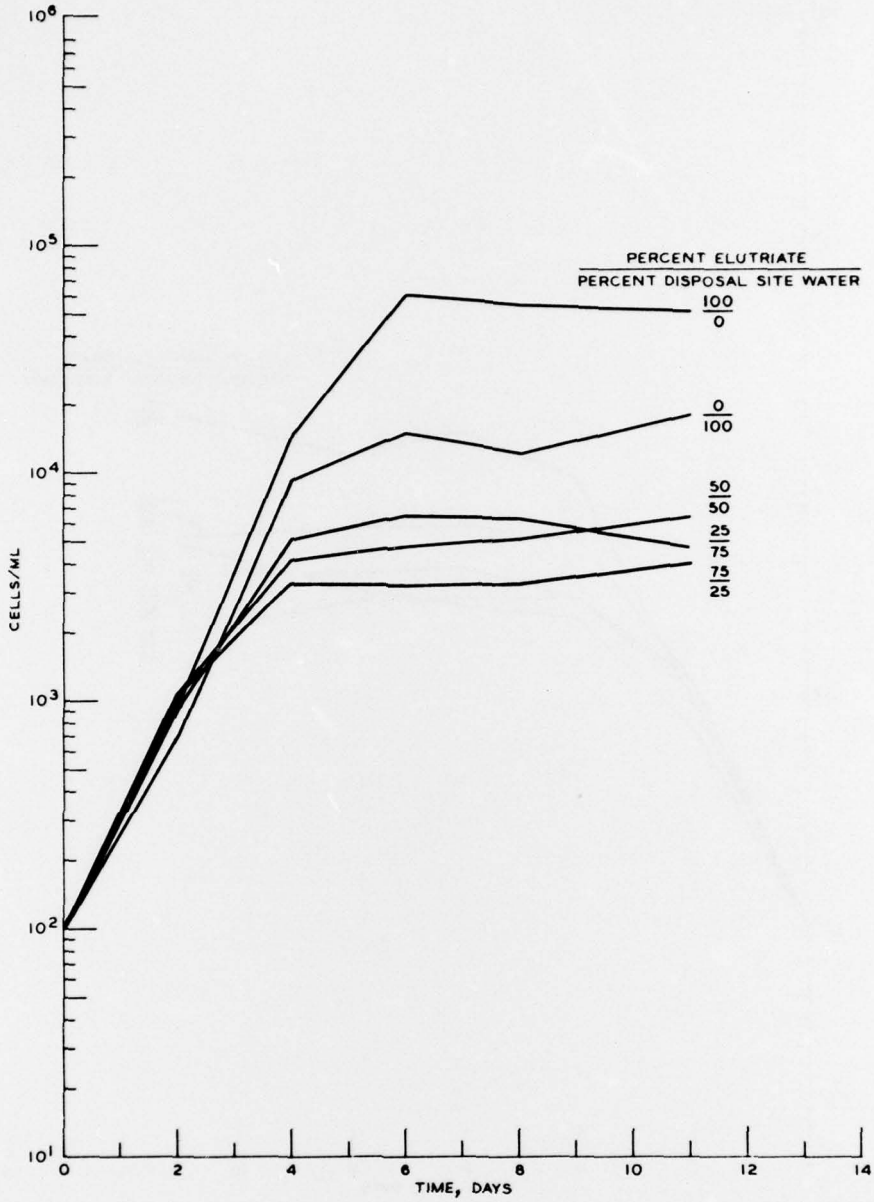

Figure B12. Growth curves for D. tertiolecta in elutriate prepared with sediment from site 3 of Arlington Channel and disposal site water collected adjacent to Arlington Channel

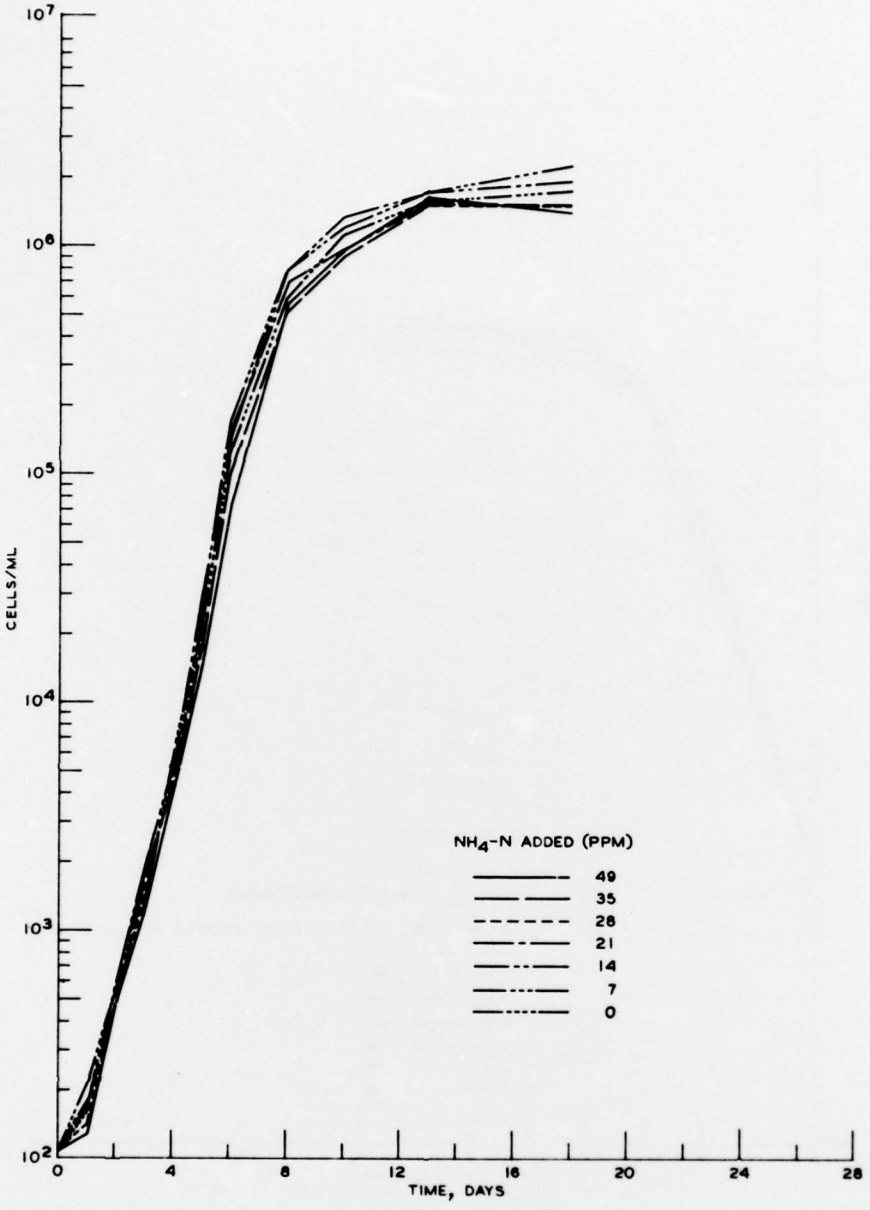

Figure B13. Growth curves for D. tertiolecta in algal assay procedure growth medium with various concentrations of ammonium-nitrogen added

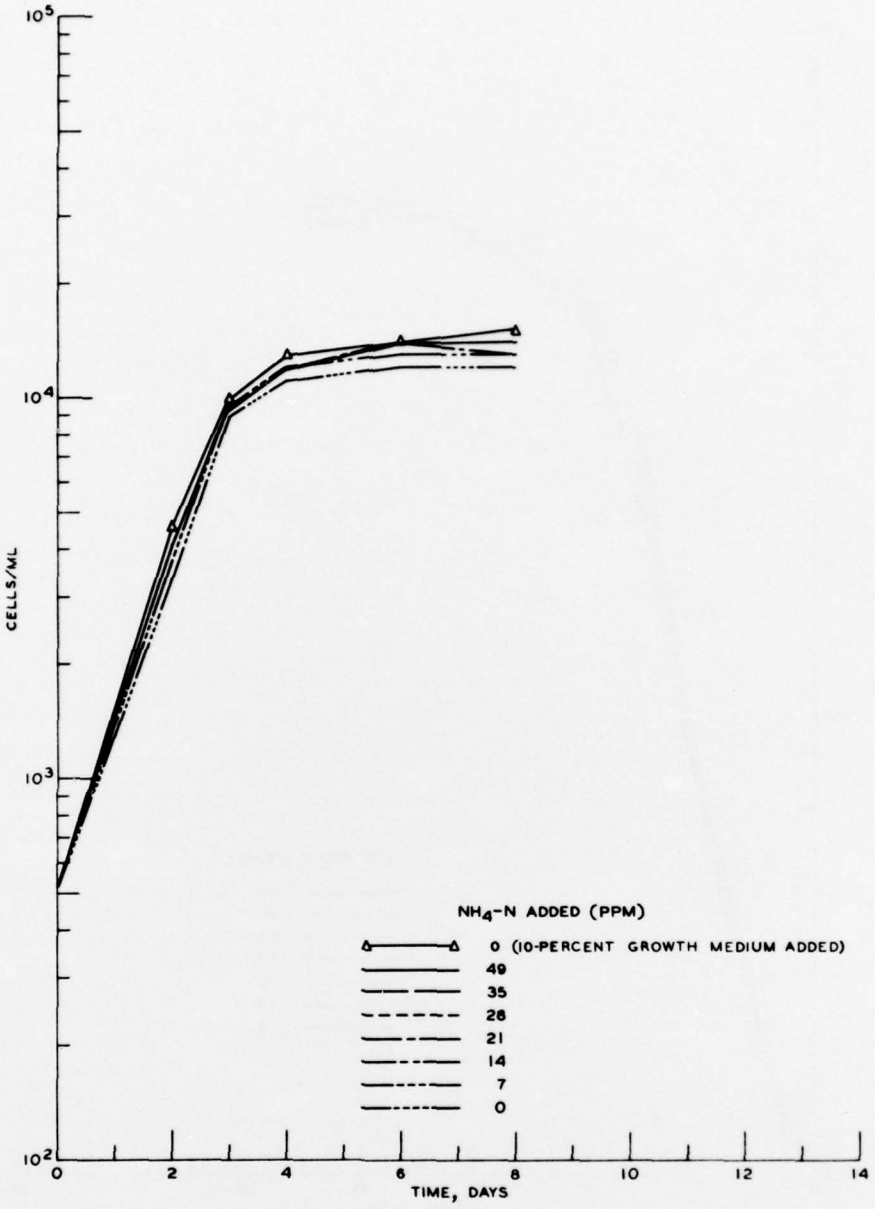

Figure B14. Growth curves for D. tertiolecta in Arlington Channel disposal site water with various concentrations of ammonium-nitrogen added

APPENDIX C: NOTATION

As	Arsenic
Cd	Cadmium
Cu	Copper
Fe	Iron
Mn	Manganese
nm	Nanometre, equal to 10^{-9} metre
Ni	Nickel
NH_3-N	Ammonium plus Ammonia-Nitrogen
NH_4-N	Ammonium-Nitrogen
NO_2-N	Nitrite-Nitrogen
NO_3-N	Nitrate-Nitrogen
NO_3-NO_2	Nitrate-Nitrite
OPO_4-P	Orthophosphate-Phosphorus
ppb	Parts per billion, equal to micrograms per litre
ppm	Parts per million, equal to milligrams per litre
ppt	Parts per thousand, equal to grams per litre
Pb	Lead
TIC-C	Total Inorganic Carbon--Carbon
TKN-N	Total Kjeldahl Nitrogen--Nitrogen
TOC-C	Total Organic Carbon--Carbon
Zn	Zinc
µl	Microlitre, equal to 10^{-6} litre
µm	Micrometre, equal to 10^{-6} metre
µW	Microwatt, equal to 10^{-6} watt

In accordance with ER 70-2-3, paragraph 6c(1)(b), dated 15 February 1973, a facsimile catalog card in Library of Congress format is reproduced below.

Shuba, Peter J

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Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C., under DMRP Work Unit No. 1E06.

Includes bibliography.

1. Bioassay. 2. Dredged material. 3. Elutriate tests. 4. Sediment. 5. Water quality. I. Carroll, Joe H., joint author. II. Wong, Karon L., joint author. III. U. S. Army. Corps of Engineers. (Series: U. S. Waterways Experiment Station, Vicksburg, Miss. Technical report D-77-3)

TA7.W34 no.D-77-3