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Project Summary

Development of Bioassay Procedures for Defining Pollution of Harbor Sediments

Donald A. Bahnick, William A. Swenson, Thomas P. Markee, Daniel J. Call, Craig A. Anderson, and R. Ted Morris

This research investigates bioassay methods which may be useful in assessing the degree of pollution of harbor sediments. Procedures studied include 96-hr toxicity tests employing *Hexagenia limbata, Daphnia magna* and *Pontoporeia affinis* as biological probes, monitoring cough frequencies of bluegill sunfish (*Lepomis macrochirus*) in interstitial water derived from sediments, chemical analyses of sediment-water systems, and chemical analysis of chironomids and *Hexagenia limbata* exposed to the sediments. Additional experiments involved investigation of the degree of removal of chemical constituents from sediments due to extraction with Lake Superior water and the use of reverse phase liquid chromatography in detecting the presence of chemical compounds with high bioaccumulation potential in the sediments.

Sediment-water systems, employing sediments from the Duluth-Superior harbor and Lake Superior, caused little toxicity in 96-hr exposure tests with *Daphnia magna, Hexagenia limbata* and *Pontoporeia affinis* although *Daphnia* was the most sensitive of these animals to toxicants.

Similar cough frequencies were found for bluegill sunfish in dechlorinated city water compared to sediment interstitial water—Lake Superior water mixtures but it was observed that broken opercular patterns occurred in the interstitial water—Lake Superior water mixtures.

Most of the sediments used in the bioassay tests would be considered polluted according to at least one chemical parameter. Chemical analyses showed the presence of low amounts of PCBs in the sediments. Most of the heavy metals primarily resided in the residual phase of the sediments.

Studies on extracting chemical species from the sediments showed that only small amounts could be readily extracted with Lake Superior water.

Evidence was found that chironomids dwelling in harbor sediments bioaccumulated PCBs and p,p'-DDE and possibly some metals.

A general toxicity index was prepared from the chemical data which indicated that animal survival in the 96-hr acute toxicity tests was generally lower using sediment systems from the more industrialized areas of the harbor.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The need for maintaining vessel accessibility to our nation's waterways requires continuous dredging of large volumes of sediment by the Corps of Engineers. Reliable, cost-effective procedures for identifying the potential effects of sediment chemical contaminants on water quality and aquatic communities are needed.

Current criteria used for the evaluation of the quality of Great Lakes harbor sediments are based largely on sediment chemical parameters. It is recognized that a comprehensive evaluation procedure should include short-term bioassay tests in evaluating the toxic effects of sediments on fish, benthos and plankton; and also tests which will assess the bioaccumulation potential of sediment chemicals in flora and fauna resulting in magnification in aquatic food chains.

This research attempts to develop procedures designed to assess potential harmful effects of harbor sediments subjected to dredging. Applicability, ease of duplication and cost effectiveness were taken into consideration in procedure development.

Sediments collected during 1977 and 1978 from six sites in Duluth, Minnesota and Superior, Wisconsin harbor area and one Lake Superior site were used in preparing systems containing water overlying a sediment substrate, containing interstitial water, or containing elutriate water. Acute 96-hr toxicity tests were carried out by exposing *Hexagenia limbata, Daphnia magna* and *Pontoporeia affinis* to certain of these systems compared to exposures to identically prepared controls.

The exposure systems consisted of sediment, water overlying the sediment, interstitial water extracted from the sediment under anaerobic conditions, elutriate water prepared from Lake Superior water mixed with sediment which was either exposed to air or kept under nitrogen prior to elutri-ate formation and Lake Superior water used to extract water solubles or colloids (generated pore water). During 1978, particulate phase elutriate water was also used as an animal exposure system.

All bioassays were conducted in an environmentally controlled area of the University of Wisconsin-Superior wet laboratory under controlled light and temperature conditions. Oversediment bioassays were designed to incorporate the mechanisms for transfer of toxic substances between sediments and benthic or planktonic communities using *Daphnia magna* and *Hexagenia limbata. Pontoporeia affinis* bioassays were used to measure acute toxicity of liquid phase elutriate water. Ninety-six hr toxicity tests employing *Daphnia magna* exposed to sediment interstitial water and elutriate water were also conducted.

Bluegill sunfish (*Lepomis macrochirus*) were monitored for cough frequencies and breathing patterns in mixtures of Lake Superior water with interstitial water extracted from the various sediments. Sixteen fish of comparable size were used in each test. The tests were conducted in systems composed of electrode chambers containing separated compartments. Each compartment was equipped with stainless steel electrodes which detected action potential resulting from muscular activity associated with breathing. The action potentials were recorded by a Gilson IMP-5H 4-channel physiograph.

The bluegill breathing patterns and cough responses were recorded for fish in dechlorinated city water and in Lake Superior-sediment interstitial water mixtures.

Sediments and interstitial waters used in bioassay tests were extensively analyzed for a variety of chemical parameters. The chemical testing of sediments included determinations of total metals, metals associated with different phases of the sediments, certain inorganic non-metal substances, particle size, pH, Eh and some trace organics (PCBs, pesticides and PAH compounds). Chemical tests on interstitial water included a number of the same chemical parameters as investigated for sediments.

Chironomids were collected from various harbor sites by isolating them from sieved sediment. These chironomids, along with *Hexagenia limbata* exposed for 96 hrs to harbor sediments, were analyzed to determine body burdens of specific organic compounds (PCBs, PAH and pesticides) and some heavy metals.

Using the results from the animal toxicity tests and the chemical analyses, attempts were made to develop a general index of toxic potential and chemical quality of harbor sediments in trying to identify correlations between sediment chemistry and animal survival for the acute toxicity tests.

The degree of removal of chemical constituents from sediments subjected to mixing with Lake Superior water was investigated by repetitive additions of Lake Superior water to sediments, separating the water from the sediments by high speed centrifugation and chemical analysis of the water phases. The total amount of each chemical released from the sediments to the water phase after numerous additions and removal of water was tabulated.

The concentrations of organic chemicals with high bioaccumulation potential contained in harbor sediments, harbor chironomids, and sediment exposed *Hexagenia limbata* were investigated using high pressure reverse phase liquid chromatography. Evidence has shown that organic chemicals with high n-octanol/water partition coefficients tend to bioaccumulate in the lipids of aquatic animals. The chemicals having high partition coefficients tend to have high retention times on a reverse phase chromatographic column. Organic solvent extracts from harbor sediments, chironomids and *Hexagenia* were injected into a high pressure liquid chromatograph employing a reverse phase column. Eluted compounds were detected by a UV detector.

Conclusions

The acute toxicity tests using sediment-water systems resulted in generally low toxicity to *Hexagenia limbata, Daphnia magna* and *Pontoporeia affinis.* The high survival of test animals indicated low levels of available toxicants in the sediments. In 96-hr bioassays, survival was found to be significantly lower for test sites compared to controls in only a few tests. The results demonstrated that the animals could be successfully maintained in the complex test systems and that the sediment-water systems caused low acute toxicity. This low toxicity in combination with low precision between replicates generally resulted in finding no significant differences in animal survival between test and control. Among the test species employed, *Daphnia magna* appeared to be the most sensitive to toxicants.

It was necessary to design the tests following approved criteria for ecological evaluation of dredging and dredge spoil disposal in marine systems. Following these criteria, controls for the bioassay tests were derived from sediments from Lake Superior and a relatively undisturbed area of the harbor. Because these sediments contained varying quantities of many toxic substances, their use negated accurate determination of the sensitivity of the bioassay procedures. It is therefore recommended that future studies aimed at identifying screening procedures incorporate more effective controls.

The use of *Daphnia magna, Hexagenia limbata* or *Pontoporeia affinis* as test organisms for potential toxic effects of sediments should be considered further. Of these species, *Daphnia magna* appears to be most suited as a test organism due to ease of culturing, sensitivity, and the large amount of information available on the response of *Daphnia* to specific chemicals. Further tests would be useful employing sediment samples having greater variation in chemical quality. Recognizing that sediments used in this study contained large quantities of potentially toxic heavy metals in unavailable or non-toxic forms, it is important to develop better understanding of the conditions which would result in transformation to available forms and the effects that such transformations woud have. Comparisons of toxicity results to other recently developed toxicant screening techniques such as algal or luminescent bacteria assays are recommended.

The average cough frequencies of bluegills in dechlorinated city water and in interstitial water from the sampling site sediments mixed with Lake Superior water were generally similar. Cough frequencies during the first 22 to 25 hrs of fish exposure were found to be elevated above the frequencies observed for the control for three of the six sites studied. Bluegill opercular activity was nearly continuous in dechlorinated city water in contrast to broken patterns of activity observed in interstitial water—Lake Superior water mixtures formed from sediments obtained from five of the six sampling sites.

Based on our observations, bluegill cough frequencies are difficult to interpret and their usefulness in determining differences in sediment quality was limited due to observed similarities in results for the various sites. The data suggests that extensive experience in conducting fish cough response tests is necessary to interpret the results, and therefore the technique has limited application as a general screening procedure.

Chemical analysis for heavy metals in the sediments revealed that the residual phase of the sediments contained the highest concentrations of most metals. However, measurable amounts (>1 mg/kg) of arsenic, cobalt, copper, nickel and zinc were found in the organic and sulfide sediment phases for nearly all the samples, and selenium and cadmium were found in these phases for some of the samples.

PCB concentrations in the sediments ranged from 0.3 to 2.1 mg/kg based on a dry sediment basis. These values are not high compared to the 10 mg/kg or above level for polluted sediments. In addition to PCBs, low levels of polycyclic aromatic hydrocarbons were found in two of the harbor sediments and low levels (1 to 15 μg/kg) of pentachlorophenol were detected.

Chemical analysis of sediment interstitial water showed that many of the chemical species were probably associated with very fine (possibly colloidal) particles in the water. The concentrations of many of the metals were much lower in filtered water samples compared to non-filtered samples.

The concentrations of chemicals found in liquid phase elutriate water did not change greatly when prepared from an exposed sediment compared to sediment unexposed to air.

Studies on determining the amounts of chemical species which would be released upon flushing the sediments with Lake Superior water showed that only about one percent or less of most of the chemical parameters (COD, Fe, Mn, Ni, Pb, Cu, Zn, Hg) was removed from the sediments by water extraction. These results indicated that the measured chemical species were not readily available to water except when associated with particulates.

Although survival of test organisms was generally high during the 96-hr toxicity tests, some correlations of survival of *Daphnia magna* in water overlying sediments (1977 tests) with chemical parameters were found. Many of these correlations involved the concentrations of metals in the sediment, in interstitial water removed from the sediment or in elutriate water formed by mixing sediment with Lake Superior water.

Some correlations were found between *Daphnia magna* survival in elutriate water—Lake Superior water mixtures and individual chemical parameters. Correlations were found between iron concentrations in interstitial water removed from the sediments and *Daphnia* survival.

On the basis of one or more chemical parameters, most of the sediment samples used in the bioassay tests would be classified as polluted according to the currently used sediment evaluation criteria. Ranking the sediments according to their concentrations of a large number of metals, inorganic nonmetals and organic chemical parameters indicated that sites located in the Superior harbor (near the Superior entry to Lake Superior) and a Lake Superior site were less polluted than sites located near the more industrialized zones in the harbor.

A general index of toxic effects, developed by considering the relative percentage of low survival for the various acute toxicity tests, showed that survival was generally lower in test systems derived from sediments in the industrialized areas of the harbor compared to the less developed areas and the Lake Superior site. Comparing the percent low survival values for the sites to rankings based on sediment chemical results indicated a positive correlation ($r = 0.80$, $P > 0.1$). A lower correlation coefficient was found between rankings based on interstitial water chemistry and the percent low survival values. These results indicate that the combined chemical test for the sediments were a fair indicator of general toxicity.

Further analysis of bioassay results and chemical characteristics of the sediments is desirable. If correlations hold for a wide variety of sediments, additional understanding of the causes of the observed toxicity results may be obtained.

Evidence was found that chironomids dwelling in the harbor sediments had accumulated PCBs and p,p'-DDE. Compared to PCB levels in the dried sediment samples, bioaccumulation factors of 11 to 18 times were found in the animal tissues. Bioaccumulation factors for p,p'-DDE in chironomids compared to dry sediment values ranged from 10 to 40.

For *Hexagenia limbata* exposed to sediments for 96 hrs, tissue/sediment bioaccumulation factors for PCBs were 4.8 (two harbor sediments) and 8.2 (Lake Superior sediment) while tissue/sediment bioaccumulation factors for p,p'-DDE were about 25 to 30.

The amounts of PCBs associated with ingested sediment in chironomid and *Hexagenia* samples were less than 5% of the total PCBs in these animals.

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The results of bioaccumulation studies of heavy metals in chironomids indicate that the measured amounts of most of the metals arose from ingested inert material contained in their digestive tracts. However, limited evidence suggested that the observed concentrations of mercury, cadmium and chromium in chironomids were due to accumulation in tissues.

Chromatograms of organic extracts from sediments, chironomids and *Hexagenia limbata*, using reverse phase high pressure liquid chromatography, showed the presence of organic compounds with high bioaccumulation potential; although no attempt was made to identify and quantitate chemicals. This method of screening samples for the amounts of bioaccumulated organic compounds is potentially useful.

Screening of sediment extracts or extracts of animals exposed to sediments using reverse phase high pressure liquid chromatography should be further investigated. In particular, the eluent fractions containing organic compounds with high lipid solubility should be studied for methods to quantitate and perhaps identify highly bioaccumuable compounds.

Donald A. Bahnick, William A. Swenson, Thomas P. Markee, Daniel J. Call, Craig A. Anderson, and R. Ted Morris are with the University of Wisconsin, Superior, WI 54880.
***Richard Anderson** is the EPA Project Officer (see below).*
The complete report, entitled "Development of Bioassay Procedures for Defining Pollution of Harbor Sediments," (Order No. PB 81-178 261; Cost: $15.50, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Environmental Research Laboratory
U.S. Environmental Protection Agency
Duluth, MN 55804