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Assessment of the Ecotoxicological Hazard of Sediments in Waukegan Harbor, Illinois

by

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Abstract

Waukegan Harbor, on the western shore of Lake Michigan 30 miles north of Chicago, received large inputs of PCBs between 1948 and 1971, much of which remains in harbor sediments. To evaluate the environmental hazard of these sediments, 24 samples were examined for PCB concentrations and acute invertebrate, community, and plant toxicity. Total PCB concentrations in sediment samples ranged from 5 to 17,251 mg kg^{-1}, in agreement with ranges reported in previous studies. All but three stations are above the "safe" level of 10 mg kg^{-1} recommended by Mason and Hanger (1980). There is a general gradient of decreasing PCB concentration with increasing distance from the head of Slip 3.

In three acute bioassays (Microtox™ bacterial test, *Selenastrum capricornutum* algal bioassay, *Panagrellus redivivus* nematode growth and development test) sediment elutriates from the 24 stations sampled showed moderate to extreme overall toxicity and at least one highly toxic response from each station. All nematode tests were run at 10% elutriate, and the levels of mortality observed at this concentration (up to 97%) are extraordinarily high. In analyses of bioassay response with PCB concentrations, only the total number of postive tests per station produced a significant correlation. While the harbor is clearly severly contaminated with PCBs, high overall toxicity is not restricted to areas with high PCB concentrations. Other factors (oils and grease, lead, aluminum, etc.) may be involved in producing these toxic responses.

PCB contamination in Waukegan Harbor sediments produced adverse effects in protozoan community bioassays. When exposed to sediment elutriates, mature communities exhibited a reduction in number of species. Colonization of fresh substrates showed significant responses at elutriate concentrations only one-fourth as strong as those required to affect mature communities.

Phytotoxicity tests that rely on direct contact between plant root systems and contaminated sediment represent an alternative approach to toxicity assessment of these solid substances. Phytoassay methods included tests of duckweed, lettuce, and millet. Nineteen of the 21 sampling stations showed significant phytotoxicity to millet ($P < 0.05$). Duckweed exhibited no adverse reaction to the sediments, and the lettuce root elongation test failed to show harmful effects from the sediment, with the exception of samples from Station E.

Because of the highly toxic nature of sediments at many stations, even some with only moderate PCB levels, a more thorough investigation of other contaminants should be performed before the cause of the toxicity in the harbor sediments can be determined. This investigation should include analyses for lead, aluminum, and oils and grease, and an expansion of the community-level bioassays to include more stations.

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Executive Summary

Waukegan Harbor is located on the western shore of Lake Michigan approximately 30 miles north of Chicago and 9 miles south of the Illinois-Wisconsin border. The shores of the harbor are lined with commercial and industrial facilities.

In 1948, the Johnson Motors Division of Outboard Marine Corporation (OMC), headquartered in Waukegan, began purchasing Pydraul, a hydraulic fluid manufactured by Monsanto Chemical Company. This fluid contained polychlorinated biphenyls (PCB) and was used in high-pressure diecast machines in OMC's factory on the shore of Waukegan Harbor. Pydraul leaked steadily from these machines into a floor drainage system that ultimately emptied into Waukegan Harbor and Lake Michigan. About 8 million pounds of Pydraul were purchased between 1948 and 1971, and OMC estimates that 900,000 pounds may have been discharged to the harbor.

Since 1929, U.S. industries like Monsanto and OMC manufactured and used PCBs because of their high dielectric constant, their high chemical and thermal stability, their non-flammability, and their low production cost. These characteristics make PCBs industrially desirable, but also enable them to accumulate and persist in the natural environment. Because PCBs are non-polar and slightly soluble in water and have a slight vapor pressure, they can be expected to be present in the air, water, and solid phases of an ecosystem. Because no substantial evidence suggests that PCBs are produced in the environment, either from natural sources or from the chemical transformation of other compounds, all environmental contamination by PCBs is inferred to result from the production and use of materials containing PCBs.

In 1971, after evidence accumulated showing that exposure to PCBs could result in hazards to human health and to the environment, Monsanto voluntarily banned sales of hydraulic fluids like Pydraul. In 1979 all PCB sales and use were banned in the United States. Samples taken by the Illinois Environmental Protection Agency in 1978 showed that high levels of PCBs had accumulated in the sediments of Waukegan Harbor; PCBs have since been detected in Waukegan Harbor by several studies.

The sediment in Waukegan Harbor was exposed to high concentrations of PCBs for about 20 years. This study sought to assess the existing ecotoxicological hazard. Although contaminants such as metals, oil and grease, and other organic compounds might also be present in sediment samples from the harbor, their effects were not separated from those of the PCBs. An accurate evaluation of the risks posed by the overall composition of the sediment is, therefore, presented in this study.

To perform an analytical and statistical ecotoxicological assessment of the sediments in Waukegan Harbor, the following hypotheses were examined:

1) The concentrations of certain pollutants in the sediments of Waukegan Harbor exceed environmental guidelines.

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2) The distribution of these concentrations can be used to determine their source.
3) The concentrations of certain pollutants can be used to predict the response of test organisms to sediment elutriates in a suite of bioassays.
4) The toxic responses observed are attributable to specific compounds or groups of compounds found in the sediments.

To test these hypotheses, the following experimental procedures were conducted:

1) The concentrations of total PCBs in the sediment samples at 24 sites were determined.
2) Acute bioassays using an elutriate from each Waukegan Harbor sediment sample were performed for all sites with the luminescent bacterium Photobacterium phosphoreum, the green alga Selenastrum capricornutum, and the free-living nematode Panagrellus redivivus. Bioassay results and total PCB values were statistically compared to determine the ecotoxicity of the PCBs.
3) Bioassays using protozoan community structure as an index of ecosystem effects were performed in situ (at Waukegan Harbor) and in the laboratory.
4) Phytotoxicity assays were conducted with duckweed, millet, and lettuce plants as an application of direct sediment testing.

Results of these initiatives are summarized below.

Total PCB concentrations in sediment samples from Waukegan Harbor ranged from 5 to 17,251 mg kg^{-1}. These values are in substantial agreement with concentration ranges reported in previous studies. All but three stations are above the "safe" level of 10 mg kg^{-1} recommended by Mason and Hanger (1980). As expected, we found a general gradient of decreasing PCB concentration with increasing distance from the head of Slip 3; however, minor variations in this gradient in areas of lower concentration do exist.

In three single species bioassays, sediment elutriates from the 24 stations sampled showed moderate to extreme overall toxicity and at least one highly toxic response. The correlation between nematode and algal bioassays was significant, but Microtox-algal and Microtox-nematode correlations were not. All nematode tests were run at 10% elutriate, and the levels of mortality observed at this concentration (up to 97%) are extraordinarily high.

In correlations of single species bioassay response with PCB concentrations, only the number of postive tests per station produced a significant relationship. While PCBs clearly play a role, high overall toxicity is not restricted to areas with high PCB concentrations. Other factors may be involved in producing toxic responses. The role of oils and grease, lead, and aluminum (all of which could be expected as waste products of local industry) should be investigated in future studies.

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PCB contamination in Waukegan Harbor sediments had adverse effects on protozoan communities. When exposed to sediment elutriates, mature communities exhibited a reduction in numbers of species. Colonization of fresh substrates showed significant responses at elutriate concentrations only one-fourth as strong as those required to affect mature communities. The *in situ* bioassays showed similar results, establishing a more direct link between contamination and environmental hazard. In all cases, photosynthetic species of protozoa were the most severely affected.

The most important finding in the phytotoxicity testing was the disparity of response among test species. Even though the three plant species used were all advanced plant life species, they displayed surprisingly different responses to the same set of sediment samples. We presume this is due to varying structure and niche among species and to the response of each organism to different contaminants in the very complex sediment mixture. The millet root elongation tests were the most effective in detecting sediment toxicity where nineteen of 21 stations showed significant phytotoxicity. In general, duckweed tests and lettuce root elongation tests failed to show harmful effects.

Because of the highly toxic nature of sediments at many stations, even at some with only moderate PCB levels, a more thorough investigation of other contaminants should be undertaken before the cause of the toxicity of the sediments can be determined. Waukegan Harbor is an industrialized area. While the PCB contamination is an obvious point-source, other industrial contaminants may well play a part. Future investigations should include extended analyses for lead, aluminum, and oils and grease, as well as an expansion of the community-level bioassays to include more stations.

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Chapter 1

General Introduction

1.1. Environmental Profile of Waukegan Harbor, Illinois

Waukegan Harbor is located on the western shore of Lake Michigan approximately 30 miles north of Chicago and 9 miles south of the Illinois-Wisconsin border. The harbor is 0.9 mile long and runs north and south in an "L" shape. Two 800-foot side channels branch to the west. Depths range from 6 feet in the side channels to 20 feet in the main navigational channel (Thomann and Kontaxis 1981).

The harbor bottom consists of three distinct layers: (1) 4 -10 feet of soft sediment/organic silt with a 40-50% moisture content, (2) a sand layer, and (3) the natural clay harbor bottom (Mason and Hanger 1980). The shores of Waukegan Harbor are lined with commercial and industrial facilities. Total discharges, runoff and industrial, to the harbor are estimated to be 0.25×10^3 m^3 per day. Approximately 0.75×10^3 m^3 per day (3 MGD) of harbor water is withdrawn to surrounding industries (Thomann and Kontaxis 1981).

In 1948, the Johnson Motors Division of Outboard Marine Corporation (OMC) began purchasing Pydraul, a hydraulic fluid manufactured by Monsanto Chemical Company. This fluid contains polychlorinated biphenyls (PCB) and was used in high-pressure diecast machines in OMC's factory on the shore of Waukegan Harbor. Pydraul leaked steadily from these machines into a floor drainage system that ultimately emptied into Waukegan Harbor and Lake Michigan. About 8 million pounds of Pydraul were purchased between 1948 and 1971, and OMC estimates that 900,000 pounds may have been discharged to the harbor (Mason and Hanger 1980). In 1971, after evidence accumulated showing that exposure to PCBs could result in hazards to human health and to the environment, Monsanto voluntarily banned sales of hydraulic fluids like Pydraul. In 1979 all PCB sales and use were banned in the United States (Eisler 1986). Samples taken by the Illinois Environmental Protection Agency in 1976 showed that high levels of PCBs had accumulated in the sediments of Waukegan Harbor. Table 1.1 lists levels of PCBs detected in Waukegan Harbor by several studies. The recommended freshwater aquatic life protection criterion for PCB is 0.014 $\mu g\ L^{-1}$ for a 24 h exposure (Eisler 1986). The "safe" sediment level is approximately 10 $mg\ kg^{-1}$ (Mason and Hanger 1980).

1.2. Behavior of Polychlorinated Biphenyls within the Environment

Since 1929, U.S. industries like Monsanto and OMC manufactured and used PCBs because of their high dielectric constant, their high chemical and thermal stability, their non-flammability, and their low production cost. These characteristics make PCBs industrially desirable, but also enable them to accumulate and persist in the

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natural environment (Rodgers and Swain 1983). Because PCBs are non polar and slightly soluble in water and have a slight vapor pressure, they can be expected to be present in the air, water and solid phases of an ecosystem (Murphy and Rzeszutko 1978). Because no substantial evidence suggests that PCBs are produced in the environment, either from natural sources or from the chemical transformation of other compounds, all environmental contamination by PCBs is inferred to result from the production and use of materials containing PCBs (National Academy of Science 1979).

Table 1.1 Summary of PCB concentrations in the water column and sediment of inner Waukegan Harbor

		PCB concentration in	
		water column	sediment
Study	Year	($\mu g L^{-1}$)	($mg kg^{-1}$)
Soil Testing Ser.	1976	–	<0.1 - 1.1
ENCOTEC	1976	–	62.0- 9,900
OMC	1976	0.22 - 0.51	–
ENCOTEC	1977a	–	66.0 -6,400
ENCOTEC	1977b	0.62 - 14.0	–
OMC	1978	0.40 - 1.7	–
ERG, Inc.	1979	0.015 - 0.087	–
Armstrong	1980	–	161.5 - 3,634.0
Mason & Hanger	1980	–	10.0 - 400,000

When introduced to an aquatic system, hydrophobic PCBs partition into the more apolar compartments such as the sediment or suspended particulate matter or accumulate in different trophic levels (National Academy of Science 1979, Fox *et al.* 1983, Eisler 1986). The level and configuration of chlorination of the biphenyl molecule and the organic or lipid content of the solid or organism influence the degree of association of the PCB molecules with these compartments. In Waukegan Harbor, the organic silt layer is more highly contaminated with PCBs than the sand or clay layers (Mason and Hanger 1980). Higher-chlorinated PCB isomers are less soluble in water, preferentially adsorbed by soil materials, less mobile in soil, less degradable by microorganisms, and less volatile than lower-chlorinated isomers (PLUARG 1978, National Academy of Science 1979, Griffin and Chian 1980). In general, the prime removal mechanisms of PCBs in the water column are attenuation and burial in the sediments (Eadie *et al.* 1983, Weininger *et al.* 1983). These processes can be disrupted by physical resuspension, bioturbation, biodegradation, or bioaccumulation in the water column, at the benthic boundary, or within the

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sediment layer (Lick 1981, Larsson 1985); however, the sedimentary compartment of the ecosystem remains the largest net sink for PCBs (Armstrong and Swackhamer 1983). When PCB concentrations in sediments are sufficiently high, concentrations in the water column may reach environmentally unsafe levels. If these releases are significantly greater than the rate of deposition, long-term pollution problems can result with the sediments acting as a source of PCB residues rather than as a sink (National Academy of Science 1979, DiToro and Horzempa 1983, Larsson 1985).

Growing evidence indicates that PCBs can be degraded by microorganisms in the sediment (National Academy of Science 1979, Griffin and Chian 1980). In general, lower-chlorinated isomers and the position of chlorine substitution on the biphenyl molecule affect the rate of PCB degradation (National Academy of Science 1979, Griffin and Chian 1980, Neeley 1983).

PCBs may be both bioconcentrated and biomagnified in aquatic organisms. A bioconcentration factor (BCF) that relates the concentration of a chemical in water to the concentration of that chemical in the tissue of aquatic organisms can be used to predict the amount of a contaminant that might bioconcentrate. The tissue concentration of chemicals such as PCBs, which are very slowly eliminated from fish, must be very large to reach a steady-state condition. A BCF of 30,000 (a relatively low BCF estimate for PCBs) means that a fish exposed to 1 $\mu g\ L^{-1}$ PCB in the water column can be expected to accumulate 30,000 $\mu g\ kg^{-1}$ in its tissue (Veith 1980). As recently as 1983, fish flesh samples from Waukegan Harbor still exceeded the FDA limit (2,000 $\mu g\ kg^{-1}$). Fish flesh sampled from the Harbor for the IEPA Cooperative Fish Contaminant Monitoring Program (IEPA 1984) had PCB concentrations ranging from 2,600 to 12,600 $\mu g\ L^{-1}$. For all PCBs, bioconcentration factors are generally higher with increasing exposure period and with increasing chlorination of PCB congeners (Eisler 1986).

PCBs have generally been shown to be more toxic as the level of chlorination decreases (Mayer *et al.* 1977, Eisler 1986) because the lesser chlorinated molecules are more mobile and, therefore, more available to organisms in the water column. Many of the toxicological studies of polychlorinated biphenyls have been performed with Aroclor standards or purified individual isomers (National Academy of Science 1979, Mayer *et al.* 1977, Ernst 1984). Ecosystems with high levels of PCBs are rarely contaminated with only one Aroclor or isomer. Biodegradation can alter the profile of isomers in the environment (Griffin and Chian 1980). The solubility of individual isomers can be reduced when other PCBs are present (Griffin and Chian 1980), and contaminants and degradation by-products such as chlorinated dibenzofurans can be orders of magnitudes more toxic than the original PCBs (McKinney 1976, Eisler 1986). Further, concentrations of PCBs in test solutions in the laboratory can be considerably above the true solubility of PCBs and these, therefore, laboratory toxicity values can be misleading when extrapolated to the environment (Mayer *et al.* 1977). Because all components of an aquatic ecosystem are interrelated, an assessment of the biological accumulation, toxicity and cycling of contaminants, like PCBs, is necessary to determine the full impact of an environmental hazard (Dennis 1976, Zhang *et al.* 1983).

Chapter 2

Objectives of the Study

Sediment samples from Waukegan Harbor were used to assess the existing ecotoxicological hazard. Although contaminants such as metals, oil and grease, and other organic compounds might also be present in these samples, their effects were not separated from those of the PCBs. An accurate evaluation of the risks posed by the overall composition of the sediment is, therefore, presented in this study.

To perform an analytical and statistical ecotoxicological assessment of the sediments in Waukegan Harbor, the following hypotheses were examined:

1) The concentrations of certain pollutants in the sediments of Waukegan Harbor exceed environmental guidelines.
2) The distribution of these concentrations can be used to determine their source.
3) The concentrations of certain pollutants can be used to predict the response of test organisms to sediment elutriates in a suite of bioassays.
4) The toxic responses observed are attributable to specific compounds or groups of compounds found in the sediments.

To test these hypotheses, the following experimental procedures were conducted:

1) The concentrations of total PCBs in the sediment samples at 24 sites were determined.
2) Acute bioassays using an elutriate from each Waukegan Harbor sediment sample were performed for all sites with the luminescent bacterium *Photobacterium phosphoreum* , the green alga *Selenastrum capricornutum*, and the free-living nematode *Panagrellus redivivus*. Bioassay results and total PCB values were statistically compared to determine the ecotoxicity of the PCBs.
3) Bioassays using protozoan community structure as an index of ecosystem effects were performed *in situ* (at Waukegan Harbor) and in the laboratory.
4) Phytotoxicity assays were conducted with duckweed, millet, and lettuce plants as an application of direct sediment testing.

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CHAPTER 3

SAMPLING AND ANALYSIS OF PCBs FROM WAUKEGAN HARBOR SEDIMENT

3.1. Materials and Methods

Sediment samples were collected on 24-25 November 1985 and 3 June 1986 from Waukegan Harbor either by a hand corer or Ponar dredge. The sediment from each collection station was mixed for homogenity, put into glass sample jars, and stored at 4°C until analysis. The designated stations are shown in Figure 3.1.

3.1.2. Determination of PCB levels in Waukegan Harbor sediments by the perchlorination method.

Analyses of the Waukegan Harbor sediments for total PCBs were performed in the laboratory of J.B. Risatti at the Illinois State Geological Survey. Detailed methods are described in a report by W. Sheridan and J.B. Risatti (1987), and Figure 3.2 summarizes the procedure. Twenty-five grams of sediment from each station were extracted, concentrated, then cleaned on florisil. Approximately 210 ml of florisil eluate were generated of which 1.0 ml was used for perchlorination (Steinwandter and Brune 1983, Steinwandter 1984). After the reaction, the sample was cleaned on silica gel, and a 1.0 µl aliquot was injected into the gas chromatograph.

3.2. Results and Discussion

Figure 3.1 shows total PCB concentrations for 18 stations with values ranging over several orders of magnitude, from 5 to 17,251 mg kg^{-1}. We did not identify stations where concentrations were as high (400,000 ppm) as those detected by Mason and Hanger (1980), but our values exceeded those found in other studies (Table 1.1). At all but two stations (U and X), the "safe" level recommended by Mason and Hanger (1980) was exceeded. It is clear that, if this level were used as a criterion, most of the sediments of Waukegan Harbor would not be classified as "safe."

The highest values are from stations in or near Slip Number 3, and those from Stations J and K (those nearest the suspected principal point source when PCBs were being discharged) exceeded 10 000 mg kg^{-1}. Some slight variation notwithstanding, PCB concentrations tended to decrease from the head of Slip Number 3 out toward the entrance to the harbor and Lake Michigan proper.

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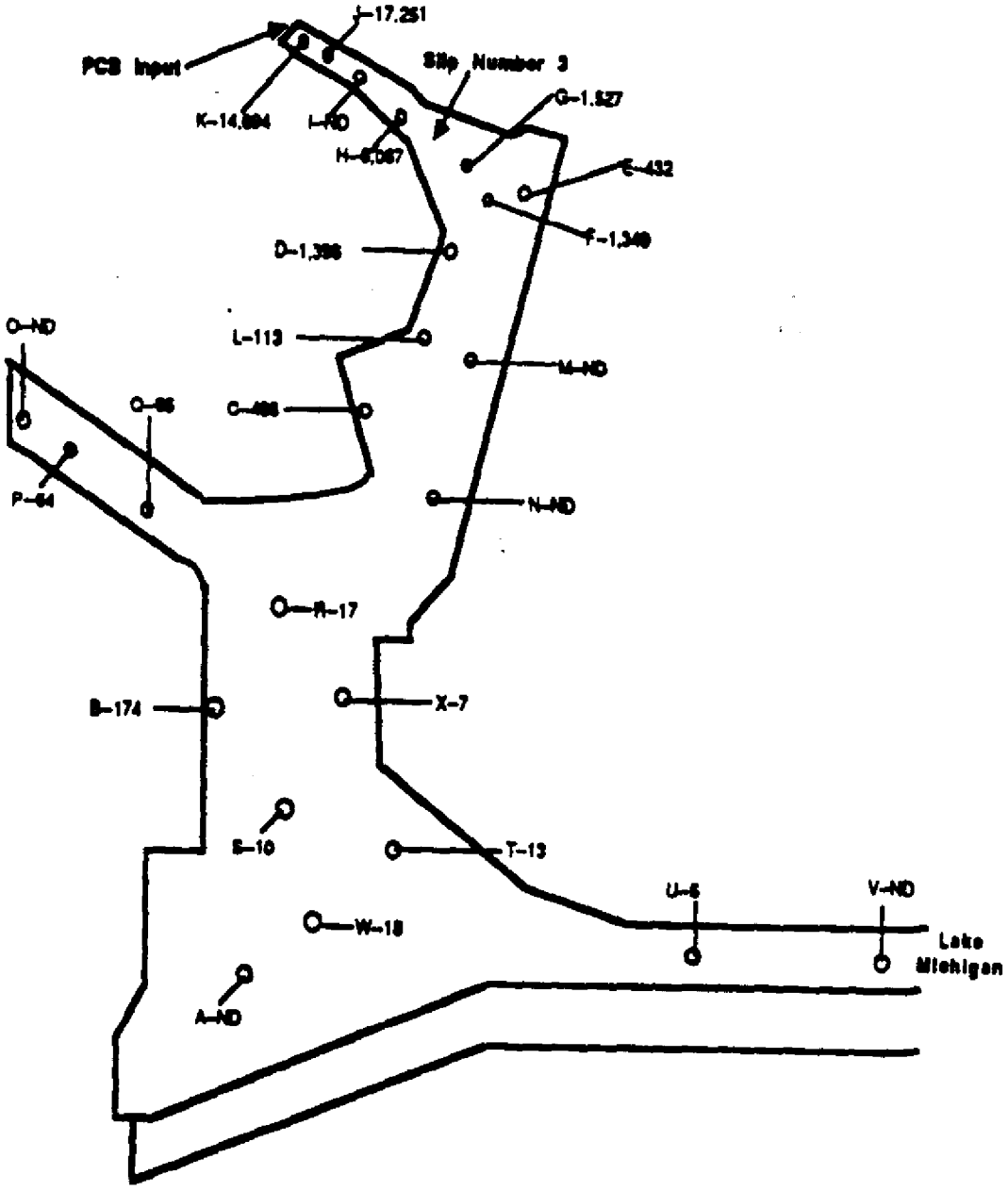
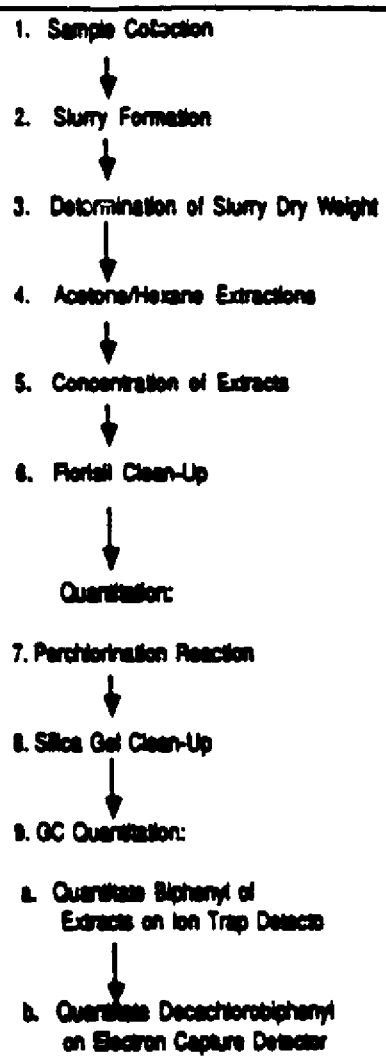


Figure 3.1 Levels of PCBs (ppm) in Waukegan Harbor sediments.

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Figure 3.2. Procedure of analysis of PCBs from Waukegan Harbor



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Chapter 4

Single Species Toxicity Tests with Waukegan Harbor Sediment

PCBs in Waukegan Harbor could be released to the water column by several routes. Microbial action in the sediment might reduce higher-chlorinated molecules to more water-soluble congeners. Organisms living at the interface of sediment and water are capable of resuspending and cycling contaminated sediments. Phytoplankton and other plants might accumulate contaminants from the sediments or water column and release them when plant cells rupture or die. Measurements of these processes are beyond the scope of the current study.

Physical processes that disturb the sediment can also lead to resuspension of settled contaminants. Disruptive processes in an aquatic environment include violent water movement caused by passing boats, storms, or strong winds. To simulate these processes, the elutriate test, a water leach using one part sediment to four parts leaching water, was used. This technique, developed as an accurate method to predict which components of the sediment will be released to the water column, has been in use since 1973 and has been evaluated under an extremely wide range of conditions in marine, estuarine, and freshwater systems (Engler 1980). Bulk analysis (solvent extraction which strips contaminants from the sediment) cannot be used to predict the concentrations of chemicals that will be found in the water column and available to the organisms there. For the same reason, bulk sediment analysis is a better predictor of for the exposure of organisms at the benthic (sediment-water) interface (Hoke and Prater 1980). Bioassays with the filtered liquid-phase from the elutriation test have been shown to project the earliest measure of the toxicity of the sediment (Engler 1980). Liquid-phase elutriates from 24 sediment samples in Waukegan Harbor were used to determine biological response to PCB contamination.

Concentrations of PCBs in Waukegan Harbor sediments proved to be exceptionally high; however, the chemical characterization of the sediment does not in itself prove an ecological threat to the Harbor. Laboratory experiments which expose standardized organisms to the elutriates from the sediment can aide in assessing the hazard associated with the contamination.

The many organisms that make up the biotic component of the aquatic ecosystem vary in basic structure, organization, and metabolism. The effect of a contaminant or group of contaminants cannot be predicted for the entire ecosystem if only one organism is used in the biological assays. To gather the most information on ecosystem toxicity, a determination of the susceptibility of as many component organisms as is economically and reasonably possible should be performed. A comprehensive hazard assessment requires acute toxicity data, using a variety of species occupying several trophic levels (LeBlanc 1984). While such short-term

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effects as increased mortality or reduced function are the most severe effects of environmental contamination, more subtle long-term effects such as carcinogenisis, mutagenesis, and the disruption of normal developmental activities may present a major risk for organisms associated with the contaminated environment and their progeny. Assays which help assess long-term risks are also important in ecotoxicological assessment (Samoiloff *et al*. 1980).

The assay organisms chosen for the ecotoxicological assessment of Waukegan Harbor sediments include the luminescent marine bacterium *Photobacterium phosphoreum* (Microtox ™), the freshwater green alga*Selenastrum capricornutum*, and the free-living nematode worm *Panagrellus redivivus* .

The Microtox™ assay measures luminescence of *P. phosphoreum* . Inhibition of this luminescence is considered a toxic response. Results of Microtox™ assays has been compared to those of standard assays using rainbow trout (*Salmo gairdneri*), fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), sheepshead minnow (*Cyprinidon variegatus*) and cladoceran (*Daphnia magna*) for a variety of pure compounds and complex environmental samples. In most cases, Microtox™ results showed equal sensitivity to the compounds tested (Bulich *et al.* 1981, Curtis *et al.* 1982, Quereshi *et al.* 1982). Bulich *et al.* (1981) concluded that the Microtox™ EC_{50} data are comparable with 24- to 96-hour fish data. *D. magna*, *S. gairdneri*, and *P. promelas* were reported by DeZwart and Sloof (1983) to be 2.54, 2.04, and 1.99 times more sensitive than *P. phosphoreum* , respectively.

The *S. capricornutum* assay measures the photosynthesis of an algal culture. Inhibition of photosynthesis is considered a toxic response. The sensitivity of *S. capricornutum* was compared with the cladoceran *D. magna*, *L. macrochirus*, the saltwater alga *Skeletonema costatum* , the marine zooplankton *Mysidopsis bahia*, and *C. variegatus* by LeBlanc (1984) for 19 non-pesticide organic compounds. Generally, *S. capricornutum* was equally or more sensitive than *D. magna*, *L. macrochirus* , and *C. variegatus* . *S. capricornutum* was found by DeZwart and Sloof (1983) to be, on average, 1.09 times more sensitive than *P. phosphoreum* (Microtox™) in a comparison using 15 compounds.

These findings indicate that the Microtox™ and *S. capricornutum* tests are comparable to the more conventional freshwater assays and could be considered superior if the ease, speed, and cost-effectiveness of the protocols were also compared.

The *Panagrellus redivivus* developmental assay is capable of detecting lethal, semilethal, inhibitory, mutagenic, or stimulatory environmental conditions (Samoiloff *et al.* 1983). Nematodes, in general, are unlikely candidates for strictly acute test protocols because they are one of the hardiest groups of organisms in the ecosystem. Laboratory-cultured organisms are, however, more sensitve to changing environmental conditions (Samoiloff and Bogaert 1984). The *P. redivivus* protocol

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includes a measurement of survival but also continues to follow the developmental progress of a test population of worms. The nematode must complete three molts from the smallest juvenile stage to the adult stage. At each molt, the worm entrains itself to the environmental conditions, molting only if conditions are favorable for continued growth. Under environmental stress, a population of *P. redivivus* contains a higher proportion of juvenile stages than is found in an unstressed environment (Samoiloff and Bogaert 1984). Using this assay, the effects of long-term exposure to an environmental sample can be measured (Samoiloff *et al.* 1980). Acute mortality, acute sub-lethal effects, chronic mortality, chronic sub-lethal effects, and phenotoxic effects can be differentiated or combined into one composite parameter called fitness that represents the overall health of the tested population. Like the Microtox™ and algal tests, this protocol is rapid, simple, and cost-effective.

Each of the bioassays was performed on elutriates from samples of 24 sites in Waukegan Harbor. Results were compared statistically between the assays and with the bulk sediment concentrations of PCBs determined in each sample.

4.1. Materials and Methods

4.1.1. Sampling

Twenty-four sediment samples were collected in sufficient volume to perform each of the three bioassays except for stations K and V, where sampling techniques failed to produce enough sediment to provide adequate elutriate for the *S. capricornutum* assay.

4.1.2. Elutriation (U.S. Army Corps of Engineers 1976)

One part mixed sediment was added to 4 parts distilled water in an acid-washed glass container. To suspend the sediment in the water, air was bubbled through the system for two hours. After a settling period of approximately 4 hours, the elutriate was filtered through a glass fiber filter (nominal porosity: 1.2 µm) and then diluted appropriately for the bioassays.

4.1.3. *Photobacterium phosphoreum* (Microtox™) bioassay (Bulich 1977)

An aliquot of the 100% concentration of elutriate from each station was used with the Beckman Microtox Analyzer and the 2:1 dilution series protocol as described by Bulich (1982) and Beckman, Inc. (1982). Each test was performed for two replicates of each of 4 dilutions (45, 22.5, 11.25, 5.63%) and a blank (diluent + bacteria). Luminescence loss was calculated for a 15-minute, 15°C test and a linear regression performed. An EC_{50} value (the concentration at which 50% luminescence inhibition was observed) was obtained from the regression equation.

4.1.4. *Selenastrum capricornutum* bioassay (Boss *et al.*, in press)

In this bioassay a photosynthesis inhibition curve for each elutriate was calculated. An aliquot of elutriate, six dilutions of elutriate (5, 10, 20, 40, 60, 80 %) and a control (diluent + nutrient + algae) were used for each test and results were expressed as percentages of control photosynthesis. The algal culture was incubated at 25°C for

at least 6 days before the test. A 20% dilution of the algal culturing medium was used as a diluent for the elutriate. Four replicates of 100 mL of each elutriate dilution were inoculated with 2 mL of viable *S. capricomutum*. The algae were allowed to acclimate to the elutriate concentrations for 20 hours at 25°C under a constant light source. After the acclimation period, 5 µCi of sodium bicarbonate ^{14}C was introduced to each flask. After 4 hours of exposure to the radioisotope, a 4 mL aliquot of each sample was acidified with 1 drop concentrated HC1 and all unincorporated ^{14}C was bubbled off. The radioactivity of the samples was measured using a Packard Tri-Carb 2000CA Liquid Scintillation Analyzer. The percent photosynthetic inhibition was calculated by dividing each sample radioactivity (dpm) by the mean of the control sample's radioactivity (dpm) and multiplying by 100. A linear regression comparing elutriate concentration and percent photosynthetic inhibition was performed for each station. The EC_{50} value was taken to be the elutriate concentration at which 50 percent photosynthetic inhibition occurred, as described by the regression equation.

4.1.5. *Panagrellus redivivus* bioassay (Samoiloff *et al.* 1980)

The *Panagrellus redivivus* bioassay was performed for each sediment sample at the laboratories of BioQuest International in Winnipeg, Manitoba. This rapid, simple, long-term toxicity assay is described in detail by Samoiloff *et al.*. (1980). Briefly, the assay utilizes the four post-embryonic stages of the nematode, each characterized by a specific size range. Animals of the smallest post-embryonic stage are introduced to the contaminant. Growth from one stage to the next requires normal physiological and informational processes and is inhibited when these processes are blocked or inhibited. The stage distribution of test and control animals represents the primary set of data from which toxicity is determined. The stage distribution can be analyzed to provide evidence for lethality, semilethality, and inhibition. A composite parameter, "fitness," can be calculated for all tests. Low fitness denotes strong toxic response. In the present study only one concentration (10%) was tested at each of the 24 sites.

4.2. Results and Discussion

4.2.1. The bacterial bioassays

The Microtox™ test yielded 21 significant ($P \leq 0.05$) toxic responses, 87.5% of the 24 stations tested (Table 4.1). The lowest EC_{50} detected was a 21% concentration of elutriate, and 7 of 24 stations (29.2%) had EC_{50}s of less than 50%. At five stations a statistically significant toxic response was observed but 50% inhibition was not obtained. In these cases, the calculated EC_{50} values were greater than 100%. These stations, however, would probably have EC_{20}s (the concentration corresponding to a 20% inhibition) of less than 100%.

The stations showing strong toxicity were not restricted to Slip Number 3 and vicinity, where PCB concentrations are highest. Some very toxic sediments were collected near the center of the main basin of the harbor, at stations S (10 ppm of PCBs) and W (18 ppm).

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Table 4.1. Summary of Microtox™ response parameters for sediment elutriates at 24 stations in Waukegan Harbor.

Station	Slope[a]	EC50[b]	% response[c]
A	-0.22	218.46	NS[d]
B	-0.15	349.36	NS[d]
C*	-1.04	46.77	46.56
D*	-0.64	82.30	29.06
E*	-0.26	194.82	11.56
F*	-2.66	20.93	113.00
G*	-0.96	53.05	42.73
H*	-0.52	94.22	23.67
I*	-1.76	36.22	67.49
J*	-0.95	52.34	42.92
K*	-0.65	74.17	29.82
L*	-1.42	33.89	65.25
M*	-0.49	97.67	22.49
N*	-0.78	62.52	35.58
O	-0.11	477.86	NS[d]
P*	-0.84	61.18	37.18
Q*	-0.65	65.27	27.76
R*	-0.44	113.22	19.72
S*	-1.71	29.89	76.43
T*	-0.94	54.67	41.72
U*	-0.34	151.61	15.02
V*	-0.19	277.12	8.26
W*	-1.11	42.89	51.23
X*	-0.41	121.20	18.36

[a] slope from the dose-response curve (inhibition)
[b] 50% effective concentration, or EC50

[c] %response = 100 − $\frac{\text{\%luminescence at 45\% elutriate}}{\text{\%luminescence control}}$

[d] variance in linear regression not attributed to tested parameters
* statistically significant inhibition ($p \leq 0.05$) which permits calculation of a %response

NS = not significant
NA = not available.

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4.2.2. The algal bioassays

At 12 of 22 stations sampled (55%), a significant inhibition of the photosynthetic rate of *Selenastrum capricornutum* was observed (Table 4.2). The lowest EC_{50} value (38.9%) was from station J, site of the highest PCB concentration, but less PCB-contaminated stations also produced strong toxic responses (e.g. A and S).

Table 4.2. Summary of algal response parameters for sediment elutriates at 24 stations in Waukegan Harbor.

Station	Slope[a]	EC50[b]	% response[c]
A	-0.72	38.5	91.79
B	-0.15	206.67	NS[d]
C	-0.56	52.49	89.74
D	-0.83	92.87	85.31
E	-0.73	226.49	NS[d]
F	0.01	-3729.00	NS[d]
G	-0.80	47.34	91.04
H	-0.71	50.56	83.85
I	0.62	-88.00	NS[d]
J	-0.85	39.86	102.34
K[e]	NA	NA	NA
L	-1.48	96.67	78.66
M	9.60	5.33	NS[d]
N	3.90	-52.91	NS[d]
O	-2.20	73.83	103.56
P	1.25	-362.41	NS[d]
Q	-2.50	159.94	NS[d]
R	-0.71	527.18	NS[d]
S	-0.86	54.20	89.02
T	-0.86	84.27	91.24
U	-1.00	82.04	89.25
V[e]	NA	NA	NA
W	-0.38	110.28	NS[d]
X	-0.86	63.97	86.71

[a] slope from the dose response curve (photosynthetic inhibition)
[b] 50% effective concentration, or EC50, estimated from the the regression equation

[c] %response = 100 − $\frac{\%\text{photosynthetic inhibition at 100\% elutriate}}{\%\text{control photosynthesis}}$

[d] variance in linear regression not attributed to tested parameters
[e] insufficient volume of sediment collected for performance of test

NS = not significant
NA = not available.

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4.2.3. The nematode bioassays

The free-living nematode *Panagrellus redivivus* showed a toxic response to sediment elutriates from all 24 stations (Table 4.3). Given that the test concentration was only 10%, the toxic responses observed are truly remarkable. At two stations, response relative to controls was above 90%. Percent response values were above 20% for all stations.

Table 4.3. Fitness (Samoiloff 1980) and percent response for the four-d *Panagrellus redivivus* bioassay[a] for 24 stations in Waukegan Harbor.

Station	Fitness	% response[b]
A	73	27
B	64	36
C	52	48
D	48	52
E	56	44
F	42	58
G	59	41
H	66	34
I	78	22
J	68	32
K	10	90
L	23	77
M	31	69
N	23	77
O	22	78
P	34	66
Q	22	78
R	31	69
S	31	69
T	31	69
U	3	97
V	16	84
W	15	85
X	22	78

[a] The test material was a 10% concentration of a sediment elutriate from each of 24 stations in Waukegan Harbor. All responses were statistically significant ($p \leq 0.05$)

[b] %response = 100 − fitness

MDNS 225297

4.2.4. Comparisons among the three acute bioassays

The three acute bioassays performed were compared on the basis of percent response for all stations. Table 4.4 summarizes these data.

Table 4.4. Percent response values for three acute bioassays of sediment elutriates from 24 stations in Waukegan Harbor.

Station	% RESPONSE		
	Microtox	Algal	Nematode
A	NS	92	87
B	NS	NS	36
C	47	70	49
D	28	85	52
E	12	NS	44
F	113	NS	58
G	43	91	41
H	24	83	34
I	67	NS	22
J	43	102	32
K	30	NA	90
L	85	77	77
M	22	NS	88
N	36	NS	77
O	NS	104	78
P	37	NS	86
Q	28	NS	78
R	20	NS	89
S	76	88	88
T	42	91	89
U	16	88	97
V	8	NA	84
W	51	NS	85
X	18	67	78

NS = not significant
NA = not available.

Because the three acute bioassays involve vastly different levels of biological organization, the toxic effects they measure are also very different. No one bioassay can detect all types of toxic effects, which is why multiple bioassays are recommended in screening procedures (Cairns 1984, Cairns and Pratt 1985). One would therefore not expect that a bacterial, an algal and a nematode bioassay would be strongly correlated. When the percent response data for the three tests were compared by non-parametric correlations (Kendall's tau, Spearman's rho), a significant relationship was found between the algal and nematode tests ($p \leq 0.05$).

A more meaningful way to look at the overall toxicity of sediment elutriates is to

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compare their general "toxicity value" (Williams *et al.* 1986) for each bioassay. Reducing the data to fewer classes, this method smooths out small variations among stations so that major differences are readily apparent. Table 4.5 summarizes the toxic responses found in Waukegan Harbor. Eighty percent of the responses measured (56/70) were at least moderately toxic.

Table 4.5. Number of stations in 5 categories of toxicity value (after Williams *et al.* 1986; as measured by % response) in each of 3 acute bioassays of Waukegan Harbor sediment elutriates.

Toxicity Value	Microtox	Algal*	Nematode	Total
0-No response	3	10	0	13
1-Weakly toxic (6-10% resp.)	1	0	0	1
2-Moderately toxic (11-40% resp.)	11	0	5	16
3-Highly toxic (41-80% resp.)	5	0	5	10
4-Extremely toxic (81-100% resp.)	4	12	14	30

*2 stations not assayed

This assessment scheme allows us to use a common basis, the % response, to compare composite response of the stations to the three bioassays. In Table 4.6, a composite toxicity index is computed for each station. The minimum score possible for a toxicity index is 0: no assay produced a toxic response. The maximum possible score is 12: all assay results were extremely toxic. The mean score of the Waukegan Harbor stations was 7.83. Eleven stations had composite toxicity indices under or at the median of 8 while 13 stations had indices above. Two stations, L and S, scored the maximum value; no stations scored the minimum value. All stations produced a toxic response in at least one assay.

4.2.5. Bioassays and PCB concentrations

If we compare the toxicity index data from Table 4.6 with the PCB concentrations from Figure 3.1, we find that elutriates from some stations (e.g. S, T, and U) with low PCB concentrations were nevertheless highly toxic to the bioassay organisms. In non-parametric correlations between bioassay responses and PCB values, the only significant correlation was with the total number of positive tests per station ($p \leq 0.05$). Thus PCBs were directly related to overall toxicity, but not to the individual bioassays, a finding that suggests that other chemical factors might be involved in producing the toxic responses.

The various relationships and correlations should not obscure the fact that elutriates

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from all 24 station produced statistically significant toxic responses in at least one of the bioassays. In most screening procedures, this finding alone would be cause to examine this hazard more closely.

Table 4.6 Toxicity values (% response) for sediment elutriates from 24 stations in Waukegan Harbor for each of 3 acute bioassays [a]

Station	Microtox	Algal	Nematode	Composite Index
A	0	4	2	6
B	0	0	2	2
C	3	4	3	10
D	2	4	3	9
E	2	0	3	5
F	4	0	3	7
G	3	4	3	10
H	2	4	2	8
I	4	0	2	6
J	3	4	2	9
K	2	0	4	6*
L	4	4	4	12
M	2	0	4	6
N	2	0	4	6
O	0	4	4	6
P	2	0	4	6
Q	2	0	4	6
R	2	0	4	6
S	4	4	4	12
T	3	4	4	11
U	2	4	4	10
V	1	0	4	5*
W	3	0	4	7
X	2	4	4	10
Mean	2.25	2.00	3.38	7.63

[a] 0 = no response; 1 = weakly toxic; 2 = moderately toxic; 3 = highly toxic, 4 = extremely toxic

* composite index based on scores from Microtox™ and nematode assays only

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Chapter 5

Protozoan Community Bioassays

Toxicity tests with single species have provided the majority of data used to evaluate the environmental hazard of chemicals (National Research Council 1981). As appreciation of the complexity of ecosystems has increased, so has concern about possible bias in hazard assessments based solely on the response of single species in isolation (Giesey and Odum 1980, Cairns 1984, Odum 1984).

The microbial community that colonizes artificial substrates includes a variety of taxa ranging from bacteria through protistans to small metazoans. This community is a composite of the communities that inhabit natural substrates (Henebry and Cairns 1984). Protozoan communities established on artificial substrates in natural systems are ideal units for toxicity studies (Cairns *et al.* 1985). Stable replicate communities (20-60 species) develop on the substrates within 3-21 days and are easily transfered intact from the field to the laboratory. Tests using these communities can be carried out rapidly (1 d. for acute, 14-28 d. for chronic) with minimal space and without elaborate apparatus (Cairns *et al.* 1980, McCormick *et al.* 1985). The use of these communities is scientifically valid since protozoa encompass several trophic levels (Pratt and Cairns 1985) and represent important components of aquatic food chains in both freshwater and marine ecosystems (Barsdate *et al* 1974, Goldman 1983). In addition, most protozoan species exhibit a nearly cosmopolitan distribution that allows the results of toxicity tests with protozoan communities to be applied to almost any system. Colonization experiments that examined the effects of nutrient loading in the Flint River (Georgia) at the ecosystem level demonstrated that protozoan communities reflected differences in water quality more accurately than other taxonomic groups examined, including algae, macroinvertebrates, and fish (Pratt 1983).

The objective of this study was to evaluate the responses of protozoan communities to contaminated sediments from Waukegan Harbor. We hypothesized that (1) indigenous communities in contaminated areas of the harbor would differ structurally from those at contaminant free sites; (2) elutriates of contaminated sediments would cause structural and functional changes in laboratory bioassays with protozoan communities.

5.1. Materials and Methods

5.1.1 *In situ* colonization of artificial substrates by protozoa

We compared the structure, colonization dynamics, and functional group composition of indigenous protozoan communities at an assumed noncontaminated site with those at the contaminated site (Station K of Slip Number 3) in Waukegan Harbor (Fig. 5.1) to directly evaluate the ecotoxicological effect of PCB-contaminated harbor sediments.

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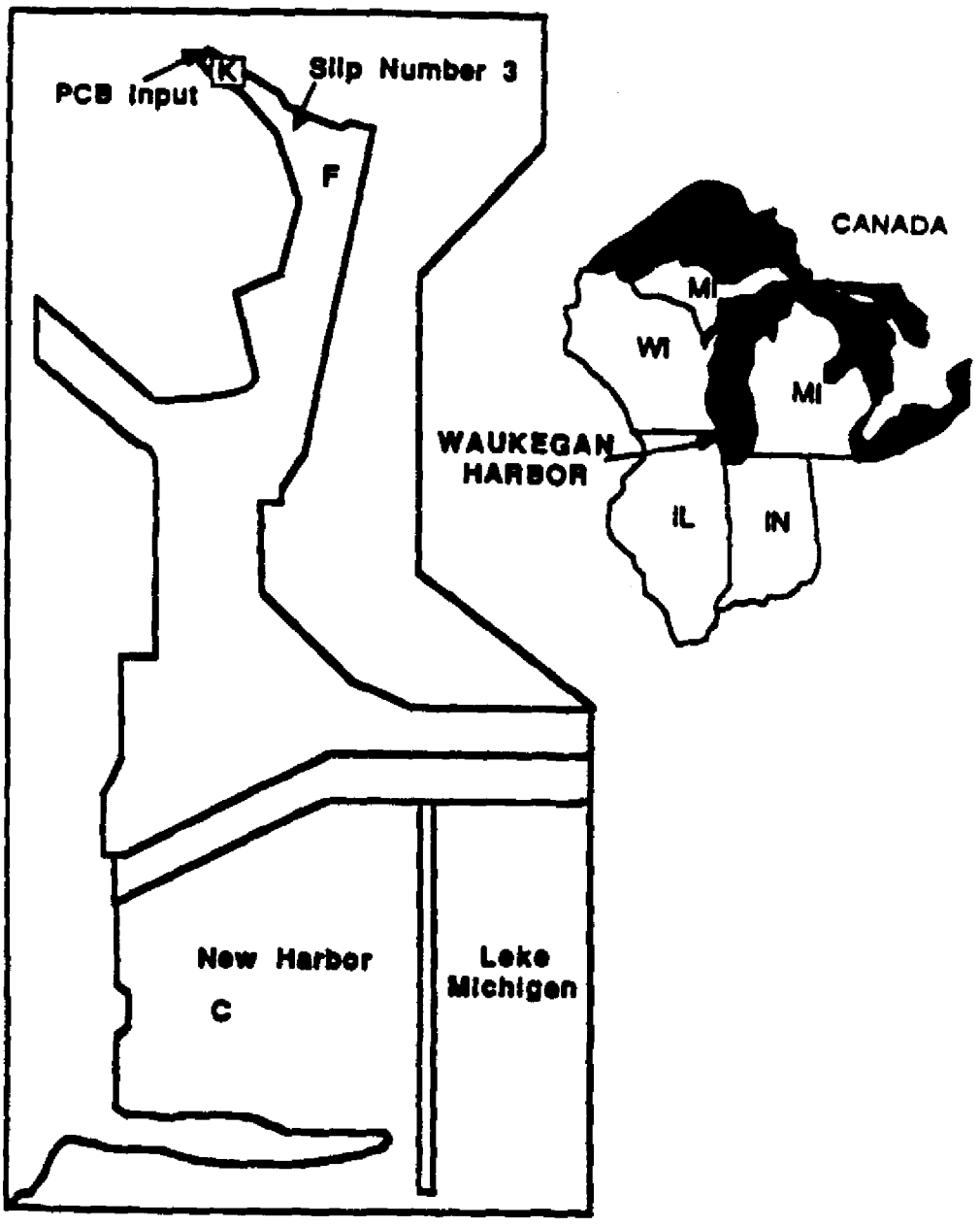


Figure 5.1. Contaminated (K and F) and uncontaminated (C) stations in Waukegan Harbor.

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The contamination-free site, South Harbor, a public boat harbor, was separated by a breakwater from the contaminated area and had no history of toxic contamination (Fig. 5.1).

We evaluated colonization dynamics at each site by anchoring 15 identical polyurethane foam (PF) block artificial substrates (7.5 x 6.5 x 5 cm) in the upper portion (50 cm below the surface) of the water column, as has been done in other studies using PF block substrates in polluted waters (Cairns *et al.* 1979). Substrates were also placed in the lower portion (20 cm above the sediment surface) of the water column at each site because of the known contamination in the sediments. Three replicate substrates were removed from the upper and lower portions of the water column on an expanding time schedule (i.e., on days 1, 3, 7, 15, 21, and 28). Each substrate was sampled by squeezing it over a clean collecting vessel to remove 135±10mL water and debris. The contents were allowed to settle, and the number of colonizing species was determined by repeated subsampling and microscopic observation. Taxa were identified to genus and species when possible. These methods and their repeatability are described in detail in Cairns *et al.* (1976) and Cairns *et al.* (1979).

5.1.2 *In situ* bioassays

Mature communities established on PF substrates anchored in South Harbor were transfered to the upper and lower portions of the water column at Station K. After one and two weeks, three substrates were collected from the upper and lower portions of the water column, and changes in species richness and functional groups were evaluated, as described in the section on protozoan colonization.

5.1.3 Laboratory bioassays

Sediment elutriates from Stations K and F, in areas of high (54,960 ppm) and lower (183 ppm) PCB concentration, respectively (Mason and Hanger 1980) were used in a series of laboratory bioassays with mature protozoan communities on artificial substrates collected from two sites in Champaign County. The sites, chosen for their location near the Illinois Natural History Survey (INHS) toxicology laboratory were INHS Pond 2 (0.08 ha) and Lincolnshire Lake (6.3 ha); both are systems with well-documented histories (Gorden *et al.* 1981 for INHS pond 2, University of Illinois Limnology Class 1980 for Lincolnshire Lake). Collected communities were acclimated to a 16 h light (~1500 lux), 8 h dark regime and to ambient laboratory temperatures (24-26°C) for 48 to 96 h in 20-L filtered (1.2 µm porosity) dilution water obtained from the substrate collection site. For each bioassay three substrates were exposed to a concentration of elutriate and three substrates (controls) to filtered pond water in separate 1000 mL acid-washed beakers. The test and control systems were exposed to the light and temperature regime to which they had been acclimated. After 24 h and 48 h PF substrates were removed from beakers and evaluated for species diversity, etc., as in the colonization experiments.

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Changes in photosynthetic and respiration rates were evaluated by transferring twenty mature communities from Pond 2 or from Lincolnshire Lake directly into separate 300 mL glass stoppered bottles (BOD bottles). To measure photosynthesis, three bottles containing communities in a concentration of elutriate and three bottles containing communities in filtered pond water (controls) were exposed to light continuously. Dissolved oxygen (D.O.) in the bottles was measured with a YSI model 51B dissolved oxygen meter (equipped with a probe and an electric stirrer designed for use with BOD bottles) at the start of the experiment and at 4, 8, 24, and 48 hr. Photosynthesis was evaluated as the gain in D.O. in the bottles. To measure respiration, six bottles containing mature communities, three in elutriate and three in filtered pond water, were kept in complete darkness; D.O. was measured at the intervals and by the method previously described. Respiration was evaluated as loss in D.O.

The effect of elutriates on colonization rates was evaluated by procedures similar to those of Cairns *et al.* (1980) and Cairns and Pratt (1985), in which small, artificial *islands* were colonized by protozoa from known source pools (*epicenters*). Our epicenters were protozoan communities that had been allowed to develop on PF substrates in INHS Pond 2. Static test systems consisted of 30-L plastic containers filled with elutriate (filtered pond water in controls) and containing 6 initially sterile PF substrate islands one-fourth the size of the epicenters (Fig. 5.2).

Islands and epicenters were added after filling the containers with filtered pond water. Epicenters and islands were anchored with monofilament line so they would not touch each other or the walls of the container. Six test containers (three with elutriate and three controls) were placed randomly under fluorescent lighting to provide a base level of photosynthesis (unmeasured) and to prevent nonrandom colonization by phototactic species. Light intensity was ~1500 lux, and was maintained on a 16L:8D schedule; temperature was 24-26°C. Dissolved oxygen was measured regularly in each tank and never fell below 80% saturation. An island from each tank was removed for sampling after 1, 3, 7, and 15 days (and after 21 days in controls). Epicenters were removed and examined for protozoa at the conclusion of the experiment. Contents of the substrates were sampled and examined for species diversity, etc., as previously described.

5.1.4 Water Chemistry

Common parameters of water quality (pH, alkalinity, hardness, etc.), nutrients (soluble and total phosphorus, total Kjeldahl nitrogen, ammonia, etc.), and dissolved and particulate organic carbon and trace elements were measured in samples from each of the four PF substrate colonization sites. Samples were collected and analyzed using standard methods (APHA *et al.* 1985).

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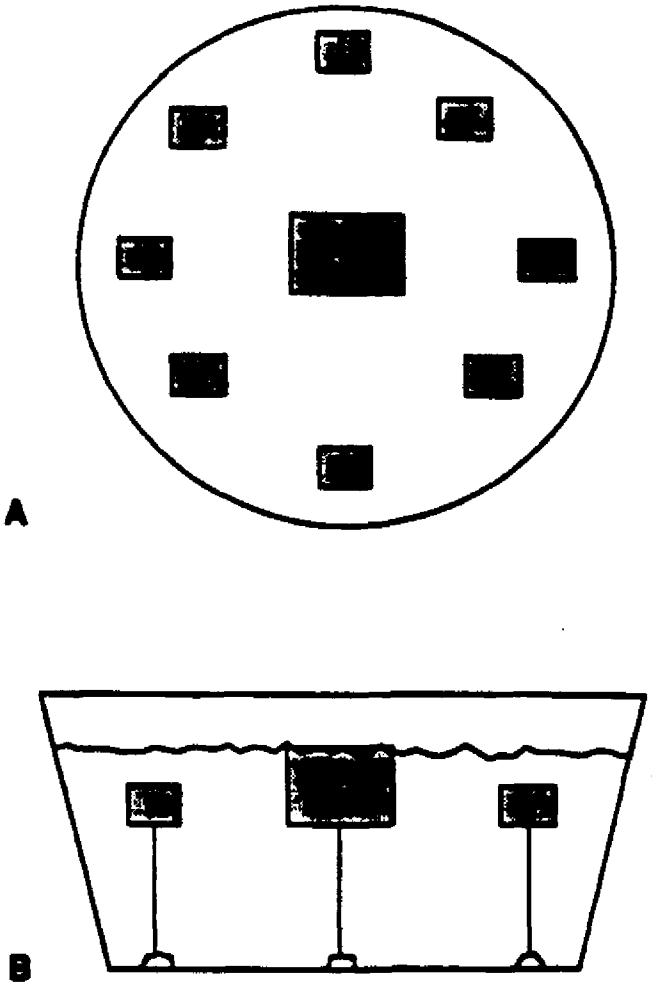


Figure 5.2. Top (A) and lateral (B) views of 30-L test systems used in island (I)/epicenter (E) colonization experiments. Not to scale.

5.2. Results and Discussion

5.2.1 Colonization rates at contaminated and noncontaminated sites

A maximum number of protozoan species was reached within 21 days on PF substrates in the top portion of the water column of the polluted harbor (Fig. 5.3A). Species were still accumulating at the end of the 28-day sampling period in South Harbor (Fig. 5.3D). The maximum number of species (30-35) recorded from substrates was about the same in both harbors.

Variation in the colonization rates of PF substrates located in the top portions of the water column in the clean and polluted harbors can be explained by differences in water chemistry, particulary differences in nutrient levels. Substrates in nutrient-poor, relatively unproductive (oligotrophic) lakes are colonized at slower rates but ultimately support higher numbers of protozoan species than in nutrient-rich, relatively productive (eutrophic) waters (Plafkin *et al* 1980, Henebry and Cairns 1984, Pratt *et al.* 1985). The rate of protozoan species accumulation on artificial substrates has been directly related to concentrations of nitrogen in the water column (Plafkin *et al.* 1980). Concentrations of all forms of nitrogen were about twice as high at Station K in the polluted harbor than in South Harbor (Table 5.1). Water column concentrations of soluble trace elements were not in the acutely toxic range at either site (Table 5.2). Increased nutrient availability in the polluted harbor appeared to stimulate colonization of substrates in the top portion of the water column and may have negated the inhibitory effects of toxic contamination in the sediments. Substrates in the bottom portion of the water column of the contaminated harbor, where colonization was inhibited, were probably exposed to greater concentrations of toxic materials (rationale in section 5.2.2).

5.2.2 Differences in the structure of indigenous protozoan communities

The accumulation of species (Fig. 5.3A), the total abundance of protozoa (Fig. 5.3B), and the phototroph abundance (Fig. 5.3C) in the communities on PF substrates located at the bottom (closer to the sediments) of the polluted harbor were significantly inhibited. In South Harbor, no significant differences were found between top and bottom substrates for any of these attributes of community structure (Fig. 5.3D -5.3F).

Although we have no direct evidence, protozoa on the bottom substrates in the polluted harbor may have been exposed to higher concentrations of resuspended sediment and attached contaminants. Everard and Denny (1985) have demonstrated that concentrations of contaminants may be three times higher in water immediately above polluted sediments than higher in the water column because of adsorption of toxics to resuspended fine particulate matter.

Reduced light levels may have limited phototroph abundance on bottom substrates, but, we have no evidence of higher turbidity in the polluted than in the clean harbor. However, the presence of dense stands of rooted submersed aquatic vegetation

MONS 225306

(*Potamogeton spp.*) at the polluted site suggests that lack of adequate solar energy was not the limiting factor for photosynthetic protozoans.

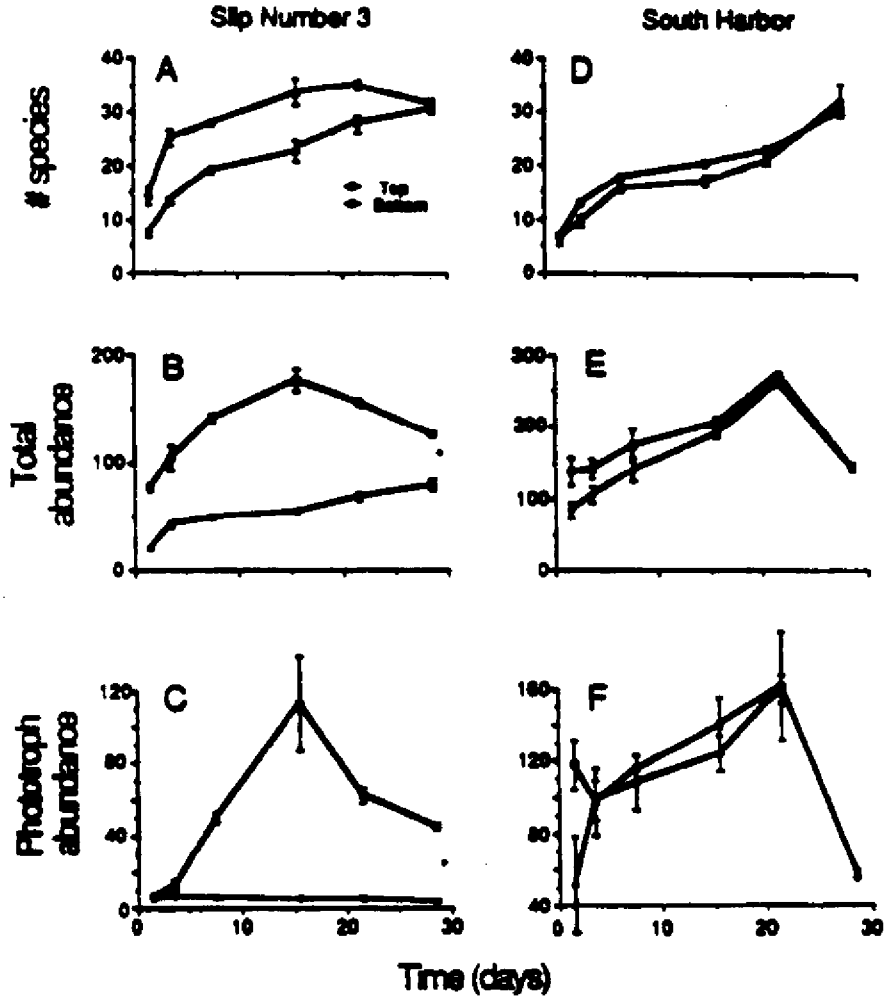


Figure 5.3. Colonization of PF block substrates by protozoa in Slip Number 3 (Station K)and South Harbor (Station C). Values are means of triplicates; error bars are standard deviations. Stars (*) indicate significant differences from controls.

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Table 5.1. Water chemistry at sites of colonization of artificial substrates by protozoans.[a]

Parameter	South Harbor	Waukegan Station K	INHS Pond 2	Lincolnshire Lake
pH [b]	7.8	7.15	9.4	8.8
Tot. Alk.	115	116	104	152
Spec. Cond. [c]	295	284	292	322
EDTA Hardness	192	88	125	113
Total Phosphorus	0.04	0.06	0.04	0.07
Soluble Orthophosphate	0.01	0.01	0.02	0.03
Nitrate-N	0.21	0.19	0.03	0.03
Nitrite-N	0.01	0.02	0.01	0.01
Total Kjeldahl-N (unfiltered)	0.44	1.18	0.40	0.64
Total Kjeldahl-N (filtered)[d]	0.40	0.88	0.40	0.40
Ammonia	0.09	0.27	0.09	0.13
Organic-N (unfiltered)	0.35	0.92	0.31	0.51
Organic-N (filtered)[d]	0.31	0.61	0.31	0.27
Total Nitrogen (unfiltered)	0.66	1.40	0.44	0.68
Total Nitrogen (filtered)[d]	0.62	1.09	0.44	0.44
Sulfate	24.5	28.2	44.1	59.6
Total Carbon	38.4	41.0	26.9	47.7
Inorganic Carbon	27.5	28.7	16.5	34.8
Total Organic Carbon	10.9	11.3	10.4	12.9
Particulate Organic Carbon	2.5	0.1	0.3	0.2

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Table 5.1. (Continued)

Parameter	South Harbor	Waukegan Station K	INHS Pond 2	Lincolnshire Lake
Dissolved Organic Carbon	8.4	11.2	10.1	12.7
Chloride	13.5	14.7	14.3	24.3

[a] All values in mg/L unless otherwise specified

[b] pH units

[c] µmhos cm^{-1} at 25° C

[d] Whatman GF/A (1.0 um pore size)

Table 5.2. Trace elements in water column at sites of colonization of artificial substrates by protozoans.[a]

Element	South Harbor	Waukegan Station K	INHS Pond 2	Lincolnshire Lake
Al	0.148	0.071	0.063	0.418
As	<0.014	<0.014	<0.014	<0.014
B	<0.002	<.002	0.349	<0.002
Ba	0.021	0.021	0.015	0.099
Be	0.001	<0.001	0.001	<0.001
Ca	33.7	35.0	9.8	37.4
Cd	<0.001	<0.001	<0.001	<0.001
Co	<0.002	0.002	<0.002	0.002
Cr	0.021	0.02	0.027	0.044
Cu	0.006	0.012	<0.005	<0.005
Fe	0.128	0.130	0.065	0.538

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Table 5.2. (Continued)

Element	South Harbor	Waukegan Station K	INHS Pond 2	Lincolnshire Lake
K	0.642	3.93	0.655	1.84
Mg	11.0	11.3	14.4	24.4
Mn	0.011	0.019	0.022	0.091
Mo	<0.006	<0.006	<0.006	<0.006
Na	5.32	8.83	31.8	11.0
Ni	0.006	<0.006	0.008	0.009
P	<0.054	<0.054	<0.054	<0.054
Pb	<0.011	<0.011	<0.011	<0.011
Sb	0.018	<0.011	<0.011	0.017
Se	<0.021	0.022	<0.021	0.035
Si	0.498	0.432	1.23	1.85
Sn	<0.041	<0.041	<0.041	<0.041
V	<0.025	<0.025	<0.025	<0.025
Zn	0.013	0.027	0.011	0.017

[a] All values are in mg/L

5.2.3 *In situ* bioassays

Number of species (Fig. 5.4A) and total abundance of protozoa (Fig. 5.4B) decreased from weeks 1 to 2 in mature substrate communities transferred from South Harbor to the water column at Station K of Slip Number 3. After exposures of 1 and 2 weeks in the polluted harbor, phototrophic abundances were significantly lower on bottom substrates (Fig. 5.4C); total abundance was significantly reduced on top and bottom substrates after two weeks (Fig. 5.4B).

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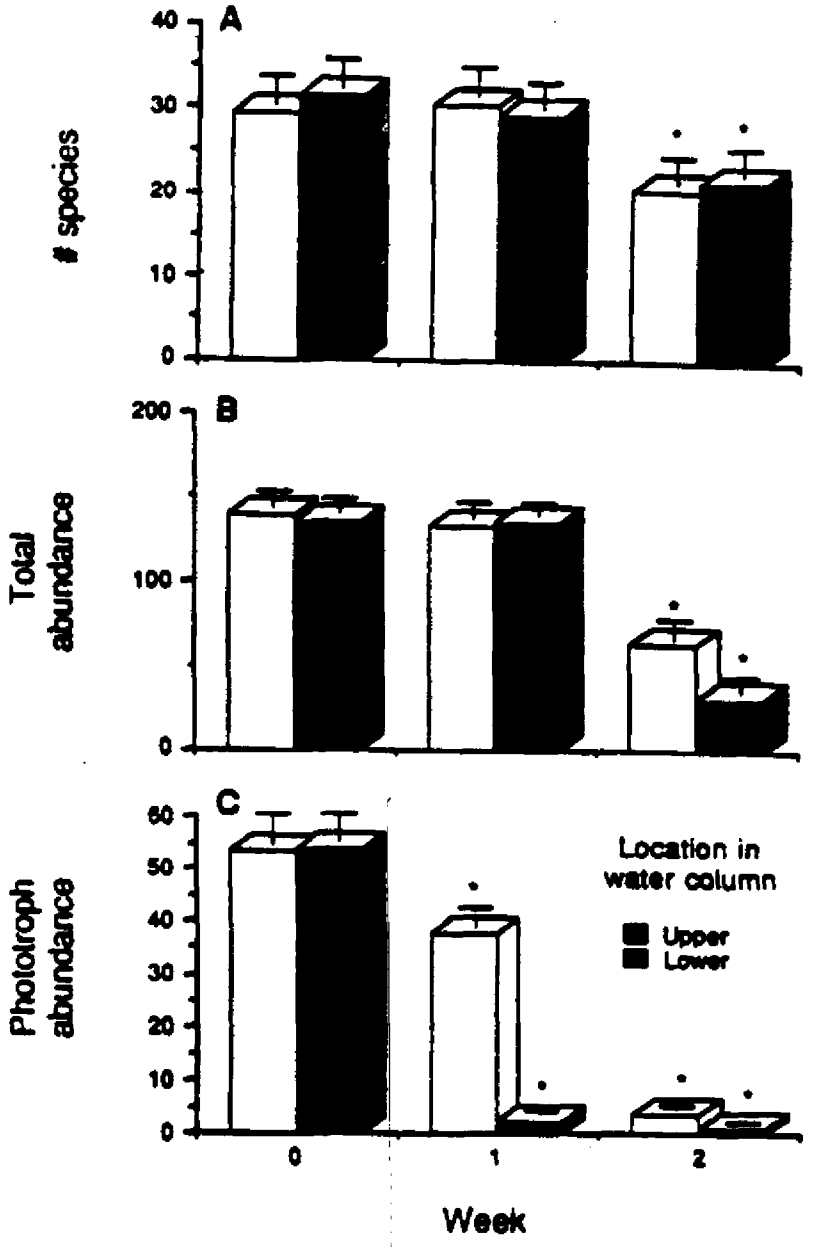


Figure. 5.4 Structural changes in protozoan communities transferred from South Harbor (Station C) to Slip Number 3 (Station K). Values are means of triplicates: error bars are standard deviations. Stars (*) indicate significant differences from controls.

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Mature communities and ecosystems may be resistant to displacement by toxic stress (Odum 1981). The *in situ* tests (up to 2 weeks exposure) suggest that longer-term exposure of substrate communities to pollutants results in species reductions that may not be detected in short-term bioassays (24-48 hr, or even 1 week). The reduction in phototroph abundance during the first week, particularly in communities transferred to the bottom of the polluted harbor, lends additional support to the theory that contaminants in harbor sediments had a greater and more immediate impact on phototrophic species than on other feeding types.

5.2.4 Effect of Station F elutriate on community structure

Mature protozoan communities on PF substrates from Lincolnshire Lake were exposed to a series of concentrations of unfiltered elutriate from Station F. Numbers of species on substrates exposed for 24-hours tto 100% concentration of elutriate were significantly lower than on substrates (controls) not exposed to elutriate (Fig. 5.5A). Numbers of phototrophic species were significantly reduced in 10% elutriate (Fig. 5.5B). These results support those of the *in situ* colonization experiments and bioassays, since numbers of phototroph species were inhibited by concentrations of elutriate lower than the concentation required to reduce total numbers of protozoan species.

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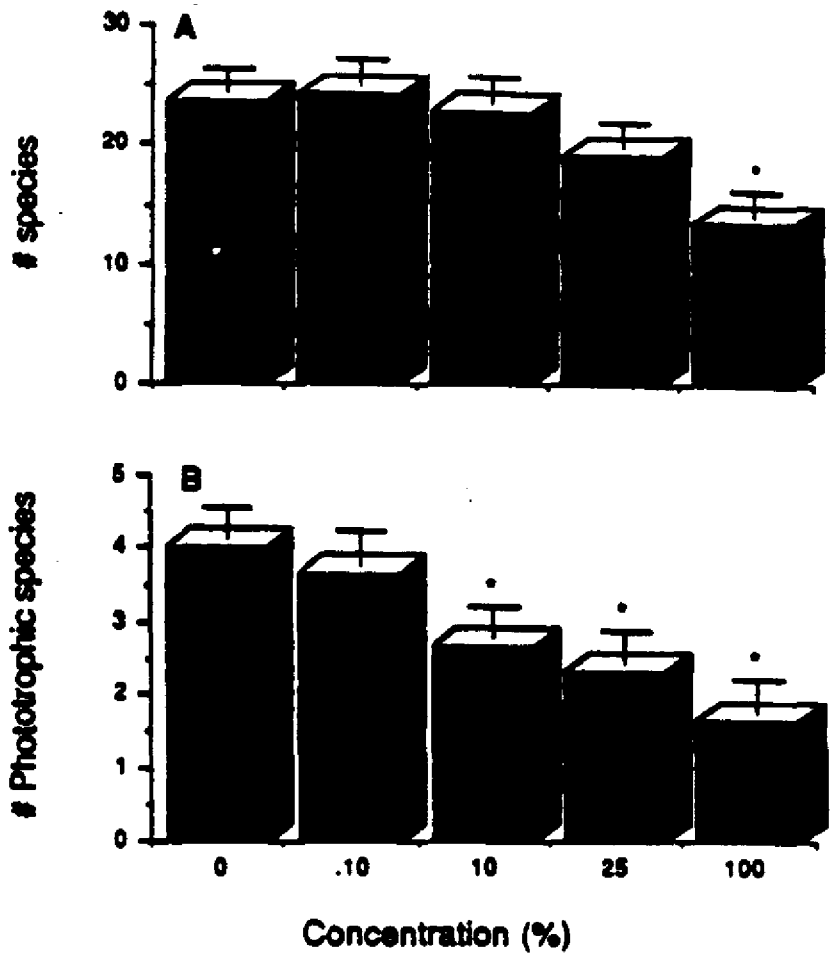


Figure 5.5. Structural changes in protozoan communities from Lincolnshire Lake after 24-h exposure to Station F elutriate. Values are the means of triplicates; error bars are standard deviations. Stars (*) indicate significant differences from controls.

Mature substrate communities from Lincolnshire Lake were exposed to 0% (controls) or 100% concentrations of filtered elutriate from Station F. The elutriate was filtered to conform to the bacterial, algal and nematode sediment bioassays. No differences in numbers of species between control and test substrates were found after either 24- or 48-hour exposure to elutriate (Table 5.3). Total abundance was reduced at 48 hr, and phototroph abundance was significantly lower at 24 and 48 hr (Table 5.3). These results are similar to those of the *in situ* colonization experiments, where factors in the polluted harbor (probably increased levels of nutrients) initially stimulated numbers and abundances of species in protozoan communities but inhibited phototrophs.

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Table 5.3. Structural changes in protozoan communities from Lincolnshire Lake after exposure to filtered Station F elutriate. [a]

	Control		100% elutriate	
Parameter	24 h	48 h	24 h	48 h
No. species	20.7(1.5)	20.3(1.5)	20.3(1.1)	21.1(2.8)
Total abundance	304(14.6)	301(12.6)	315(15.0)	251.3(20.7)*
Phototroph abundance	126.0(9.5)	109.0(7.5)	47.3(6.0)	24.7(3.5)*

[a] Values means triplicates; standard deviations in parentheses. Stars (*) indicate significant differences from controls.

5.2.5 Effect of Station K elutriate on community structure

Mature substrate communities from Lincolnshire Lake were exposed to 100% concentrations of filtered elutriate of sediment from Station K. Numbers of species and total abundance were significantly reduced in the elutriate (Table 5.4); phototroph abundance was not significantly impacted. When mature protozoan communities from INHS Pond 2 were used in identical experiments, numbers of species, total abundance, and phototroph abundance were significantly reduced (Table 5.4).

Table 5.4. Structural changes in protozoan communities from Lincolnshire Lake (LL) and INHS Pond 2 after 24-hours exposure to filtered Station K elutriate. [a]

	Control		100% Elutriate	
Parameter	LL	Pond 2	LL	Pond 2
No. Species	13.7(0.6)	24.7(1.2)	6.7(1.5)*	18.7(1.1)*
Total Abundance	59.7(25)	426.7(7.0)	36.3(3.5)*	123.3(6.6)*
Phototroph Abundance	7.7(1.1)	130.1(15.1)	4.3(0.6)	50.3(5.3)*

[a] Values are means of triplicates: standard deviations are in parentheses. Stars (*) indicate significant differences from controls.

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Lincolnshire lake, our first source of protozoan communities for the laboratory bioassays, contained a quantity of <10-µm diameter norganic silt particles. Adsorption of toxic contaminants to these silt particles may have reduced the amount in solution. Phototrophs would be more protected than other feeding types when contaminants adhere to particulates because they use dissolved nutrients and are most affected by soluble forms of toxicants. Other feeding types--algivores, bactivores-detritivores, nonselective omnivores, and predators (Pratt and Cairns 1985)-- feed on particulate matter and would ingest contaminants on particles. Many heterotrophic protozoa obtain nutrition by a combination of ingestion of particles and uptake of solutes (Dive 1981) and would be exposed to dissolved and adherent toxics.

Lincolnshire Lake and the PF substrates suspended in it became progressively more silty during the study, and the lake was finally considered inappropriate as a source of protozoan communities, because it differed markedly from South Harbor. The protozoan communities on the silty substrates from Lincolnshire Lake were also dissimilar from those found in South Harbor. For example, communities from Lincolnshire Lake contained few phototrophic protozoans (an abundant group on substrates from South Harbor), the group that appeared to be most sensitive to contaminants in Slip Number 3 sediment. Protozoan communities from INHS Pond 2 were therefore used in most of the remaining bioassays. Because substrates from INHS Pond 2 contained only small amounts of silt, not as much elutriate would be taken out of solution as in bioassays with substrates from Lincolnshire Lake. The communities from Pond 2 also contained about 19 times the abundance of phototrophs as communities from Lincolnshire Lake (similar to the phototroph abundance in communities from South Harbor); thus, the toxic contaminants were more likely to have a measurable effect on the communities.

5.2.f. Effect of Station K elutriate on community function

At a concentration of 65% Station K elutriate, oxygen production by mature microbial communities from INHS Pond 2 was significantly reduced (Fig. 5.6A); oxygen consumption was not significantly affected by the elutriate. Neither oxygen production nor consumption was significantly influenced in 32% elutriate (Fig. 5.6B). When mature protozoan communities from Lincolnshire Lake were exposed to 50% elutriate from Station K for 24 hours, oxygen production was significantly reduced (Fig. 5.7A). A 48-hr exposure to 50% elutriate had no significant effect on either oxygen production or consumption (Fig. 5.7B).

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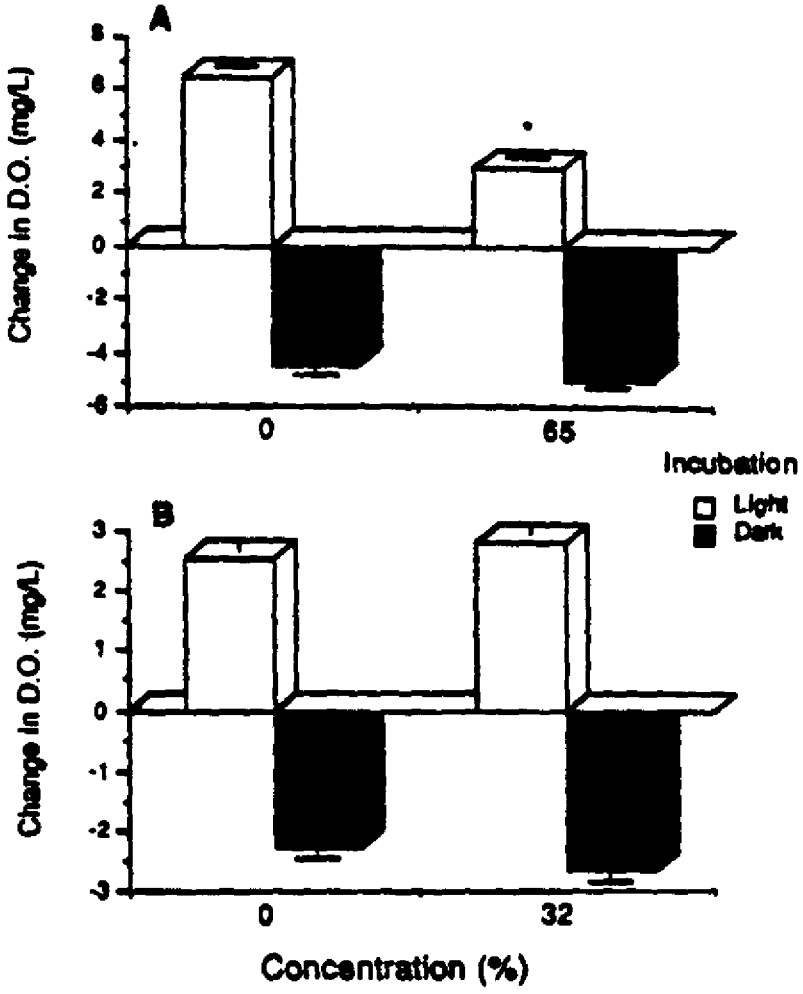


Figure 5.6. Liberation and consumption of dissolved oxygen by microbial communities from INHS Pond 2 during 24-h light and dark bottle incubations with filtered elutriate from Station K. Values are means of triplicates; error bars are standard deviations. Stars (*) indicate significant differences from controls.

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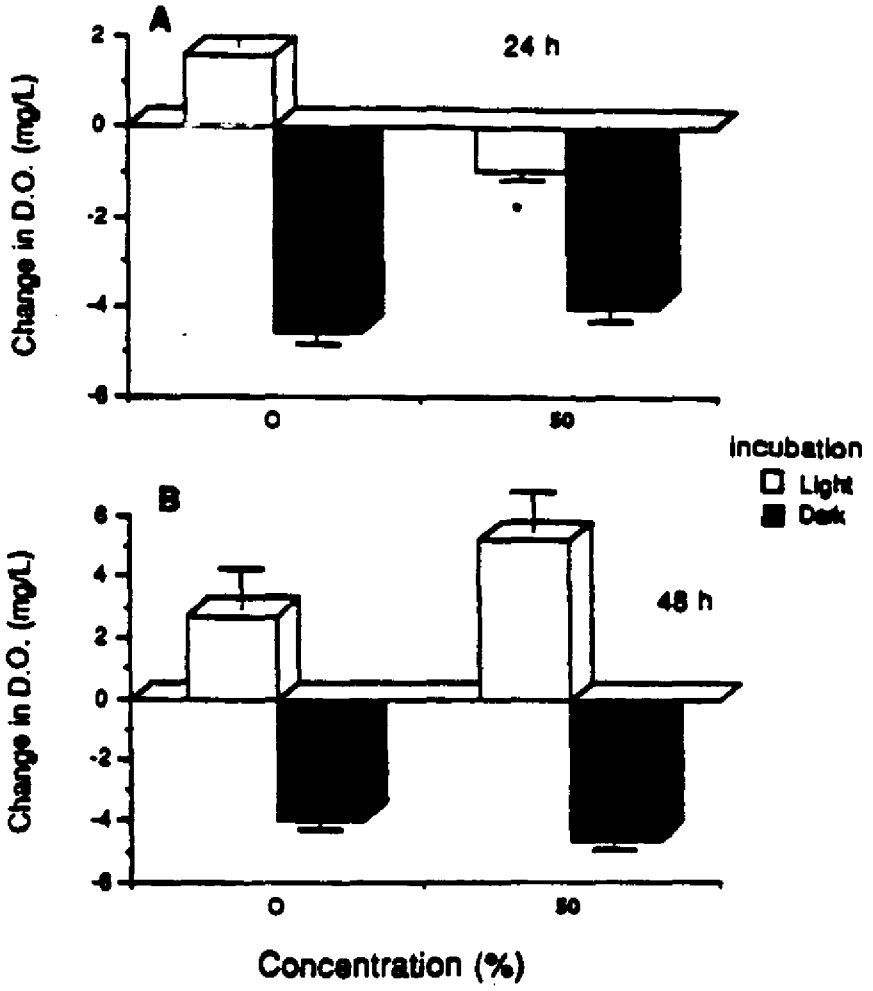


Figure 6.7. Liberation and consumption of dissolved oxygen by microbial communities from INHS Pond 2 during light and dark bottle incubations with filtered elutriate from Station K. Values are means of triplicates; error bars are standard deviations. Stars (*) indicate significant differences from controls.

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The inhibition of photosynthesis parallels the reductions in phototroph diversity and abundance in bioassays where changes in community structure were the endpoints. At 32% elutriate, both photosynthesis and respiration were slightly (but not significantly) stimulated in Pond 2 communities; and at 48 h Lincolnshire Lake communities exposed to elutriate produced more oxygen than did the controls. These findings support the concept that silt in Lincolnshire Lake may have reduced exposure of phototrophs to dissolved contaminants. Nutrients in the sediment from Slip Number 3 plus favorable conditions in the laboratory systems (reduced turbidity, continuous exposure to light, warmer temperatures) may have stimulated photosynthesis and respiration in protozoan communities.

In experiments involving the colonization of barren islands, epicenters from INHS Pond 2 were placed in different concentrations of filtered Station K elutriate. The number of species that colonized PF substrate islands and the rates at which species accumulated on islands decreased as elutriate concentration increased (Fig. 5.8). After 21 days the number of species in each concentration of elutriate was significantly reduced over that in the control. This type of concentration related to a reduction in number of colonizing species has been demonstrated for several toxic compounds (Cairns *et al.* 1980, Niederlehner *et al.* 1985). The colonization bioassay appears to be sensitive and reliable; the only major problem is the expertise and time necessary to obtain the results. The colonization assay could be a very useful secondary level test employed after initial screening tests.

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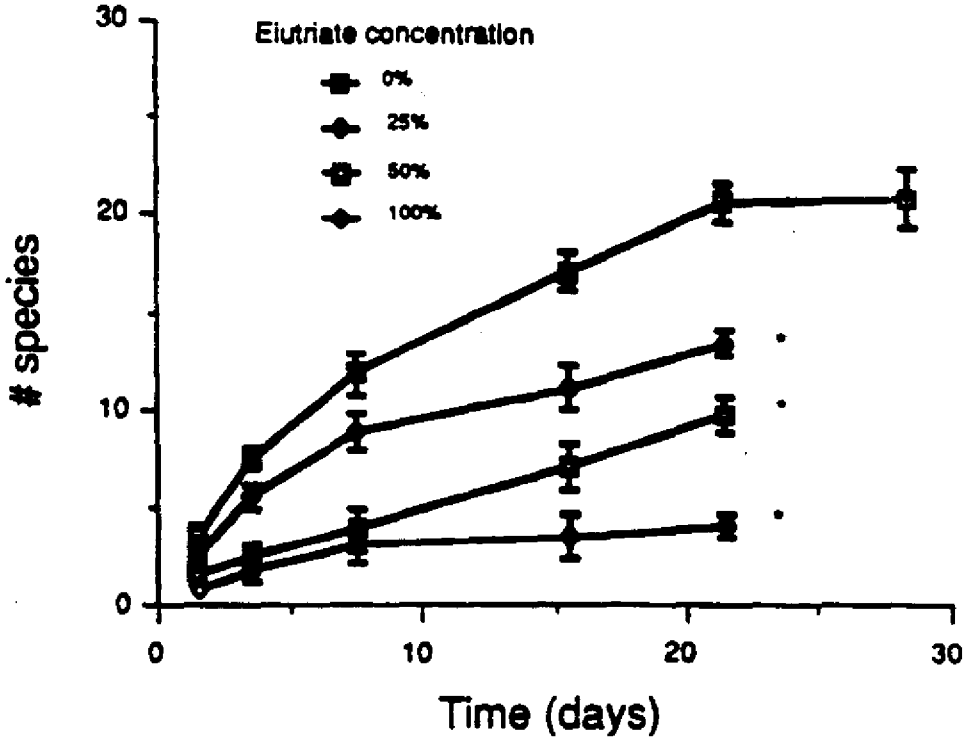


Figure 5.8. Colonization of barren islands by mature INHS Pond 2 protozoan communities during exposure to Station K elutriate. Values are means of triplicates; error bars are standard deviations. Stars (*) indicate significant differences from controls.

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5.2.7 Ecotoxicological significance

We do not need to exptrapolate from laboratory data to predict the impact of contamination on certain organisms in Waukegan Harbor Slip Number 3, because *in situ* tests were conducted with indigenous species. Contamination in the harbor did alter the structure of indigenous protozoan communites. Laboratory bioassays confirmed the results of field testing, and photosynthetic species were most affected in both settings.

The information provided by this series of protozoan tests is more complex than that provided by single species bioassays. The results can probably be used with greater reliability to predict the impact of sediment contamination on actual communities and ecosystems. However, caution must be exercised in conducting these experiments and in interpreting the data. For example, colonization of barren substrates located in the top of the water column was more rapid in the contaminated harbor than in the uncontaminated harbor; but, laboratory tests showed that colonization was inhibited by elutriates of sediments collected from the contaminated harbor. What appear to be contradictory results are explained by the fact that soluble nitrogen levels were higher in the contaminated harbor than in the uncontaminated harbor. In the top portion of the water column of the contaminated harbor, the stimulation of protozoan colonization provided by high nitrogen levels was apparently greater than the inhibitory effects of the toxic contaminants. The colonization of barren substrates by protozoa was inhibited in the lower portion of the water column of the polluted harbor where levels of toxic contaminants (on fine suspended particulate matter) were probably higher. In the laboratory tests, concentrations of soluble nutrients should have been similar in control and test systems because filtered pond water was used in both. Therefore, we can be more certain that differences in colonization rates were caused by the presence of toxic materials in the sediment elutriate rather than by basic differences in water quality (as was probably the case with the *in situ* colonizations).

The importance of colonization success, one of the most sensitive levels of community response, may not be immediately obvious; but, it is a functional measure at the community level of organization. It reflects the ability of the community to replicate and organize itself and is somewhat analogous to reproduction in a single species (Niederlehner *et al.* 1985). Concentrations of substances affecting the colonization process in protozoan communities may have severe effects on other components in the ecosystem. Rapid and continual recolonization and succession are common in microbial communities, occurring with a frequency of days rather than months or years. Individual species must traverse a relatively hostile environment during colonization, and they are directly exposed to toxic materials in the water. This exposure is much like what occurs in traditional bioassays, where single species are tested in systems with no refuge and no interaction with other species that might reduce stress on the organisms being tested. The colonization tests may be thought of as 20-30 simultaneous single species bioassays involving organisms of several trophic levels.

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Several papers have provided a theoretical framework for the observation that mature communities show increased stress tolerance (Mellinger and McNaughton 1975, Odum 1981). Evidence also suggests that communities are cybernetic systems with intrinsic homeostatic mechanisms (Patten and Odum 1981) and that they may be able to adjust to some effects of stress without loss of species. Thus, communities display structural inertia (as defined by Cairns 1976) that single species cannot. Acute tests monitoring *structural* changes at the community level would be expected to show consistently less sensitivity to toxic stress. In our experiments a 25% concentration of Station K elutriate significantly reduced the numbers of protozoa colonizing barren islands, but numbers of species in mature communities were significantly reduced only in 100% elutriate.

The main disadvantages to current tests using protozoan communities to assess ecotoxicological hazard are the taxonomic expertise needed, the time required to identify species, and the complexity of the resulting data. In this preliminary study, the full complement of protozoan bioassys could be performed at only two stations. However, the laboratory bioassays showed that reductions in total species abundance (biomass) occur at concentrations of elutriate that reduce numbers of species in mature communities. Evaluation of biomass on the artificial substrates might be simplified by using measurements of ATP, carbon, or amino-acid content. Measurement of chlorophyll content when combined with one of the methods for total biomass might be used to calculate an autotrophic index. Community photosynthesis, an easily measured variable, could serve as a sensitive measure of an important functional process. Use of protozoan communities in ecotoxicology, therefore, need not be limited by lack of taxonomic expertise.

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Chapter 6
Phytotoxicity Tests

6.1 The Extraction Procedure *versus* Phytotoxicity Tests

6.1.1 Limitations of the Extraction Procedure

The currently accepted method for determining sediment toxicity is the Extraction Procedure (EP), published in 43 Federal Register 58958. This procedure uses ambient water as a medium for the extraction of water-soluble substances. The filtrate is used in toxicity tests with such organisms as fish, daphnids, and algae. EP has been tested extensively by various investigators, especially Lee and associates and Prater and associates (Lee *et al.* 1975, Prater and Anderson 1977, Jones and Lee 1978, Epler *et al.* 1980, Hoke and Prater 1980, Bahnick *et al.* 1981, Laskowski-Hoke and Prater 1981).

The extraction step and the subsequent separation step are costly and time consuming, and experimental errors are often introduced during these steps. For example, Epler *et al.* (1980) found that the extraction efficiencies of calcium, chromium, and nickel varied, with the coefficient of variation ranging from 2 to 94 %. The mean coefficients of variation were 14, 36, and 42 % for calcium, nickel, and chromium, respectively. These experimental errors are over and above those associated with the parallel step in bioassays.

Many researchers suggest that sediment toxicity tests are still in the stage of early development. Bahnick *et al.* (1981) conducted a 96-hr bioassay with *Hexagenia limbata*, *Daphnia magna*, and *Pontoporeia affinis* and performed chemical analyses of sediments. They also examined Chironomids and *Hexagenia limbata* exposed to the sediments for their uptake of chemicals. The results of chemical extraction showed that only small amounts could be readily extracted. Prater and Anderson (1977) indicated that a correlation could be drawn between the percent mortality of test organisms and chemical data derived from bulk samples. Further study showed that the mortality of the fathead minnow was significantly correlated with the elutriate levels of chloride (Hoke and Prater 1980). A more recent study was made to correlate 96-hr sediment bioassays with chemical analysis of bulk and elutriate samples. Of the 76 bivariate correlations performed, 14 were considered potentially meaningful (Laskowski-Hoke and Prater 1981). Hannan *et al.* (1976) analyzed 24 elements from sediment and soil samples and correlated algal bioassay results with results of the standard EP. They concluded that the standard EP has limited value and that more meaningful data could be obtained from bioassays.According to Epler *et al.* (1980), the potential problems associated with the extraction step relate to the introduction of acetic acid, which has a significant phytotoxicity.

6.1.2 Feasability of Phytotoxicity Tests

A direct method of assessing marine sediment toxicity by using oyster larvae was reported by Chapman and Morgan (1983). A major difference between this method

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and EP is that the test species were placed in a sediment-water mixture, and the extractions and separation were omitted. This direct approach is difficult in toxicity tests using such indicator organisms as fish, daphnids, and bacteria. It is, however, readily applicable to plant species.

Plant organisms extend their roots into sediment and respond to its toxicity. This direct method requires no extraction and separation steps, and the advantages are enormous. First, the experimental errors associated with the extraction and separation steps are bypassed. Second, results are obtained more economically. Third, phytoassays provide an alternative test method especially suited for organic-laden sediment samples containing herbicidal compounds.

A wide range of recent research indicates that aquatic macrophytes may be strong candidates as test organisms for determining sediment chemistry.

Fekete *et al.* (1976) reported that the growth of common duckweed was directly related to the phosphorus content of sediment samples. Its growth under anaerobic conditions was much greater than under aerobic conditions, a finding that indicated e greater release of available phosphorus under anaerobic conditions. A chemical determination of total phosphorus in the sediments was also made but was found to be unreliable for predicting amounts of phosphorus available to duckweed.

Mayes *et al.* (1977) studied the role of roots in the uptake of the non-essential trace metals cadmium and lead. Plants were grown in two lakes, one a control and the other treated. Plants grown in the same water but in sediments from different sources had significantly different concentrations of the two metals.

Harding and Whitton (1978) determined the heavy metal budget of the Derwent Reservoir, Northern England. They found that the heavy metals cadmium and lead deposited rapidly in sediments. Macroscopic plants grew only occasionally in this reservoir, due perhaps in part to its heavy metal toxicity. Of the two most common submerged species, *Nitella flexis* probably accumulated almost all its metal content directly from water; *Glyceria fluitans* derived its heavy metals from sediment.

Common duckweed (*Lemna minor*), a free-floating aquatic macrophyte, has been used in experiments at the Illinois State Water Survey for several years (Wang 1986a 1986b). Results indicate that duckweed is as sensitive to heavy metal toxicity as fish species, and sometimes more so. For example, the water quality criterion for the Zn ion was identical whether obtained from tests of faunal species or duckweed: criteria for Cd and Ni ions derived from duckweed tests were more stringent than those derived from tests of faunal species (Wang, 1986a). Duckweed exhibits an EC_{50} (fifty % effect concentration) value 3 to 4 orders of magnitude more sensitive for pesticides than that exhibited by bluegill and daphnid (Bishop and Perry 1981).

The root elongation method can be considered the early-life-stage test of higher plants, and is part of terrestrial ecological assays for the Level 1 Bioassay (Brusick and Young 1982). Wong and Bradshaw (1982) studied the root elongation of rye grass under the

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influence of metal ions. They ranked the metals tested in the following order, from most to least toxic: Cu, Ni, Mn, Pb, Cd, Zn, Al, Hg, and Fe. The U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, and the Organization for Economic Cooperation and Development recommend the use of lettuce, radish, wheat, cucumber and red clover as test species (Ratsch 1983, Fletcher *et al.* 1985, Thomas *et al.* 1986). In Illinois, millet is commonly found in the Illinois River valley (Bellrose *et al.* 1979) and has been used in tests for phenol and chlorophenol toxicity. The millet root elongation test was found to be more sensitive than the biomass test (Wang 1985a, 1985b).

A comparative toxicology based on the root elongation method has been published (Wang 1986d). Cucumber, lettuce and millet seeds were compared concurrently. Phenol and chlorophenols were used. Results showed that millet seeds were consistently more sensitive to phenolic toxicity than cucumber and lettuce seeds and exhibited toxic effects in a regular and predictable fashion, much preferable to the irregular patterns shown by cucumber and lettuce seeds. In the case of heavy metal toxicity, however, lettuce seeds were more sensitive than either cucumber or millet seeds (Wang 1987). The results of these studies suggest that there is no single, most sensitive, representative test organism for environmental toxicology. A prudent approach, consequently, is to run a battery of tests, using varieties of test organisms. For mixed and complex environmental samples, lettuce and millet seeds are recommended as the phytotoxicity test species (Wang 1986d, 1987).

From the preceding discussion, we can conclude that phytotoxicity tests are a potentially useful tool for assessing polluted sediment. We can also postulate that the impact of polluted sediment on the water column can be determined by using duckweed plants. In the terrestrial environment of sediment slurry, moist soil, or dredged material, the root elongation method with lettuce and millet as the test species may be a relevant approach.

6.2 Methods

Sediment samples collected from 21 stations in Waukegan Harbor were homogenized, and moisture content was determined. On the basis of moisture content, a subsample was mixed with deionized water in a ration of 111 g dry sediment L^{-1} water. The mixture was stirred vigourously for 30 minutes with a magnetic stirrer. After settling for 60 minutes, the supernatant to be used to test for phytotoxicity was pipetted out without the disturbing the precipitated portion.

For lettuce and millet tests using the growth pouch method, sediment samples were used in a smaller proportion and without the extraction step. The sediment slurry was diluted into five different concentrations, on a 50 % dilution scale.

6.2.1 Duckweed Tests

The stock culture used for duckweed tests was obtained from the ground-water recharge pit located inside the property of the Illinois State Water Survey, Peoria, Illinois. The culture had been maintained in the laboratory since 1980 and is the same

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stock used in previous studies (Wang 1986a, 1986b). Plant nutrient solution was added weekly to the stock culture at double strength (2X) the algal growth medium recommended by Standard Methods (1985), following a previous study (Wang 1986e). The culture was illuminated with constant, cool-white fluorescent light at 3300 lux. At room temperature, the duckweed plants reproduced continuously and provided test specimens year-round. The duckweed plant, identified as *Lemna minor* , has been offered as a reference species for duckweed toxicity tests.

Duckweed specimens were selected from the stock culture 24 hours before tests began. Selection critieria required that the duckweed specimens be healthy-looking and uniform, with two fronds of approximately equal size per colony. Plants that were over-sized, under-sized, or discolored were not used; neither were those with irregular shapes, insect-bite marks or other irregularities. To prevent injuries to the duckweed plants, plastic forks were used for handling instead of forceps. After selection, test specimens were kept away from light.

The duckweed bioassay experiments were performed with disposable plastic petri dishes, 60 x 15 mm. To an 18-mL sediment extract sample, 36 µL nutrient stock solution (Standard Methods 1985) was added. This solution was placed into a dish, and 30 fronds, or 15 colonies of duckweed plants, were added. After lids were placed, dishes were randomly distributed and a constant light was provided by cool-white fluorescence at an intensity of 6456 lux. A control sample was also prepared to which only plant nutrients were added, again at double strength the algal growth medium. Each experiment was conducted in triplicate. Temperature was maintained at 26-28 °C and the incubation time was 96 hours.

At the end of incubation, the number of fronds in each dish was counted with the aid of a lighted magnifying glass. Each recognizable, protruding bud was counted. The net increase in number of fronds was indicative of duckweed growth.

6.2.2 Lettuce and Millet Tests

Root elongation tests using lettuce and millet seeds were conducted in a manner similar to that described in previous reports (Wang 1985a, 1985b, 1986d, 1987) but with some modification. Lettuce and millet seeds were from the same batches used in previous studies at the ISWS lab : *Lactuca sativa* L. var. Buttercrunch (lettuce) and *Panicum miliaceum* 1984 (millet). They were stored at -10 °C.

Seeds were treated with 10 % Clorox™ solution for 20 minutes and rinsed repeatedly with deionized water. Treated seeds were tested with the petri dish method and the growth pouch method. In the first test, containers were disposable plastic dishes 100 x 15 mm. A 5-mL sediment extract was pipetted into the dishes and 90-mm, Whatman #1 filter paper was placed on the extract. (Note: no plant nutrients were added in any of the root elongation tests.) Ten treated seeds were placed on the filter paper. In this test, deionized water was used as the control, and each test was performed with six replicates. After 120 hours of incubation in the dark at 24-25 °C, the root elongation of each seed was measured to 1 mm.

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6.2.3 Chemical Determinations

The pH, alkalinity, and hardness of the sediment extracts were determined according to Standard Methods (1985). The moisture content of the sediment samples was calculated after the samples had been heated to 105 °C, cooled, and weighed.

6.3 Results and Discussion

6.3.1 Sediment Characteristics

Characteristics of the sediment are given in Table 6.1. All pH values fell in the range 7.00 to 7.56, except at station W, which showed pH 6.90. Solid content was greater in the first batch of samples (from 51 to 79 %) than in the second batch (31 to 61 %). Alkalinity content ranged from 76 to 140 mg L^{-1} as calcium carbonate. Hardness content ranged from 220 to 460 mg L^{-1} as calcium carbonate.

Table 6.1 Sediment Characteristics of Waukegan Harbor

Station	Depth (ft)	pH	Solid content (%)	Alkalinity[a] (mg L^{-1})	Hardness[a] (mg L^{-1})
A	15	7.52	79.4	89	332
B	15	7.56	74.8	114	286
C	10	7.38	71.3	123	312
D	18	7.52	67.0	102	372
E	18	7.18	54.5	111	350
F	19	7.27	50.8	121	370
G	16	7.46	68.5	79	457
H	14	7.56	74.8	93	263
J	12	7.42	66.9	102	307
L	18	7.02	31.0	123	239
M	20	7.00	30.8	127	233
N	22	7.17	35.2	89	217
O	21	7.07	51.5	119	241
P	23	7.18	46.5	123	255
Q	25	7.35	43.8	140	251
R	24	7.16	37.8	114	249
S	19	7.18	44.0	131	247
T	25	7.01	37.9	127	219
U	25	7.46	60.9	123	235
W	22	6.90	34.4	114	223
X	19	7.15	32.8	123	227

[a] expressed as mg L^{-1} $CaCO_3$

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6.3.2 Duckweed Tests

The Waukegan sediment samples as noted earlier were mixed with deionized water. The supernatant portion was enriched with plant nutrients and tested with duckweed plants. The controls contained nutrient concentrations but no sediment. For comparison, water samples from Lake Eureka, Lake Canton, the Illinois River, and one well located inside the property of the Illinois State Water Survey were also tested. The sediment samples were tested in two batches, A to J and L to X. Each sample was tested in triplicate and designated as I, II, or III. Results are given in Tables 6.2a and 6.2b.

The results, when compared with those for the control sample, indicated that Waukegan sediment had no adverse effect on duckweed plants. Table 6.2a shows that the net increase of duckweed fronds for the control sample, was 50, with a standard deviation of 4. The t-tests indicated that none of the samples exhibited either an inhibitory or a stimulatory effect at the 95 % confidence level. In Table 6.2b, duckweed growth in the control sample was lower: the mean value was 43 duckweed fronds and the standard deviation was 2. Neither the sediment samples nor the water samples showed less growth than this. On the contrary, a significant stimulatory effect occurred, and almost all samples produced greater duckweed growth than the controls.

Although duckweed reared in the laboratory was reported to be as sensitive to heavy metal toxicity as fish species, and occasionally more so (Wang 1986a) *in situ* observations have indicated that duckweed is resistant to environmental stress, and the only macrophyte that thrived in waste water holding ponds (Rodgers *et al.* 1978). One explanation is that duckweed is a fast-growing species (Hillman and Culley 1978). Its rapid life cycle allows it to acclimate quickly to environmental stress in the field and thus develop tolerance to adverse conditions. A similar case is algal adaptation to zinc toxicity (Wang 1986c). It is unlikely, however, that duckweed acclimated during this 96-hour acute test. The more plausible interpretation is that duckweed plants are not sensitive to these sediment samples. In other words, these sediment samples caused no harmful effects to duckweed.

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Table 6.2a Increases in Number of Fronds in Duckweed Tests of Samples A to J

		I	II	III	mean	S.D.
Control		50	54	47	50	4
Waukegan	A	51	51	56	53	3
	B	42	58	41	47	8
	C	48	50	51	50	2
	D	44	53	44	47	5
	E	56	54	52	54	2
	F	56	55	53	57	3
	G	56	60	55	57	3
	H	54	47	56	52	5
	J	47	48	53	49	3
Lake Eureka		53	55	48	52	3

Table 6.2b Increases in Number of Fronds in Duckweed Tests of Samples L to X

		I	II	III	mean	S.D.
Control		42	46	42	43	2
Waukegan	L	51	51	55	52*	2
	M	53	55	55	54*	1
	N	48	51	46	48*	3
	O	51	44	53	49*	5
	P	48	46	59	51*	7
	Q	61	44	48	51*	9
	R	52	52	56	54*	3
	S	57	51	54	54*	3
	T	54	51	51	52*	2
	U	47	54	48	49*	4
	W	50	42	46	46	4
	X	48	56	47	51*	5
Lake Eureka		51	48	48	49*	3
Lake Canton		48	42	46	45	3
Illinois River	38	48	47	46	47	5
Well water		44	52	53	50*	3

* $P < 0.05$

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6.3.3 Lettuce Tests

The lettuce tests using the petri dish method were conducted concurrently with the millet tests (following section) and the duckweed tests (preceding section). These tests were conducted three times, with the tests designated in Tables 6.3a and 6.3b as Tests I, II, and III. Each test solution was tested with 60 seeds, and mean values and standard deviations are given.

The root elongation bioassay typically has high variability. The coefficients of variation for the lettuce test (approximately 30 %), obtained by dividing the standard deviations by the mean values (Tables 6.3a and 6.3b), were very close to the results (33-36 %) of previous studies (Wang 1986d, 1987).

The results of the repeated tests of the 21 sediment samples showed that the sediment samples exhibited significant ($P < 0.05$) inhibitory effect on lettuce root elongation in only four instances (one test each for samples E, O, Q, and W). After the three tests for each of these samples were averaged, it was determined that only sample E displayed inhibition effects: 10 % inhibition, significant at the 95 % confidence level.

These results suggest that the Waukegan Harbor sediments did not have harmful effects on the early-life development of lettuce, except perhaps at station E. Lettuce seed is a species recommended for phytotoxicity tests by the U.S. Environmental Protection Agency, the Food and Drug Administration, and the Organization for Economic Cooperation and Development (Fletcher *et al.* 1985, Miller *et al.* 1985, Thomas *et al.* 1986). In comparison with millet seeds, lettuce seeds have been found to be more sensitive to metal toxicity but less sensitive to phenol toxicity (Wang 1986d, 1987).

6.3.4 Millet Tests

The millet tests were conducted concurrently in the same manner as the lettuce tests. Two batches of sediment samples were tested separately and each test was repeated three times. The results are presented in Table 6.4a and 6.4b.

One measure of quality assurance is the comparison of data from this study with previously reported values. In previous control millet tests, root length was reported to be 44 $\pm$ 28 mm (Wang 1986d) and 45 $\pm$ 28 mm (Wang 1987). These values compared favorably with the control value in our study, 47 $\pm$ 27 mm. There was a slight variation between the control tests results for the first and second batches: 50 $\pm$ 29 mm and 44 $\pm$ 29 mm, respectively. The variation, however, was not statistically significant.

All of the 21 sediment samples from Waukegan Harbor, except those from two stations (N and W) were found to be harmful to millet plants at the 95 % confidence level.. The highest inhibition effect was 61 % from station E sediments; the lowest was 13 % from stations Q and R.

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Table 8.3A Mean Root Lengths (mm), Standard Deviations, and % Inhibition in Lettuce Tests of Samples A to J (Petri Dish Method)

		Test I		Test II		Test III		% mean
		Length	S.D.	Length	S.D.	Length	S.D.	inhibition
Water control		44	16	35	16	48	16	
Waukegan	A	48	12	41	17	52	16	–
	B	42	16	41	16	57	12	–
	C	44	16	48	15	54	13	–
	D	44	15	49	15	55	17	–
	E	31*	12	38	10	45	12	10*
	F	44	16	43	13	50	16	–
	G	48	13	43	11	48	12	–
	H	47	16	47	12	60	16	–
	J	45	15	44	13	48	17	–
Lake Canton water				36	15	48	15	–
Lake Eureka water				42	10	45	16	–
Illinois River water				38	11	47	11	–
Well water				34	13	47	12	–

* $P < 0.05$

Table 8.3B Mean Root Lengths (mm), Standard Deviations, and % Inhibition in Lettuce Tests of Samples L to X (Petri Dish Method)

		Test I		Test II		Test III		% mean
		Length	S.D.	Length	S.D.	Length	S.D.	inhibition
Water control	45	15	41	17	44	15		
Waukegan	L	45	15	48	15	45	15	–
	M	47	17	51	15	44	16	–
	N	41	17	50	20	48	12	–
	O	46	13	46	15	40*	16	–
	P	43	16	43	17	42	15	–
	Q	46	13	46	17	40*	16	–
	R	44	14	46	19	42	15	–
	S	44	16	46	16	40	15	–
	T	44	13	51	12	41	13	–
	U	44	16	47	17	44	12	–
	W	45	14	46	17	40*	17	–
	X	43	15	44	17	46	14	–
Lake Canton water				39	13	39*	11	–
Lake Eureka water				41	13	42	11	–
Illinois River water				40	11	38*	11	–
Well water				34	13	47	12	–

*$P < 0.05$

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Table 6.4A Mean Root Lengths (mm), Standard Deviations, and % Inhibition in Millet Tests of Samples A to J (Petri Dish Method)

		Test I		Test II		Test III		% mean
		Length	S.D.	Length	S.D.	Length	S.D.	Inhibition
Water control	53	27	51	29	46	31		
Waukegan	A	37*	22	26*	22	43	35	30*
	B	39*	22	31*	20	39	35	27*
	C	35*	25	29*	22	31*	28	36*
	D	34*	23	31*	25	37*	25	32*
	E	18*	9	14*	10	28*	21	61*
	F	37*	23	23*	18	37*	24	35*
	G	31*	23	26*	20	32*	27	41*
	H	43*	32	30*	26	42	34	23*
	J	30*	27	26*	18	40	22	38*
Lake Canton water				27*	27	45	28	25*
Lake Eureka water				14*	10	39	22	20*
Illinois River water				30*	19	48	26	45*
Well water				47	36	36*	25	14*

* $P < 0.05$

Table 6.4B Mean Root Lengths (mm), Standard Deviations, and % Inhibition in Millet Tests of Samples L to X (Petri Dish Method)

		Test I		Test II		Test III		% mean
		Length	S.D.	Length	S.D.	Length	S.D.	Inhibition
Water control	41	24	40	34	51	29		
Waukegan	L	29*	29	37	34	39*	34	20*
	M	29*	22	42	30	25*	29	27*
	N	35*	26	46	29	47	31	–
	O	31*	24	31*	29	35*	28	27*
	P	33*	23	46	32	34*	29	14*
	Q	33*	26	37	34	44	31	13*
	R	31*	24	40	27	44	25	13*
	S	26*	25	35	30	40*	29	23*
	T	31*	27	40	27	41*	32	15*
	U	34*	29	42	29	37*	27	14*
	W	39	29	39	35	46	29	–
	X	33*	25	41	29	39*	31	14*
Lake Canton water				39	29	33*	25	20*
Lake Eureka water				34	25	36*	21	21*
Illinois River water				33	24	37*	22	23*
Well water		43	33	36	32	49	37	–

* $P < 0.05$

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The distribution pattern of the inhibitory effect in the harbor is depicted in Figure 6.1. The phytotoxicity of the sediment samples is rather widespread, with sediments from the northern end of the harbor appearing to be more phytotoxic than those from the southern end. If the harbor is divided into two approximately equal parts by a line drawn south of stations C and N as shown in Figure 6.1, the average inhibition effect in the southern end (10 stations, excluding station W) was 19 % with a standard deviation of 7; the average in the northern end (9 stations, excluding station N) was 35 % with a standard deviation of 12. The "hot spot" of the harbor was apparently in the region surrounding stations E, F, and G. The near-shore stations C, D, and J also contained relatively high phytotoxicity.

6.3.5 Growth Pouch Method

Lettuce and millet tests were conducted concurrently with the growth pouch method. Only the first set of samples, A to J, were tested. Because whole sediment was used, tests were run at a much lower sediment concentration and also in a series of concentrations. Results are given in Table 6.5.

The results of the lettuce tests indicated that these sediment samples did not cause significant deleterious effects, a finding that is not surprising since at greater sediment concentrations, these sediment samples were not inhibitory to the same plant species (Table 6.3). Even the sample from station E produced no significant effect.

The results of the millet tests were uneven. Only stations A, F and G displayed significant phytotoxic effects ($P < 0.05$), as seen in Table 6.5. In contrast, all nine stations showed adverse effects in the millet test employing the petri dish method (Table 6.4). In the latter experiment, extracts from 111 g L^{-1} sediment slurry were used; while in the former, slurries had a much lower concentration as indicated in Table 6.5

6.4 Summary

The most important finding in this study is the disparity of responses among test species. Even though the three were advanced plant life species, they displayed surprisingly different responses to the same set of sediment samples.

Duckweed is a free-floating macrophyte. It has been reported to be sensitive to toxicity of heavy metals and herbicides. This plant, however, did not exhibit an adverse reaction to the Waukegan Harbor sediments.

The lettuce root elongation test is recommended by USEPA, FDA, and OECD. This species has been reported to be very sensitive to heavy metal toxicity. The test in this study, however, failed to show harmful effects of the sediment samples, except for a sample from station E.

The millet root elongation tests were highly effective in detecting sediment toxicity. Samples from 19 of 21 stations exhibited significant phytotoxicity to millet, $P < 0.05$. The maximum effect was found at station E with 61 % inhibition after three repeated tests.

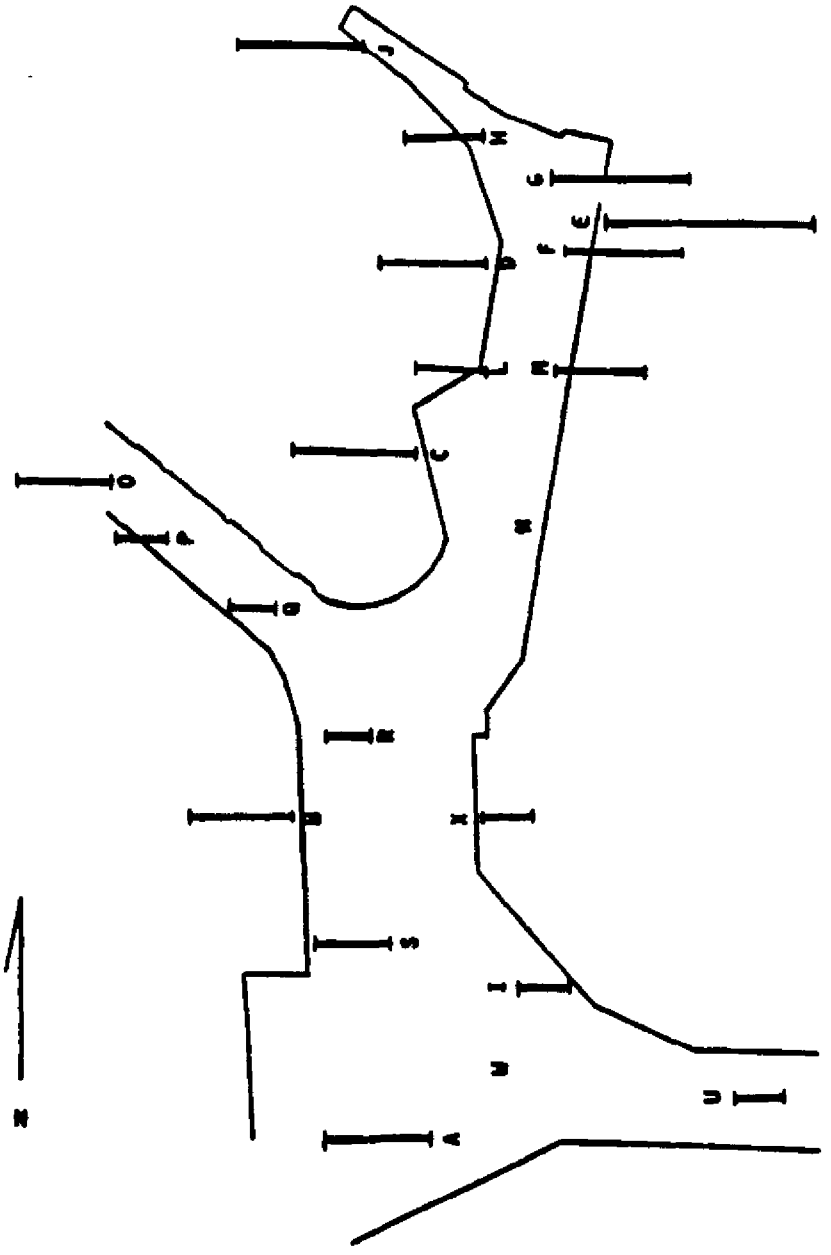


Figure 6.1 Phytotoxicity distribution in Waukegan Harbor, in relative units.

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In the millet tests using the growth pouch method, the sediment concentration was much lower than that in the petri dish tests. This lower concentration was less inhibitory to millet root elongation.

Table 6.5 Mean Root Lengths (mm), Standard Deviations in Lettuce and Millet Tests of Samples A to J (Growth Pouch Method)

		Sediment concentration $g L^{-1}$	Lettuce Length	S.D.	Millet Length	S.D.
Waukegan	A	0	46	11	85	31
		1.91	45	11	85	27
		3.82	46	11	90	27
		7.64	46	12	82	37
		15.27	49	10	80	36
		30.55	45	11	71*a	40
Waukegan	B	0	45	10	82	28
		2.80	44	10	83	29
		4.67	42	12	85	30
		7.78	42	12	90	31
		12.97	43	11	81	30
		21.62	41	9	86	33
Waukegan	C	0	41	10	76	28
		2.74	38	11	85	28
		4.57	40	13	82	35
		7.62	45	12	84	27
		12.71	46	10	91	31
		21.18	45	13	82	24
Waukegan	D	0	46	14	79	29
		1.45	46	9.86	80	26
		2.91	44	11.91	84	37
		5.82	45	10	87	25
		11.64	44	12	79	32
		23.27	46	12	86	28
Waukegan	E	0	42	14	81	29
		1.95	42	12	80	30
		3.91	43	29	73	35
		7.81	46	14	79	33
		15.63	50	10	79	34
		31.25	47	13	79	28
Waukegan	F	0	47	13	76	30
		2.21	46	10	84	30
		4.43	46	14	86	29
		8.86	46	8	83	30
		17.72	47	15	78	30
		35.43	46	12	66*b	21

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Table 6.5 continued

		Sediment concentration g L^{-1}	Lettuce Length	S.D.	Millet Length	S.D.
Waukegan	G	0	46	17	86	26
		1.57	46	9	82	27
		3.14	46	12	84	33
		6.29	44	14	92	21
		12.58	46	11	83	28
		25.15	41	14	78[*c]	25
Waukegan	H	0	44	16	72	34
		0.74	46	15	74	27
		1.49	47	15	82	34
		2.97	46	16	90	28
		5.95	46	13	80	37
		11.86	47	15	86	27
Waukegan	J	0	49	14	79	23
		0.69	50	14	84	25
		1.39	46	15	92	27
		2.77	51	12	91	32
		5.54	50	15	82	39
		11.08	52	10	82	38

* $P < 0.05$
[a] 17 % inhibition
[b] 14 % inhibition
[c] 9 % inhibition

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Chapter 7

General Conclusions

7.1. PCB Concentrations in Sediment

Total PCB concentrations in sediment samples from Waukegan Harbor ranged from 5 to 17,251 mg kg^{-1}. These values are in substantial agreement with concentration ranges reported in previous studies. All but three stations are above the "safe" level of 10 mg kg^{-1} recommended by Mason and Hanger (1980). As expected, we found a general gradient of decreasing PCB concentration with increasing distance from the head of Slip 3; however, minor variations in this gradient in areas of lower concentration do exist.

7.2. Single Species Bioassays

In three acute bioassays, sediment elutriates from the 24 stations sampled showed moderate to extreme overall toxicity and at least one highly toxic response. The correlation between nematode and algal bioassays was significant, but Microtox-algal and Microtox-nematode correlations were not. All nematode tests were run at 10% elutriate, and the levels of mortality observed at this concentration (up to 97%) are extraordinarily high.

7.3. PCB Toxicity

In correlations of bioassay response with PCB concentrations, only the number of postive tests per station produced a significant relationship. While PCB's clearly play a role, high overall toxicity is not restricted to areas with high PCB concentrations. Other factors may be involved in producing toxic responses. The role of oils and grease, lead, and aluminum (all of which could be expected as waste products of local industry) should be investigated in future studies.

7.4. Community-level Bioassays

PCB contamination in Waukegan Harbor sediments had adverse effects on protozoan communities. Mature communities lost species when exposed to sediment elutriates. Colonization of fresh substrates, a functional parameter, was even more sensitive, showing significant responses at elutriate concentrations only one-fourth as strong as those required to affect mature communities. The *in situ* bioassays showed similar results, establishing a more direct link between contamination and environmental hazard. In all cases, photosynthetic species of protozoa were the most severely affected.

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7.5. Phytotoxicity Tests

The most important finding in this study is the disparity of response among test species. Even though the three plant species used were all advanced plant life species, they displayed surprisingly different responses to the same set of sediment samples. Presumably, this is due to varying structure and niche among species and to the response of each organism to different contaminants in the very complex sediment mixture. The millet root elongation tests were the most effective in detecting sediment toxicity.

7.6. Recommendations

Because of the highly toxic nature of sediments at many stations, even at some with only moderate PCB levels, a more thorough investigation of other contaminants should be undertaken before the cause of the toxicity of the sediments can be determined. Waukegan Harbor is an industrialized area. While the PCB contamination is an obvious point-source, other industrial contaminants may well play a part. Future investigations should include extended analyses for lead, aluminum, and oils and grease, as well as an expansion of the community-level bioassays to include more stations.

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Chapter 8
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