DEVELOPMENT OF A TEST SYSTEM FOR SCREENING TOXIC SUBSTANCES: A COMPARISON USING ORGANIC SUBSTANCES

by

Carolyn Lee Thomas

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APPROVED:

R. C. Hoehn, Chairman

G. D. Boardman

S. G. Hornor

A. L. Buikema

J. T. Novak

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Blacksburg, Virginia

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Committee Chairman: Robert C. Hoehn
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(ABSTRACT)

The purpose of this research was to develop a test system for screening toxic substances by predicting their aquatic ecosystem effects. The system studied was a static, one liter microcosm with a diverse species assemblage. The microcosm was composed of biotic inoculum, chemically defined medium and sediment. The biotic inoculum contained primary producers, grazers, carnivores and decomposers. The chemical medium used was Taub #82 modified by adding sodium bicarbonate. Three different types of sediment were studied: sand, clay and clay plus sand.

Four organic chemicals: phenol, triethylene glycol (TEG), quinoline and naphthoquinone were evaluated with this test system. The toxicities of TEG, quinoline and naphthoquinone were compared for each sediment type. Toxicity was evaluated in terms of the chemicals' effects on primary productivity and heterotrophic activity though other effects are also noted. The toxicity of the

chemicals in this study was compared to those from other toxicological evaluations based on threshhold toxicity values (EC20 and LC50).

The screening test system evaluated in this study did not demonstrate significantly different threshhold toxicity values than the other screening systems to which it was compared. The ranking of the toxicants based on EC20 values was different for the two ecosystem properties, net production and heterotrophic activity. Naphthoquinone concentration exhibited no correlation between ecosystem property values and therefore, could not be ranked. Phenol exhibited the greatest toxicity to net production immediately after the toxicant addition. Quinoline was most toxic to net production over the longer time scale. TEG exhibited the least toxicity to net production, however, TEG exhibited higher toxicity to heterotrophic activity than either quinoline or phenol.

Although the type of sediment used in the microcosms did not change the relative toxicities of the chemicals, the microcosms with clay sediment always were observed to exhibit lower net production and higher variability.

Nonparametric statistical analyses are recommended for microcosm studies because of the lack of normally distributed data. Confidence limits of 80% are recommended because of the need for biologically conservative estimates of ecosystem toxicity.

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DEDICATION

This work is dedicated to my mother

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I. INTRODUCTION

The objectives of this research were to develop a test or screening system for toxic substances and to compare the results from testing four potential toxicants to other screening system's tests of the same or similar toxicants. The screening system evaluated in this research was a one liter, static microcosm with a species assemblage derived from natural sources and sediment. The other screening systems in this comparison included bioassays on algae, zooplankton and fish, Leffler's (1982) one-liter microcosms with derived species assemblage and no sediment and Taub's (1982) three liter microcosms with the defined species assemblages from single species cultures and sediment microcosms. The ecosystem properties used to evaluate toxicity were net production and heterotrophic activity.

An additional goal of this research was to compare parametric and nonparametric statistical interpretations of results, because of the frequent occurrence of biological data that is not normally distributed.

The following hypotheses were tested:

(i) The three screening systems compared do not demonstrate significantly different toxicity ranking for the same potentially toxic substances: therefore, there is no need for site and/or region-specific

screening tests.

(ii) The three, screening systems compared do not demonstrate significantly different toxic concentrations (EC20 or LC50) for the same potentially, toxic substances; therefore, a generalized screening system could be used for determining criteria for standards of organic chemicals.

(iii) The sediment type in an aquatic microcosm does not have a significant effect on ranking of potentially toxic chemicals based on threshhold toxicity values.

The hypotheses tests were based on the following qualifications:

(i) The development of criteria using the effective concentration that results in a twenty percent deviation from the control's mean (EC20) (Leffler, 1982). The criteria were developed for acute and chronic effects based on the time after the toxicant addition and on each of the three sediment types.

(ii) A comparison of the EC20 values for the screening systems tested in this research, the Leffler (1982) EC20 values suggested for the same toxicants, and the LC50 and EC20 values suggested by single-species

bioassay research for these test chemicals.

(iii) Toxicants were compared by using the rankings based on the relative toxicity of the four chemicals. The rankings were based on EC20 values and LD50 values for acute and chronic toxicity (when available).

(iv) The three sediment types were compared by using their rankings based on the chemical's toxicity.

The rationale influencing the experimental design of this research project came from many sources, primarily those in the Department of Energy Symposium volume titled: Microcosms in Ecological Research, edited by Giesy (1980), and those in the National Academy of Sciences report titled: Testing for Effects of Chemicals on Ecosystems (1981). The need for an ecosystem level test system for potentially toxic substances has been stated repeatedly not only by research scientists but also by industrial and government agencies such as EPA and FDA (Draggan and Reisa, 1980). Subsequent to these two reports, there had been an increase in the number of different ecosystems suggested as screening tools for potentially toxic chemicals. Two appeared to meet most of the criteria suggested by Hammons (1981) for ecotoxicological test systems: Leffler's (1981)

multispecies, no sediment, one-liter microcosms and Taub and Crow's (1980) multispecies, sediment, three-liter microcosms.

Industry and government agencies need a simple, short-term (i.e. 12 weeks) experimental protocol for testing ecotoxicological effects of potentially toxic substances. This screening system must be, first, simple so that it can be duplicated in the many industrial research labs across the nation and, second, short-term to promote rapid turnover of results to reduce industrial product development and testing time.

The comparisons made in this study will help further development and adoption of a standard, ecotoxicological test-system by the Environmental Protection Agency and other Federal and state agencies. One of these comparisons, the effect of sediment-type on the relative toxicities of compounds, had been researched earlier by Taub and Crow (1980), but a more detailed comparison was needed before conclusions concerning the need for unique criteria based on the receiving waters sediment could be made.

The four toxicants; phenol, triethylene glycol, quinoline and naphthoquinone; were four organic compounds differing in ring structure and constituent groups. The testing of a variety of organic structures provides predictive information about the possible toxicity of

compounds with similar organic structures. The relative toxicities of the four different organic structures will also help in predictions of the need for regulatory control of compounds of similar structure.

Since the Toxic Substances Control Act was passed in 1976, the Environmental Protection Agency has been funding various research projects to develop an aquatic ecosystem test system that could be used to screen potentially toxic chemicals and prevent many from being released to the environment. The National Academy of Sciences (1981) has stated that although the single species bioassay tests do provide useful information, they provide no information about either interactions among different species or about the effects of the functional processes of ecosystems. In 1981, Oak Ridge National Laboratories and the Environmental Protection Agency published a report (Hammons, 1981) that stated that comparison of "relative sensitivities of laboratory systems is an important research problem". Two Environmental Research Laboratories of the Environmental Protection Agency (Duluth and Corvallis) were conducting screening system comparison tests (Yount, 1984 and Stay, 1984) at the time of this writing.

State agencies, such as the Virginia State Water Control Board, are presently requiring only single-species, bioassay tests but indicate a need for an ecosystem toxicity test system (Va. WPCA workshop, April 1984).

The conclusions derived from this research provide useful, regulatory criteria for four potentially toxic chemicals based on 1) EC2Ø values; 2) a ranking of four representative organic-chemical structures on the basis of relative toxicity; 3) a comparison of toxic-substance screening systems; and 4) a comparison of parametric and nonparametric statistical analyses.

II. LITERATURE REVIEW

Introduction

The concern for the introduction of toxic chemicals into our environment may have developed from the publication of Rachel Carson's book Silent Spring in 1962. Later research by Moore (1966) indicated the far-reaching and long-lasting effects of DDT in our environment. At that time, no one imagined the immensity of what came to be known as the "toxics problem" (Moriarity, 1983). According to Woodwell (1981), four million chemicals have been registered with the American Chemical Society since 1965, 63,000 of which are in use worldwide today, and the chemical industry markets 1000 new synthetic chemicals yearly (Maugh, 1978). Portnoy (1978) and the National Cancer Institute (1978) estimated that 60 to 90 percent of all cancers may be due to environmental factors such as exposure to natural and synthetic toxic substances. However, we must go beyond merely addressing human health hazards and include potential environmental hazards as well (Draggan and Reisa, 1980).

In 1976 Congress addressed these concerns for human health and environmental health by enacting the Toxic

Substance Control Act (TSCA), which provides for funding for research to help predict and control the ecological effects of toxic chemicals. The list of known problems caused by toxic substances is long, but the list of unknown toxic substance problems may be unbounded (Cornaby, 1981). Guthrie and Perry (1980) said that testing and regulating the growing list of both new and old chemicals and their possible interactions in the biosphere seems an impossible assessment and a hopeless task for an environmental toxicologist. Thus, the field of ecotoxicology is burgeoning. Moriarity (1983) defined ecotoxicology as the science of the ecological effects of poisons or pollutants.

The priority now, just as it was in 1980 (Stephenson 1980 and Stern 1980), is the development of protocols for determining ecosystem effects and the setting of testing priorities for the most toxic of chemicals. In 1981, according to the National Academy of Sciences Report (NAS, 1981), the test methods for health and environmental effects involved the choice of laboratory species chosen from a group of organisms that were easily reared and tested in the laboratory. Little attention was paid to their relevance for use in testing environmental effects (NAS, 1981).

Stern (1980) and Hammons (1981) provided lists of the necessary attributes of a system designed to test ecological effects. Some of the qualities they listed are

wide-spectrum applicability, potential standardization, replicability, sensitivity to toxicants, rapid turnover of results, and low cost.

Microcosms

Aquatic microcosms may meet all of the necessary criteria mentioned above by Stern (1980) and Hammons (1981) to some degree, depending on what one accepts as the working definition of a microcosm. Giesy (1980) defined microcosms as "artificially bounded and replicable subsets of naturally occurring environments." Microcosms may be simple or complex, large or small, completely controlled or subject to natural conditions; but they are a bridge between single-species tests and complex field studies (Giesy and Odum, 1980). Giesy and Odum (1980) stated further that microcosms should minimize unexplained variability without reducing realism but should not attempt to mimic natural ecosystems precisely.

On the other hand, the National Academy of Science (1981) suggested that gnotobiotic multispecies systems, such as those of Taub and Reid (1982), are not microcosms because they are not scale-models of natural ecosystems. Harte et al. (1980) stated that microcosms can be both generic and specific in the information the microcosms provide about ecosystems.

Microcosms have been suggested as screening tests for evaluating the toxicity of chemical substances (Hammons, 1981; Hendrix et al 1981; and Giddings, 1983). A generic, aquatic ecosystem (such as was used in this research) is most useful in screening chemicals for their ability to disrupt ecosystem processes (Hammons, 1981 and Gidding, 1983). Pritchard and Bourquin (1983) referred to microcosms as "qualitative predictors" of toxic chemical fate and effects. Giesy and Odum (1980) described the disadvantages of using microcosms as being too synthetic, too insensitive, and in some cases, too complex. They listed impossible uses of microcosms, i.e. world-wide models.

On the other hand, using microcosms has many advantages over using natural ecosystems for ecotoxicological testing. One advantage is the generic nature of microcosms as Hammons (1981a) describes them, which allows predictions useful for many different ecosystems. As Taub and Crow (1980) stated, the extrapolation of data from a microcosm to a natural ecosystem is no worse than the extrapolation from one natural ecosystem to another.

Characteristics of Microcosms

Replicability of results and the potential for

numerous replicates, both characteristics of microcosms, make their use in toxicity testing appealing. Many studies have addressed these characteristic with encouraging results (e.g. Harte et al., 1980 and Brockway et al., 1979). The coefficient of variation between replicates in microcosms usually ranges between 10 and 30 percent. Functional properties, such as production, usually show lower variability than structural properties, such as population shifts (Pritchard and Bourguin, 1984).

The sensitivity of microcosms to toxicants has been demonstrated many times (e.g. Giddings et al., 1982; Brockway et al., 1979). The question that is asked more frequently concerns the comparative sensitivity of single-species toxicity tests and microcosm toxicity tests. Taub and Read (1982) documented a number of instances in which the microcosms were more sensitive to a particular toxicant than the single species tests. One excellent example of the greater sensitivity is the effect of triethylene glycol on the microcosm community and single-species bioassay organisms. Triethylene glycol is considered so nontoxic it is used as a solvent for organic chemicals being tested in single-species bioassays, but when it was used as a solvent in microcosm tests (Taub and Read, 1982 and Leffler, 1981), even low levels were toxic.

Microcosm Design

Microcosm test systems have been designed with a wide variety of characteristics, depending on the experimental question being considered, the environmental site being modeled or both (Pritchard and Bourquin, 1984). Hammons (1981b) described many of these aquatic and terrestrial test systems. Microcosms have been used of all sizes ranging from 200 mL (Pritchard et al, 1979) to 200 L (Harte et al., 1980). The medium used in microcosms can be either water from the aquatic system being simulated (Brockway et al., 1979) or chemically defined media (Taub and Read, 1982). Some microcosms contain sediment (Pritchard et al., 1979) and some do not (Leffler, 1981). The biotic inoculum in microcosms may be either an excised portion of the environment that is being simulated (Brockway et al., 1979), a derived inoculum from a stock culture in which populations fluctuate regularly (Leffler, 1981), or a mixture of species from single-species cultures (Taub and Read, 1982). Some microcosms are static (Leffler, 1981) and some are continuous flow (Hansen and Garton, 1982).

A characteristic of aquatic microcosms that may cause errors is actually a characteristic of the chemically defined medium used in many aquatic microcosm test systems developed by Taub and Dollar (1964) and Taub and Crow (1980). These media do not include any inorganic carbon (HCO_3^- or $CO_3^=$) an omission that could lead to erroneous

conclusions concerning toxicity based on algal-species shifts. King (1970) and King and Novak (1974) suggested that carbon dioxide limitations in aquatic ecosystems may occur in ecosystems with low alkalinity and high nutrients and cause changes in algal species succession and algal community composition. Moss (1972 and 1973) documented the carbon dioxide minimum requirements of many algal species and showed that blue green algal species have a much lower requirement for carbon dioxide than green algal species. Fraleigh and Dibert (1982) found that similar carbon limitations occur in microcosms with low inorganic-carbon levels. The most recently developed medium suggested by Taub and Read (1982) contains no inorganic carbon source, so the use of this medium may allow a carbon dioxide limitation when the diurnal pH fluctuation reaches its usual high levels.

Ecosystem Level Properties

The ecosystem-level measurements used to characterize microcosms have been widely discussed and Schindler et al. (1980) suggested distinct, measurable properties such a primary production, community respiration, nutrient cycling, oxidation-reduction gradients, and organic matter transformations. Pritchard and Bouquin (1984) described these properties as ecosystem level behaviors that reflect

the integration of many internal processes and components and their interactions. The use of ecosystem-level measurements are really what make microcosms useful as ecological test systems. When shifts in populations or species composition are used in characterizing toxic effects, then the generic nature of microcosms is compromised.

Toxicants

The four organic toxicants that were tested in this research were: phenol, triethylene glycol, quinoline and naphthoquinone. Organic compounds were chosen because of their potential toxicity, their increasingly larger potential in contamination of our natural water, and the relative paucity of information about their ecosystem effects. Heavy metal toxicants have been studied in much more detail than organic chemicals and yet the number of and quantity of organic chemicals that could enter the environment is potentially much larger (Guthrie, 1980). The choice of these four chemicals was based on two factors, one of which involves the "benchmark concept", as Hague et al., (1980) defined it, and the other, availability of toxicity data from other studies of these chemicals (Leffler, 1981; Taub and Read, 1982). The benchmark concept, as Goring (1975) proposed, involves the

selection of one particular chemical from a class of compounds, the evaluation of its environmental effects and measurements of its chemical properties. Other chemicals in the group are then characterized by the data concerning the benchmark compounds. The characteristics of the "benchmark compound" are used to build a profile of the class of that chemical. When a new chemical is to be produced by the chemical industry, the chemical would be matched to one of the classes of chemical compounds, and an environmental profile for the new chemical would be predicted (Hague et al., 1980).

The four organic chemicals chosen for this research represent four different classes of compounds. The phenol represents single ring compounds with one substituent group. Quinoline represents a double-ring compound with a nitrogen atom in one of the rings. Naphthoquinone is also a double ring compound with two, double-bonded substituent groups. Triethylene glycol is an aliphatic hydrocarbon.

Phenol has been extensively studied as a bacteriocidal compound and, in fact, is used as a benchmark chemical by which to judge other disinfectants' effectiveness (Tortora et al., 1982). Phenol has been receiving scrutiny more recently as a by-product of synthetic fuel production and has been tested extensively for toxicity in single-species, bioassay tests in twenty-gallon, aquatic microcosms and in pond microcosms as reviewed by Buikema et al (1979). Other

uses of phenol include the manufacture of resins, medical and industrial compounds and dyes (Windholz, 1983).

Quinoline has been studied recently as a potential by product of synthetic fuel production (Cushman and McKamey, 1981). Giddings (1981) tested quinoline in single-species bioassays and Leffler (1981) tested quinoline in aquatic microcosms. Other uses for quinoline include the manufacture of dyes and resins, preparation of niacin and as an antimalarial drug (Windholz, 1983).

Naphthoquinone has been studied less than quinoline but has been suggested as a potentially toxic product that appears during synthetic fuel production (Giddings et al, 1982). Leffler (1981) reported data which tested naphthoquinone in single-species bioassays and in aquatic microcosms. Naphthoquinone is only slightly soluble so is not as likely to be a potential pollutant in aquatic systems. There are two forms of naphthoquinone: α-naphthoquinone which occurs in nature in substituted forms such as vitamin K; and β-naphthoquinone which is synthesized for use as a reagent for resorcinol production and as a chemotherapeutic, hemostatic agent (Windholz, 1983).

Triethylene glycol has an interesting history of use and study and is generally considered non-toxic to aquatic organisms; so non-toxic, in fact, that triethylene glycol is suggested by the Environmental Protection Agency as a

solvent for other organic chemicals being tested in single-species bioassays. Taub and Read (1982) and Leffler (1981) found triethylene glycol to be toxic to aquatic microcosms by causing a pH reduction four weeks after its addition. Triethylene glycol has been patented as a chemical agent for containing and treating oil slicks (Ferm, 1974). Other uses of triethylene glycol are in the manufacture of plastics to increase pliability and as a disinfectant (Windholz, 1983).

Statistics and Data Interpretation

Statistical methods of microcosm data analyses have been in need of further study (NAS, 1981; Taub and Read, 1982). Analyses of microcosms provide data that are more easily described and tested by statistical methods because of their replicability and reproducibility. As many biologists and ecologists realize, organisms and ecosystems do not always behave in a way that produces normally distributed data (Sokal and Rohlf, 1969). The lack of a normal distribution of data is an important consideration when one statistically analyzes data. All parametric statistical techniques assume a normal distribution of data and lose their efficiency at predicting central tendencies (means) of data when the data are not normal (Hollander and Wolfe, 1973). The asymptotic relative efficiencies (a

measure of the efficacy of prediction) of nonparametric techniques, are sometimes twice as efficient (Hollander and Wolfe, 1983) compared to those of parametric techniques when not normally distributed. The routine, statistical analyses followed by Taub and Read (1982) began with a one-way analysis of variance for each variable for each day and was followed by linear-contrast analyses. Both of these statistical procedures are robust, which means that non-normal data distribution will not reduce their efficiencies significantly (Sokhal and Rohlf, 1969). The condition of non-normality is a problem when parametric tests of the means are used (t-tests). The sign test and the rank sum tests are two, nonparametric methods for testing means that would be more effective (Hollander and Wolfe, 1973).

The interpretation of data from microcosm toxicity tests has been varied and in some cases cumbersome. The tendency among those in microcosm research has been to make a large number of observations with a large number of replicates. The compilation of this enormous data set into one or two numbers for suggesting regulatory criteria has been done in a number of ways. Leffler (1981) has suggested an index known as the "relative effects" index for providing these criteria. The effective concentration of the test chemical that alters the ecosystem level measurement by twenty percent (EC 20) has been used in

algal assays of photosynthesis inhibitors and in aquatic microcosms (Leffler, 1981). Millemann et al., (1984) used the EC5Ø as the threshhold concentration.

Relevancy to Environmental Quality

The research described herein should provide much needed information in the development of an ecotoxicological test system for industry and federal agencies for screening potentially toxic substances. Since the Toxic Substances Control Act was passed in 1976, the Environmental Protection Agency has been funding various research projects to develop an aquatic ecosystem test system to screen potentially toxic chemicals. The National Academy of Sciences (1981) has stated that although the single species bioassay tests do provide useful information, they do not provide information about either interactions among different species or about the effects of the functional processes of ecosystems. In 1981, the Oak Ridge National Laboratories and the E.P.A. published a report (Hammons, 1981) that stated that comparison of "relative sensitivities of laboratory systems is an important research problem".

The comparison of ecotoxicological screening systems such as were studied in this research is relevant to assessment of both environmental quality and potential

hazards.

According to Roop and Hunsaker (1985) the Environmental Protection Agency will emphasize water-quality-based control of toxic substances and will require monitoring of industrial effluent environmental health effects in EPA's National Pollutant Discharge Elimination System (NPDES).

The microcosm screening system, proposed as a result of this research, may provide the means for industry to fulfill that requirement.

III. MATERIALS AND METHODS

The experimental design of this research combined the experimental protocols for aquatic microcosms that were suggested by Leffler (1982) and Taub and Read (1982) and provide a screening system for chemicals based on ecosystem-level effects produced by potentially toxic substances in freshwater systems.

Microcosm Description

The microcosm vessel, a one-liter (1000 mL) Pyrex glass container, was open at the top to allow free exchange of gases with ambient air, a feature that enhances the persistence of simple microcosms (Taub, 1974). A 50 mL sediment volume was added to each microcosm (sediment description is in a following section), followed by the addition of 900 mL of chemically defined medium (Taub #82 (Taub and Read, 1982)) with some modifications (the chemical composition of Taub #82 will be presented later). Biotic inoculum (50 mL) was added after the sediment and medium reached a relatively constant neutral pH between 6.0 and 7.5 pH. The inoculum came from two stock, laboratory microcosms (38 L), which were initiated three months earlier and

therefore were well established. Initiation was with inoculum from a variety of sources, including lakes, ponds, streams, pure cultures and other laboratory aquaria. These stock, laboratory microcosms were reinoculated from natural sources frequently. The biotic inoculum is described in more detail later.

Microcosm Vessel

The microcosm vessels were one-liter, hard (borosilicate) glass beakers. Glass was chosen because the toxic substances being tested were organic chemicals, and adsorption and desorption of organic chemicals to and from borosilicate glass is minimal. Vessels were cleaned according to National Bureau of Standards suggestions (Struempler, 1973) and Leffler's (1982) suggestions. The vessels were washed thoroughly with laboratory detergent, then rinsed five times with tap water, five times with 200 mg/L hypochlorite solution (strong base), five times with distilled water, five times with 10 percent hydrochloric acid (strong acid), and five times with distilled water.

One criticism of microcosm research has been that the surface to volume ratio in these systems is unrealistic; however, the surface to volume ratio of 1:1 cm /cm is within the range of some common aquatic

systems such as farm ponds or glacial lakes (Wetzel, 1983). The surface area of the vessel wall and sediment layer would provide a better indication of actual substrate space available for colonization, but it is difficult to determine in microcosms and natural systems. The vessels used in this study were similar to the microcosm vessels used by Leffler (1982).

Sediment Description

The study was divided into two phases that were based on the different types of sediment being studied. Three types of synthetic sediment were used in this study, a sand sediment, a clay sediment and a sand and clay mixture sediment. The sediment studied in phase one was a sand sediment quite similar in composition to the "standard sediment" Taub and Crow (1980) recommended. The sand sediment was 99.5 percent by weight silica sand and 0.5 percent organic matter. The organic matter was 50 percent ground, crude chitin and 50 percent powdered cellulose. The average sand particle diameter was approximately 0.3 mm, which classifies it as medium-size sand according to the soil scientists (Brady, 1974). This diameter for sediment particles is relatively large, and the sand settles quickly when disturbed.

Two sediment types were studied in phase two of this

research, sediment with clay and a clay and sand mixture. The clay sediment was comprised of kaolinite clay (EPK clay from Feldspar Corp., Edgar, FL) and organic matter (chitin and cellulose, as was added to the sand sediment). Clay was chosen because of its relatively small particle size (approximately Ø.Ø6 mm diameter). Kaolinite clay was chosen because of its nonexpanding character and low cation exchange capacity (3-15 meq/1ØØ g) compared to other silicate clays (Brady, 1974). The particle size of Ø.Ø6 mm diameter was relatively small and two hours were required for complete settling. The medium (as described later) has a divalent-cation concentration sufficient to settle this type of clay particles.

The second sediment type studied in Phase 2 was a mixture of equal volumes of the sand and clay described previously. Organic matter was added in amounts and composition identical to the first two sediments described. The sand-clay sediment had a wide range of particle sizes from Ø.Ø6 mm to Ø.3 mm in diameter.

The three sediments were acid washed and rinsed with distilled water until neutral pH was reached, then they were oven-dried before the organic matter was added. The organic matter was added in equal amounts to all sediments in powdered forms.

The most prominent difference in these three

sediments was the particle size, but other physical and chemical properties varied with the sediment type and could not be distinguished as a variable agent apart from the particle size variable. These characteristics; such as cation-exchange capacity, double-layer thickness, and particle surface character are directly influenced by particle size and are thus unique for a soil type (Hillel, 1982). The differences among these sediment types involves more than just particle size differences and these differences are all considered part of that sediment types influence.

Chemical Medium Description

The water medium used in these microcosms was synthetic and chemically defined. The use of synthetic medium in microcosms is often criticized, however its standardization is necessary to ensure accurate replication and to provide the opportunity for comparisons to be made among various microcosm research laboratories. The medium used in these microcosms was a modified Taub #82 medium (Taub and Read, 1982). Taub's medium has been and still is commonly used in microcosm research (Beyer, 1962; Gordon et al, 1969; Leffler, 1977; Hendrix et al. 198Ø). Taub #82 medium has the proper ratios of cations and anions to allow growth of most

algal species, including blue green algae and diatoms, and to allow the growth and reproduction of most planktonic and benthic animal groups. Microcrustaceans, including cladocerans, grow and reproduce easily in this medium, as do other diverse animal groups such as protozoans and nematodes. The detailed medium composition is found in Table 1. The modification of Taub #82 medium was the addition of sodium bicarbonate ($NaHCO_3^-$). The reason for this modification was a concern about the probability of carbon dioxide limitation occurring in the microcosms especially during peak oxygen production periods. According to Lehman et al. (1975) and King and Novak (1974) a significant correlation was found between the average, dissolved carbon dioxide concentration in an aquatic ecosystem and the algal group dominating the aquatic ecosystem. The lower the carbon dioxide concentration, the more dominant the blue-green algal community (Moss, 1973). With the addition of bicarbonate (HCO_3^-) another source of inorganic carbon is available for disassociation to form carbon dioxide; addition of bicarbonate also serves to minimize the pH variations that occur in aquatic ecosystems. Because bicarbonate (HCO_3^-) occurs naturally in most aquatic ecosystems, this modification made the microcosms more realistic.

Stock solutions of the salts listed in Table 1 were

Table 1. Chemically defined medium composition (Taub and Read, 1982), used in aquatic microcosms.

Medium T82 Composition*

Compound	Element	Concentration	
		mM	mg/L
$NaNO_3$	N	Ø.5	7.Ø
$MgSO_4 \cdot 7H_2O$	Mg	Ø.1	2.43
KH_2PO_4	P	Ø.Ø4	1.23
	K		1.23
NaOH	Na	Ø.Ø99	2.27
$CaCl_2 \cdot 2H_2O$	Ca	1.Ø	4Ø.Ø
NaCl	Na	1.5	34.5
$Al_2(SO_4)_2 \cdot 18H_2O$	Al	Ø.ØØ48	Ø.26
$Na_2SiO_3 \cdot 9H_2O$	Na	Ø.8Ø	36.8
	Si		22.4
$NaHCO_3$	HCO_3^-	1.Ø	12.Ø
	Na		23.Ø
Trace Metals, etc:			
$FeSO_4 \cdot 7H_2O$	Fe	1.12	Ø.Ø625
EDTA	EDTA	1.42	Ø.4145
H_3BO_3	B	Ø.75	Ø.ØØ8
$ZnSO_4 \cdot 7H_2O$	Zn	Ø.Ø25	Ø.ØØ15
$MnCl_2 \cdot 4H_2O$	Mn	Ø.25	Ø.Ø135
$Na_2MoO_4 \cdot 2H_2O$	Mo	Ø.Ø25	Ø.ØØ24
$CuSO_4 \cdot 5H_2O$	Cu	Ø.ØØ5	Ø.ØØØ32
$Co(NO_3)_2 \cdot 6H_2O$	Co	Ø.ØØ25	Ø.ØØØ15

*Note: modified by the addition of $NaHCO_3$ to provide a source of inorganic carbon.
Hardness = 1Ø9.9 mg/L as $CaCO_3$
Alkalinity = 6Ø mg/L as $CaCO_3$
NO_3-N to PO_4-P ratio is 12.5 (nM).
pH adjusted to 7.Ø

prepared according to specifications suggested by Leffler (1982) and Taub and Read (1982) and stored at 4°C until the microcosms were initiated. When the microcosms were initiated, the appropriate proportions of the stock solutions were mixed with distilled water in a 20 L carboy, then dispensed to the microcosm vessel after the pH had been adjusted to a neutral pH (See Table 1).

Biotic Inoculum

The biotic inoculum for the test microcosms consisted of a diverse assemblage of aquatic organisms (excluding fish) resembling the biota common in small ponds. The inoculum came from two 38 L, laboratory microcosms, called "stock" microcosms. These stock microcosms were initiated by the addition of Taub #82 medium, the sand sediment with organic matter, and the inoculum, combined at least three months earlier in order for the organisms to adjust to laboratory environmental conditions (Leffler, 1982). During this adjustment period, some species died, and new or modified food webs developed concomitant with the adjustments of species interactions and population dynamics in the stock microcosms. The sources of the biota in the stock microcosms included natural sources such as lakes, streams, ponds and artificial sources such as pure

cultures and older stock microcosms. The stock microcosms were reinoculated regularly with small volumes of samples from various sources to increase genetic diversity and were maintained at a constant volume by the frequent addition of distilled water (approximately one liter twice a week) to replace evaporative losses.

In addition the stock microcosms were maintained under the same environmental conditions as the test microcosms and were cross-inoculated frequently with the other stock microcosm to produce similar aquatic ecosystems.

A survey of the types of organisms found in these microcosms indicated that the stock microcosms met the minimum criteria for biotic diversity suggested by Hammons (1981), Taub and Crow (1980) and Leffler (1980). These criteria include the following types of organisms: grazer-edible, unicellular green algae, diatoms, filamentous green algae, blue-green algae, grazing macro-invertebrates, benthic detritivore macro-invertebrates, bacteria, fungi, and protozoa species. The stock microcosms included a much wider variety of organisms than suggested, such as nematodes, rotifers, and gastrotrichs.

The test microcosms were inoculated with 50 mL (5 percent of the test microcosm volume) from the well-mixed, stock microcosms after the medium and

sediment equilibrated in the microcosm vessel. The test microcosms were cross inoculated biweekly during the pretest (before toxicant was added) period by mixing equal volumes of samples from each test and stock microcosm in a large container and then adding equal volumes from the container (approximately 25 mL) back to each microcosm. Cross inoculation helps to decrease the potential for founder-species differences among microcosms, according to Leffler (1982).

After the toxic substances were added to the microcosms, cross inoculation could not be continued because the treatments were different and because the statistical assumptions concerning replicates in certain statistical analyses were different.

Weekly reinoculation from the stock microcosms continued throughout the remainder of the study. Frequent reinoculations from the stock microcosms provided opportunities for recolonization by organisms killed by the toxic substance, and simulated reasonably well the characteristic of natural aquatic ecosystem's continual recolonization. The test microcosms were allowed to mature for six weeks prior to the addition of the test chemicals. The duration of the pretest time interval was the same as that suggested by Leffler (1982) and Hendrix et. al.(1981). Leffler (1982) suggested that major changes measured after six weeks were due to

external perturbation (such as a toxicant) and that the microcosms reach their normal operating range in this period of time. Normal operating range is the 95 percent confidence bounds of an ecosystem measurement.

Environmental Conditions During Growth

The microcosms were situated in a temperature controlled room. The room temperature was constant at 20 ^{o}C $\pm$ 5^{o}C, and the water temperature was 22^{o}C $\pm$ 5^{o}C. The microcosms (stock and test) were arranged randomly on two tables covered with white paper, under six, six-foot light banks with two Vita Light (Duro-Test Company) fluorescent bulbs in each. The microcosms were arranged in such a way to provide even and consistent lighting for all microcosms. The average light intensity at the microcosm water surface was approximately 45 micro-Einsteins/sec-m (a measure of light intensity), which is in the range of light intensities used or prescribed in most microcosm research (Giddings, 1982; Leffler, 1982; Taub, 1982). To reduce the potential light or temperature gradient the microcosms were rearranged in a random manner every three weeks. No significant difference was noted due to position in growth chamber.

These carefully controlled environmental conditions

were necessary for the short time scales that make microcosm testing of toxic substances so attractive. Admittedly, the daily variations in light intensities at the water surface and air temperatures, both of which normally peak at mid-day, were not simulated in growth chambers. These fluctuating growth conditions are abiotic in nature, however, and are not controlled by the biotic component. Therefore, because our major interest was in the biotic component, manipulating the abiotic environment did not invalidate the information obtained but rather enhanced the quality of information by allowing increased replication.

Test Chemicals

The four test chemicals were organic compounds, chosen because of organic compounds' potential toxicity, their increasingly larger potential in contamination of our waterways, and the relative paucity of information about their ecosystem-level effects. The comparative nature of this research required that the selected chemicals must have been evaluated previously in other microcosm studies and that single-species, bioassay data be available, preferably for aquatic species. With these criteria in mind the following compounds were chosen: phenol, naphthoquinone, quinoline and triethylene glycol.

These four also represent three distinct, structural types of organic compounds, therefore, data collected from studies of these should be helpful in predicting toxicity of similarly structured organic compounds. The compounds represent a single ring with one substituent group (phenol), a double ring with nitrogen in one ring (quinoline), a double ring with double-bonded oxygens on one ring (naphthoquinone), and an aliphatic hydrocarbon (triethylene glycol). Microcosm and/or single-species-bioassay data concerning these four compounds are available (Leffler, 1982; Milleman et al., 1984; Taub, 1982).

The toxicants were obtained from the following sources: phenol-Mallinckrodt Inc., quinoline-Fisher Scientific Products, naphthoquinone, and triethylene glycol-Eastman Kodak Corporation. The four levels of the compounds tested were the same for all compounds except naphthoquinone, i. e. 10 mg/L, 100 mg/L, 1000 mg/L, and 10,000 mg/L. Naphthoquinone levels were lower because of the high toxicity of this toxicant (Leffler ,1982). The naphthoquinone levels were 0.01 mg/L, 0.1 mg/L, 1.0 mg/L, and 10 mg/L. The first three toxicants' concentrations (TEG, quinoline and phenol) may appear to be unusually high, however, they do overlap with other microcosm researcher's test concentrations and were necessary to show a gradient of effects that overlapped the

no-observable-effects level. To promote the solubility of these compounds in the water column, a carrier compound (acetone) was mixed with the chemical first, then the compound, dissolved in the carrier, was added to the microcosm. Acetone was chosen as a carrier because of its solvent characteristics and its low toxicity at the concentrations at which it was used (Leffler, 1982). The final acetone concentration was Ø.1 ml/L in each test microcosm where a toxicant was added and each carrier-compound control. In order to ensure that any carrier-compound effects were known, acetone was added to four microcosms in concentration added to the test microcosms. In the instances where a significant difference was noted between the control microcosm (no carrier nor toxicant added) and the carrier control microcosm, the carrier control microcosm data were substituted for the control-microcosm data in the analysis. The test compounds were only added once after the microcosms had been initiated. No effort was made to maintain the initial concentrations of the test chemical.

Four replicate microcosms for each test-chemical concentration, carrier control, and control were set up in Phase 1 of this research. At least three replicate microcosms were set up for each test chemical concentration in Phase 2. Leffler (1982) suggested five

replicates of each test chemical concentration. Because of time limitations, however, only three replicates during Phase 2 and four replicates during Phase 1 were involved. Time limitations were dictated by the length of time it took to analyze for a particular constituent in all microcosms on any given day. It was desirable to minimize the time interval between the first and last analysis. Phase 1 and 2 required measurements to be made on 74 microcosms. At 1-2.5 minutes per measurement, three hours was considered the maximum time interval acceptable.

Phase 1 involved the addition of the four chemicals at four concentrations, but in Phase 2 only three of the original four were tested (phenol was omitted) and only the three lowest concentrations were tested. As described earlier, another variable was added in Phase 2 (sediment type), so the number of toxicants and treatment levels had to be reduced to minimize the length of time for analyses.

The test chemical concentration in each microcosm was measured at the time of addition, three weeks after the addition, and at the end of the experimental period (seven to eight weeks after the addition) in order to document the actual concentration in the water column. The determination of organic-compound concentrations (actual toxicant levels) were made by gas chromatographic

analyses. The details of the chromatographic analyses are presented in detail later in this section.

Microcosm Measurement and Sample Procedure

The parameters monitored in these microcosms covered a wide range of ecosystem level properties and accounted for the important characteristics of aquatic ecosystems. At a workshop at Oak Ridge National Laboratories (Hammons, 1981) several microcosm researchers concluded that the following properties of aquatic ecosystems are important in assessments of ecosystem's damage by toxic substances: autotrophic activity, heterotrophic activity, the physical-chemical characteristics of the ecosystem and nutrient cycling characteristics. Analyses of taxonomic characteristics were strongly suggested with the understanding that taxonomic characteristics are not ecosystem properties but instead are community properties.

In this study, autotrophic activity, heterotrophic activity, and the physical-chemical characteristics of the microcosms were the primary focus in analyses. These measurements can be made more rapidly on large numbers of test systems and the time from the initiation of an experiment to the development of conclusions from the experiment will be shorter, a necessary characterisitic

from an applied point of view.

The measurements made to characterize these parameters were as follows: autotrophic activity--diurnal dissolved oxygen measurement; heterotrophic activity--C-14 glucose-mineralization rate; and physical-chemical characteristics--pH and alkalinity. A detailed description of the techniques used for measurement will follow.

Autotrophic activity . The autotrophic activity of the aquatic microcosms was measured by the diurnal dissolved oxygen technique developed by McConnell (1962) and Abbott (1966) and used successfully by Giddings (1982), Thomas (1978), Hendrix et al. (198Ø), Taub and Crow (198Ø), and Leffler (1982). The diurnal-oxygen method employed for determination of autotrophic activity requires three diel dissolved-oxygen measurements. One is made just before the lights go on in the morning, a second is made twelve hours later just before the lights go off, and the third measurement is made the next morning before the lights go on. The oxygen increase from dawn to dusk is an indicator of net primary production, and the oxygen decrease from dusk to dawn the second day is an indicator of community respiration. If the net production and community respiration values are added together, a rough estimate of gross production

results. Taub and Crow (1980) suggested the change in oxygen concentration from dawn to dawn is an index of autotrophy. Many researchers (Margaleff, 1968) (Odum, 1971) have suggested that the net production to community respiration ratio (P/R) is an integrative property of ecosystem health.

The technique used in these autotrophic-activity determinations involved the measurement of dissolved-oxygen concentration with a Yellow Springs Instrument (YSI), Model 33 self-stirring Biochemical Oxygen Demand (BOD) probe (Yellow Springs Instrument Company, Yellow Springs, Ohio). The probe was attached to a wooden holder, which rested on the sides of the beaker, which placed the probe in the center of the beaker with the membrane approximately four centimeters (cm) below the water surface. The probe's stirrer was activated, and measurements were recorded when the meter was stable. The membrane was changed weekly, and the meter was recalibrated every time a set of measurements was made. The probe was rinsed with distilled water twice before the probe was placed in a different microcosm.

Heterotrophic activity . The measurement of heterotrophic activity of aquatic microcosms is less well-developed than the measurement of autotrophic

activity. The diurnal, dissolved oxygen method does provide a relative, heterotrophic-activity measurement (community respiration), but Leffler (1982) suggested that the diurnal, dissolved oxygen technique was more accurate for autotrophic measurements than for heterotrophic measurement. The respiration value calculated from the diurnal, dissolved oxygen technique is community respiration and may not reflect changes in heterotrophic activity when the autotrophic community is changing also. The community respiration data from the diurnal dissolved oxygen measurements are mentioned in the results, primarily for comparative purposes because Leffler (1982) reported these values.

A second and more useful index of heterotrophic activity was measured in the test microcosms by a radiorespirometric technique that was based on a method developed by Wright and Hobbie (1966) for seawater and modified by Henry (1983) for toxicant testing in microcosms. The technique provides an index of microbial mineralization activity (carbon dioxide production from a ^{14}C-labeled substrate) and, thus, is more indicative of heterotrophic activity because the measurement is independent of the measurement of autotrophic activity. The National Academy of Sciences (1981) suggested that measures of microbial mineralization and assimilation are important measures of ecosystem behavior (i.e. ecosystem

health). The radiorespirometric technique has not been used extensively in microcosm research but may fulfill a need for better measurements of heterotrophic activity. The advantages of this technique include the small sample size required (15 mL), which is an important consideration in one-liter microcosms, and the improvement of heterotrophic activity characterization by measures independent of autotrophic measurements. The technique also can be standardized as long as controls and replicates are included in the protocol. One disadvantage of this technique is the potential for wide variability (Henry, 1983). The number of steps involved in the procedure provides many opportunities for the variability to increase. The coefficient of variation found in this research for the procedure was 25 percent, and the coefficient of variation for the procedure and the replicate treatment variability (due to differences in the microbial population) were fifty percent. These coefficient of variation values are high for the method, but they are still lower than the large, natural variability of microbial populations.

The protocol followed in this research was based on one outlined by Henry (1983). A 15 mL unfiltered sample was taken from each microcosm and added to a 50 mL flask (Kontes Glass Co., Vineland, N.J.). This was followed by the addition of 0.15 μCi of radioactively labeled glucose

(C-14). The volume of the ^{14}C glucose added was Ø.3 mL. The radioactive glucose was purchased from ICN (lot# 156501A, ICN Corp., Irvine, CA.) and had a specific activity of 250 μCi /mmol. The isotope was diluted with sterile, distilled water to a concentration of Ø.5 μCi per mL. The flask was sealed with a specifically designed, serum stopper, which was fitted with a plastic, center-well containing a pleated, 2 cm x 5 cm strip of Whatman No.1 chromatography paper (Fisher Scientific Co., Pittsburg, PA.). The flasks (four at a time) were placed on a shaker table and shaken gently for 15 minutes plus or minus two minutes (15 $\pm$ 2 min) in the dark.

The flasks were removed from the shaker table after the allotted time, and the samples were acidified by the addition of 1.Ø mL of sulfuric acid (2N) with a syringe equipped with a 25-gauge, 3.8 cm needle to stop the heterotrophic activity and cause the dissolved carbon dioxide (CO_2) to escape from the solution. Immediately after the acid addition, a CO_2 trapping agent (carbosorb from Packard Instrument Co., Downer's Grove, IL) was injected from a syringe with a 25-gauge 3.8 cm needle onto each pleated filter paper in the center wells of the flasks. The flasks were then returned to the shaker table and were shaken gently in the dark for two hours

plus or minus one-half hour (2 h ± Ø.5 h). At the end of this period the flasks were removed from the shaker table, the serum stoppers were removed and the filter papers transferred with tweezers to a polypropylene, scintillation vial (Fisher Scientific Co., Pittsburg, PA) containing 15 mL of Permafluor V scintillation fluor (Packard Instrument Co., Chicago, IL). The vials were securely capped and stored in the dark at 21.Ø C ± 3 C for 24 h ± 3 h. After 24 h the disintegrations were counted in a Beckman LS-23Ø Beta Spectrometer (Beckman Instruments Inc., Fullerton, CA.). Two channels were used when counting; channel A was set to monitor the tritium (H-3) window and Channel B was set to monitor the wide, C-14 window. The channel ratio was calculated (B/A) in order to monitor the quench characteristics.

All samples were counted along with an acidified control in which the acid was added to stop all heterotrophic activity before the substrate was added. A C-14 standard was counted each time in order to measure the scintillation counter's efficiency that day. Because of the relative comparative nature of this research all treatments were compared to controls, any variation in the quench, the efficiency (approximately 94 percent) or other protocol procedures affected the controls and the treatments equally so it was not necessary to take these variances into account, nor was it necessary to convert

counts per minute to disintegrations per minute. Calculations were made on the percent deviation in the counts per minute (cpm) of the treatment from the control, using the following formula (Leffler, 1982):

$$\% \text{ dev.} = \frac{\text{mean cpm of cont} - \text{mean cpm of treat}}{\text{mean cpm of control}} \times 100$$

Physical-chemical characteristics . One of the variables that was indicative of the physical-chemical characteristics of the microcosms was pH. The pH of an aquatic ecosystem has been described as one of the "master variables" because pH is affected by both the abiotic and biotic environment. (Schindler, et al 1980, Wade et al 1980). The pH was important also in assessing the potential for CO_2 limitation to occur, as was mentioned earlier in the chemical medium section. Diurnal, measurements of pH were made each time dissolved oxygen was measured (twice a week). The pH probe was attached to a wooden holder that rested on the sides of each beaker as measurements were made. The test microcosms were stirred with the stirrer on the dissolved oxygen probe. The pH meter used in this analysis was an Accumet pH meter model 144 and the pH probe was a Fisher combination electrode (Fisher Scientific Co., Pittsburg,

PA). The meter and the probe were recalibrated with pH 7 and pH 10 standards twice daily when measurements were made. The actual high and low pH values in each diurnal set were used in the analyses of results, as were the pH changes while the lights were on and while they were off.

The alkalinity of the test microcosms was measured two or three times during each phase of this research to assess the inorganic carbon available to the primary producers and to assess the similarity among replicates. Alkalinity and pH values provide a good estimate of the amount of dissolved inorganic carbon ($CO_3^{=}$, HCO_3^{-}, and CO_2) that is available to the primary producers. Alkalinity was measured in a 15 mL sample by the titration method, and the indicator bromocresol green (APHA, 1980). The buret used for dispensing the titrant (0.02 N H_2SO_4) was a 15 mL buret calibrated in 0.01 mL increments.

Conductivity was measured two or three times during each phase to evaluate the similarity among replicates. These conductivity measurements were made with a Yellow Springs Instrument (YSI, Yellow Springs, Ohio) S.C.T. meter, model 33.

Water temperature in six to eight microcosms was measured each time dissolved oxygen and pH were measured. The YSI dissolved oxygen meter and probe, which also had a temperature probe was used. Air temperature in the growth chamber was measured continually by a recording,

temperature gauge (Taylor Instrument Co., Asheville NC).

Orthophosphate concentration in all of the microcosms was determined once during phase 2, primarily to assess the similarity among replicates. The analyses were performed using the combined-reagent method as described in Lind (1979) but modified for a 15 mL sample. The reagent volume was 2.5 mL. The spectrophotometric analyses were performed with a Bausch and Lomb Spectronic 20 (Bausch and Lomb Inc., Rochester, NY) at a wavelength of 690nm.

Toxicant concentrations were determined with a Hewlett Packard Gas Chromatograph model 5880A (Hewlett-Packard Corp., Avondale PA). The chromatographic column used for analyses was the Supelco SE 30 (catalog number 1-2793, Supelco., Inc., Bellefonte, PA). The temperature settings are listed in Appendix 1. These analytical conditions were determined through consultation with McNair (personal communication, 1984) and through the analyses of standards prepared for each toxicant. These conditions produced the best well defined peaks. All controls were analyzed each time for each combination of conditions.

Samples for chromatographic analyses were taken from the microcosms in 15 mL aliquots, filtered with a glass fiber filter, and acidified with phosphoric acid to a pH of 2.0. The samples were frozen and stored at 0 C until

analysis (APHA, 1980). An additional study was done to determine potential decrease in toxicant concentration due to storage time (seven months maximum) and to sample-preparation procedure. In these studies, standards of all toxicants were prepared including seven different concentrations of the toxicants (5 mg/L - 20,000 mg/L). In the study of the influence of sample preparation procedure the standards were prepared at each stage of the sample preparation procedure. One group of standards was filtered and acidified, a second group was only filtered, and a third group was neither filtered nor acidified.

Community Composition . The taxonomic analyses described above were not the primary emphasis of this research and, therefore, were not extensive. Observations were made throughout the experimental period, and during phase 1, gross taxonomic observations were recorded once. Observations were made throughout Phase 2, and gross taxonomic observations were recorded twice.

Statistical Analyses

Means and standard deviations of all parameters were calculated using the Statistical Analysis System (SAS Institute, 1982) available at Virginia Tech on the IBM

360 computer. Nonparametric statistics such as median estimators and dispersion calculations, were made using the "Univariate Procedure" of the Statistical Analysis System (Ray, 1983) on the IBM 360 computer. The statistics were calculated on the primary variables: production, heterotrophic activity, and maximum dissolved oxygen, and for the secondary variables, alkalinity, conductivity and orthophosphate concentration; the statistics were calculated on a Hewlett Packard model 11-C calculator. These statistics were calculated in a variety of combinations of treatment level, sediment type and number of weeks since initiation. The mean and standard deviation were calculated, 1) for each parameter, each treatment level, and each sediment at each sampling time; 2) for each parameter, each treatment level, each sediment, and pooled over all measurement times and (3) for each parameter, each treatment level and pooled over sediment type and over all measurement times. The median and interquartile range (dispersion) was calculated for net production and maximum dissolved oxygen.

The calculation of the statistics of central tendency and dispersion were followed by a two-way analysis of variance (ANOVA) procedure for parametric analysis. The ANOVA procedure was available on the SAS program. When significant effects were found for any

parameter due to toxicant concentration or sediment type and with time elapsed since initiation of the microcosms as a covariable, then the means could be compared statistically.

The means of each parameter were tested for significant differences among treatment levels, among sediment types, and among treatment level and sediment type together as a unique treatment. The paired t test was used in these analyses.

Regression analyses were performed for each parameter's percent deviation of the treatment level from the control versus that treatment level. The percent deviation was the dependent variable and the treatment level was the independent variable. Calculation of percent deviation values for each treatment level at each sampling period was made with the following equation:

$$\%\ dev_{ijk} = \frac{\text{mean of controls}_{ij} - \text{mean of treatment}_{ijk}}{\text{mean of controls}_{ij}} \times 100$$

where i = number of sampling period

j = ecosystem property measured

k = treatment level of toxicant

A typical dose response curve of a continuous variable such as percent deviation meets the statistical assumptions required for the least squares method of calculation of linear regression and subsequent desired predictions. The least squares method analyses for linear regression were done using the SAS Program for General Linear Models (GLM), and the Hewlett Packard Model 11-C calculator. A linear model for toxicant concentration (independent variable) and percent deviation from controls (dependent variable was developed for each ecosystem property for the first week after addition (acute percent deviation) and the sum of all weeks (chronic percent deviation). These models were used to predict EC20 values.

In all of these statistical analyses the control microcosms were used as a basis for comparison. No significant differences were found between the carrier control and the control microcosms. The comparison of the two sets of controls will be presented in the Results Chapter.

The linear equations generated from these analyses allowed the prediction of EC20 values which are used in toxicity testing to indicate a toxic chemical concentration that causes an effect measured by a 20 percent deflection from the control at a particular sampling time (Leffler, 1981).

The EC20 values calculated one week after the toxicant was added would be indicative of acute toxicity levels, while those calculated after the toxicant had been present in the microcosm for a longer time period would be more indicative of chronic, or long term toxicity. In much toxicity testing, these two considerations in toxicity (immediate and long term effects) are tested separately, when in actuality both types of toxicity occur together in a real world situation.

Another comparison of the ecosystem properties and their EC20 values was made. Some toxicants affect the component parts of ecosystems differently and may be directly toxic to one component of the ecosystem but not to another. As an example, a change in production would indicate a lower EC20 value for a particular toxicant than would a change in heterotrophic activities would if algae were more affected than bacteria.

Data Interpretation

The complicated nature of the experimental design in this research allows any number of narrow conclusions to be drawn. However, to make this study informative in an applied way, certain analyses must be singled out as having greater significance and usefulness than others.

The needs of industry and regulatory agencies for criteria and subsequent standards for toxic chemicals stress the importance of the EC20 values. These values provide a criterion for a regulatory agency to use in proposing a standard and can provide a preview for industry about their need for environmental controls during production and actual production costs for a new chemical. Because of the applied emphasis of this research, the EC20 values were a major part of the data interpretation.

Another calculation that has been suggested by Leffler (1981) combines all sampling times and all parameters and provides one number to represent a toxicant's relative toxicity. Leffler (1981) named this the Relative Effects Index (REI). A similar index, suggested by Webster (1975), named the total relative-stability index, was used to integrate the total impact of any perturbation on an ecosystem. However, after using these indices in other analyses, Leffler (1983) suggested that they were not useful.

Rankings of the toxicants were made using four different ranking procedures. The first was based on the calculation of EC20 values from net production values during the first week for each toxicant (acute EC20 for production). The second was based on EC20 values from net production values pooled over the treatment period (6

wks) for each toxicant (chronic EC20 for production). The third was based on EC20 values from heterotrophic activity values during the first week for each toxicant (acute EC20 for heterotrophic activity). The fourth was based on EC20 values from heterotrophic activity values pooled over the experimental period for each toxicant (chronic EC20 for heterotrophic activity). The ranking's described by this research were compared to other microcosms' and bioassay data (Leffler, 1982; Milleman et al, 1984; and Taub and Read, 1982).

IV. RESULTS

Introduction

In this chapter, there are six main sections describing the results of the research. The sections are: I. The Effects of Sediment Type on Microcosm Attributes, II. The Effects of Toxicants on Microcosm Attributes, III. Statistical Analyses Comparisons, IV. Ranking of Toxicants According to Toxicity, and V. Measurement of Ecosystem Properties Comparison.

In the section on "The Effects of Sediment Type on Microcosm Attributes", only three of the toxicants are compared: triethylene glycol, quinoline and naphthoquinone. Phenol was only added to microcosms with sand sediment, and, therefore, no comparison of sediment type effects could be made. The sand sediment was added to microcosms in phase I and the clay and clay/sand sediments were added to microcosms in phase II. In the microcosms with sand sediment, four concentrations of each toxicant were added, but in those containing the clay and the clay/sand sediments only three concentrations were added, because of time and space

considerations.

In the section on "The Effects of Toxicants on Microcosm Attributes", results describing the effects of all four toxicants are reported, and data from the microcosms with different sediment types and identical treatments (toxicant concentration) are averaged and reported as a single value for each particular treatment. Phenol, triethylene glycol and quinoline all were added at the same concentrations: 10 mg/L, 100 mg/L and 1000 mg/L in the clay and clay/sand sediment types. The concentrations added to microcosms containing the sand-sediment were 10 mg/L, 100 mg/L, 1000 mg/L and 10,000 mg/L. Naphthoquinone was added at concentrations of 0.01 mg/L, 0.1 mg/L, 1.0 mg/L and 10.0 mg/L in the sand sediment microcosms, and 0.01 mg L, 0.1 mg/L and 1.0 mg/L in the clay and clay/sand sediment microcosms. The actual concentrations are described in the results and reflect the concentration in a sample taken four hours after the toxicant was added from each treatment (toxicant concentration) as analyzed on a gas chromatograph. A range of concentrations are used to describe the treatments when production or heterotrophic activity values for microcosms with different sediment types are averaged. Although the added concentrations were the same the measured concentrations were not. The analyses for phenol and quinoline were more accurate and

precise than the analyses for triethylene glycol and naphthoquinone.

The two major ecosystem properties reported are net primary production and heterotrophic activity. The net production is calculated as the difference in dissolved oxygen concentration from dawn to dusk (12 hours of light). The heterotrophic activity is the measure of carbon dioxide production using a radio-labeled carbon source as described in the Materials and Methods.

The week number used in the graph refers to the number of weeks after initiation of the microcosms when the designation is pretreatment. The designation posttreatment week refers to the number of weeks after the toxicants were added.

When the percent deviation from control is used as a variable, the deviation of the treatment value from the control may be greater or less than the control microcosm value.

Effects of Sediment on Various Microcosm Attributes

Effects on Net Production

Controls. The highest production throughout the experimental period occurred in the sand sediment control and acetone-control microcosms. The next highest was observed in the microcosms containing clay sediment. Production reached a high point in the sand sediment

microcosms (8.2 mg/L oxygen) at pretreatment week 3 (Figure 1A). The high point in the clay and the clay/sand sediment microcosms was reached at pretreatment week 2 (5.5 mg/L oxygen) (Figure 1B & C).

The mean production in the sand-sediment controls during the entire study was 5.2 $\pm$1.8 mg/L oxygen. The mean production in clay-sediment controls during the entire study was 2.4 $\pm$1.2 mg/L oxygen. The dissolved oxygen production in acetone-controls did not differ significantly from the other controls (Figure 2). Acetone was added to the acetone controls at the same time the toxicants were added and at the same concentration that is was added with the toxicants.

Production in microcosms containing the clay/sand sediment fluctuated little throughout the experimental period (Figure 1C). However production in the sand sediment and clay sediment microcosms fluctuated significantly more than the mixed sediment (Figure 1A & B). The acetone was not added to the acetone-control microcosms until the toxicants were added to the treatment microcosms (posttreatment week 1).

Triethylene glycol (TEG) toxicity. The percent deviation of production values in the treated microcosms from those in the controls increased significantly during and after posttreatment week three (21 days after toxicant addition) in the microcosms containing sand only

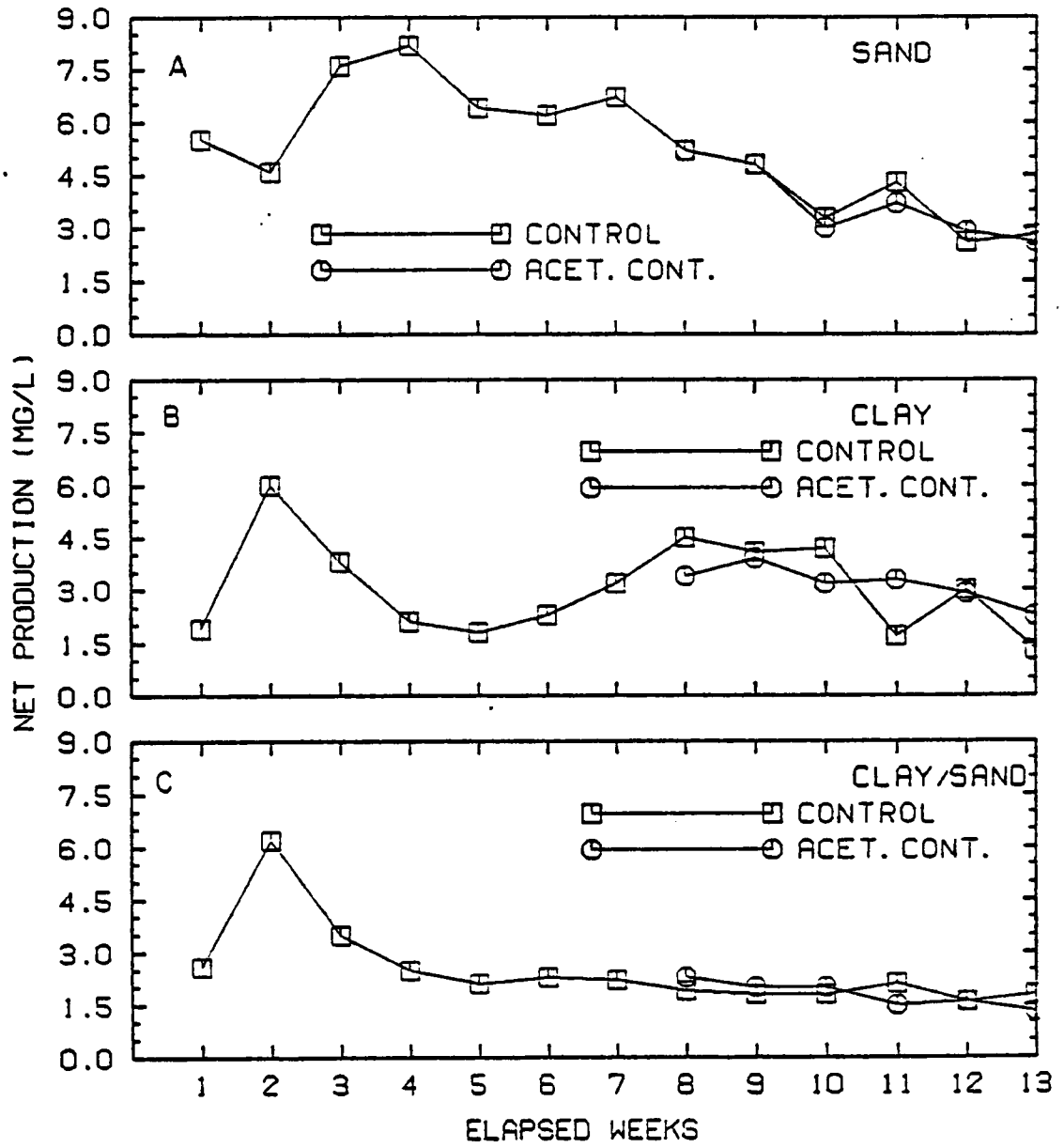

Figure 1. Mean net production in control and acetone-control (carrier) microcosms for each week during experimental period and each sediment type. Weeks 1-7 are pretreatment weeks (n=144) and weeks 8-13 are posttreatment weeks (n=4).

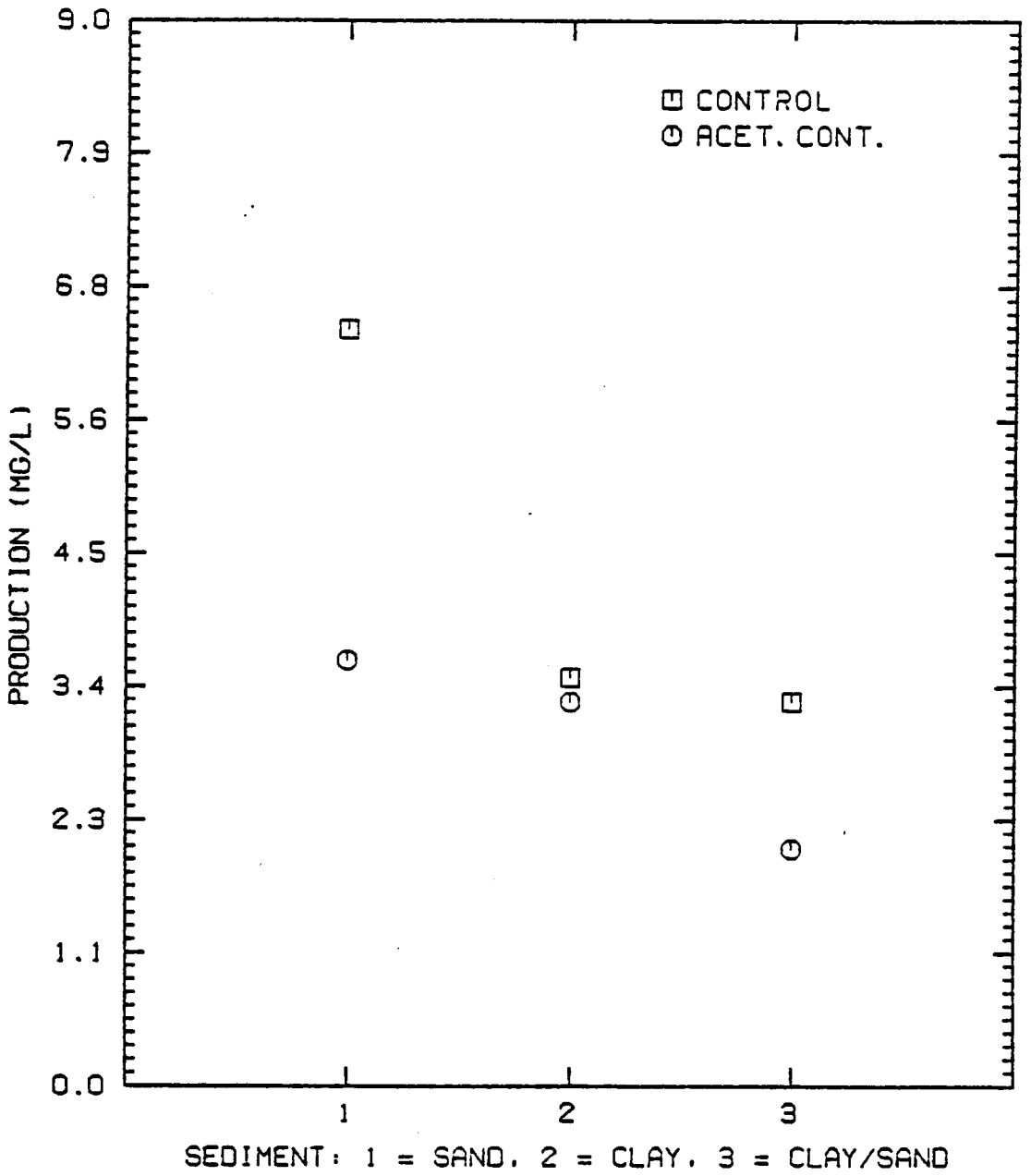

Figure 2. Mean net production in control and acetone-control (carrier) microcosms for each sediment type pooled for all weeks (n=52 for sand, and n=39 for clay and clay/sand).

and both clay and sand (Figure 3A & C) but in those with clay only the percent deviation of net production from the control was high (mean percent deviation = 23.7% to 53.8%) most of the experimental period (Figure 3B). The mean percent deviation in production of the microcosms with sand only before week three was 18.1% and 41.3% during and after week three. The mean percent deviation in production for the microcosms with both clay and sand before week three was 16.9% and 28.2% during and after week three.

The highest deviations in production within the various microcosms were as follows: sand-sediment--TEG dose, 20 mg/L, 62% on week 3; clay sediment--TEG dose, 17 mg/L, 75% on week 7; clay/sand sediment--TEG dose, 17 mg/L, 58% on week 6 (Figure 3A, B, C).

The TEG appeared to be most toxic in microcosms containing clay (mean percent deviation = 37%) while the toxicity was less in the microcosms containing sand only (33%) and with both clay and sand (25%).

The lowest dose of TEG did not always produce the lowest percent deviation from controls. The dose-response of TEG dose and production was not linear, and will be discussed in a later section.

Quinoline toxicity. In microcosms containing the three sediments the net production began recovery to control levels except in the highest quinoline dose (206

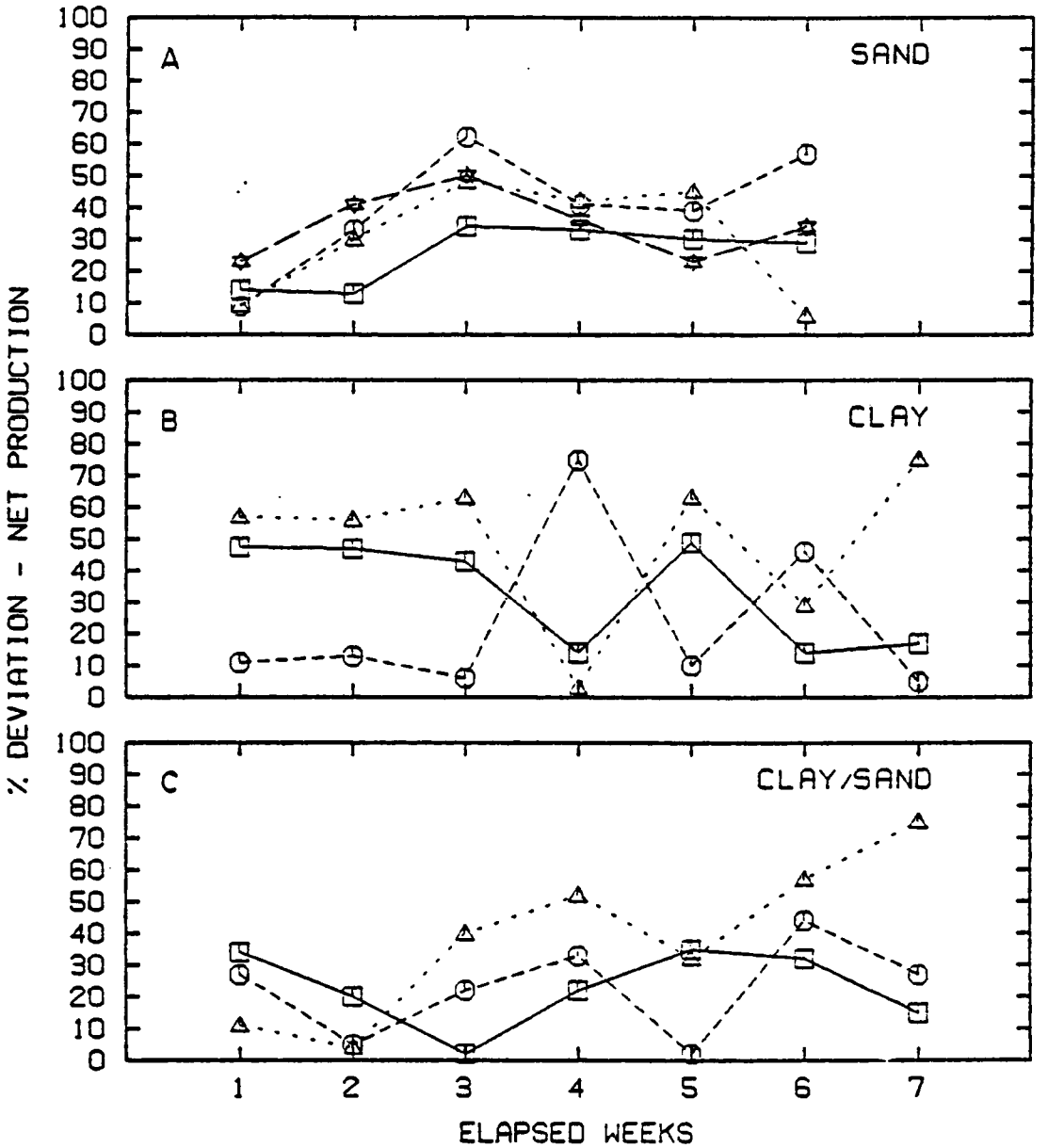

Figure 3. Mean percent deviation from the controls of net production (mg/L-O) in microcosms with TEG added for each sediment type and week after addition. Level 1 (10 mg/L) =□—□, level 2 (14-20 mg/L) =⊖--⊖, level 3 (17-42 mg/L) =△--△, and level 4 (250 mg/L) =✡--✡, (n = 3-4).

mg/L) in the microcosms containing sand (Figure 4A, B & C). Those containing sand and both sand and clay began recovering (exception noted) three weeks after dosing (Figure 4A & C) and those containing clay only began recovering one week later. However, the quinoline concentration did not decrease (refer to later section - Toxicant Effects on Production - Quinoline).

The highest deviations in production within the various microcosms were as follows: sand-sediment--quinoline dose, 206 mg/L: 101% on week 3; clay sediment--quinoline dose, 39 mg/L: 93% on week 1; clay/sand-sediment--quinoline dose, 20 mg/L: 189% on week 2 (Figure 4 A, B, & C).

Toxicity in microcosms containing sand only was greater (mean percent deviation = 46%) than those with clay only (mean percent deviation = 34%). Toxicity in microcosms containing both clay and sand was less affected by quinoline (mean percent deviation = 24%). The quinoline dose of 206 mg/L was omitted from the calculations because the microcosms with clay only and both clay and sand did not receive this high a dose.

Naphthoquinone toxicity. Naphthoquinone toxicity was more evident in microcosms containing clay only (mean percent deviation = 35%) and those containing both clay and sand (mean percent deviation = 29%) than in those containing sand alone (mean percent deviation = 15%)

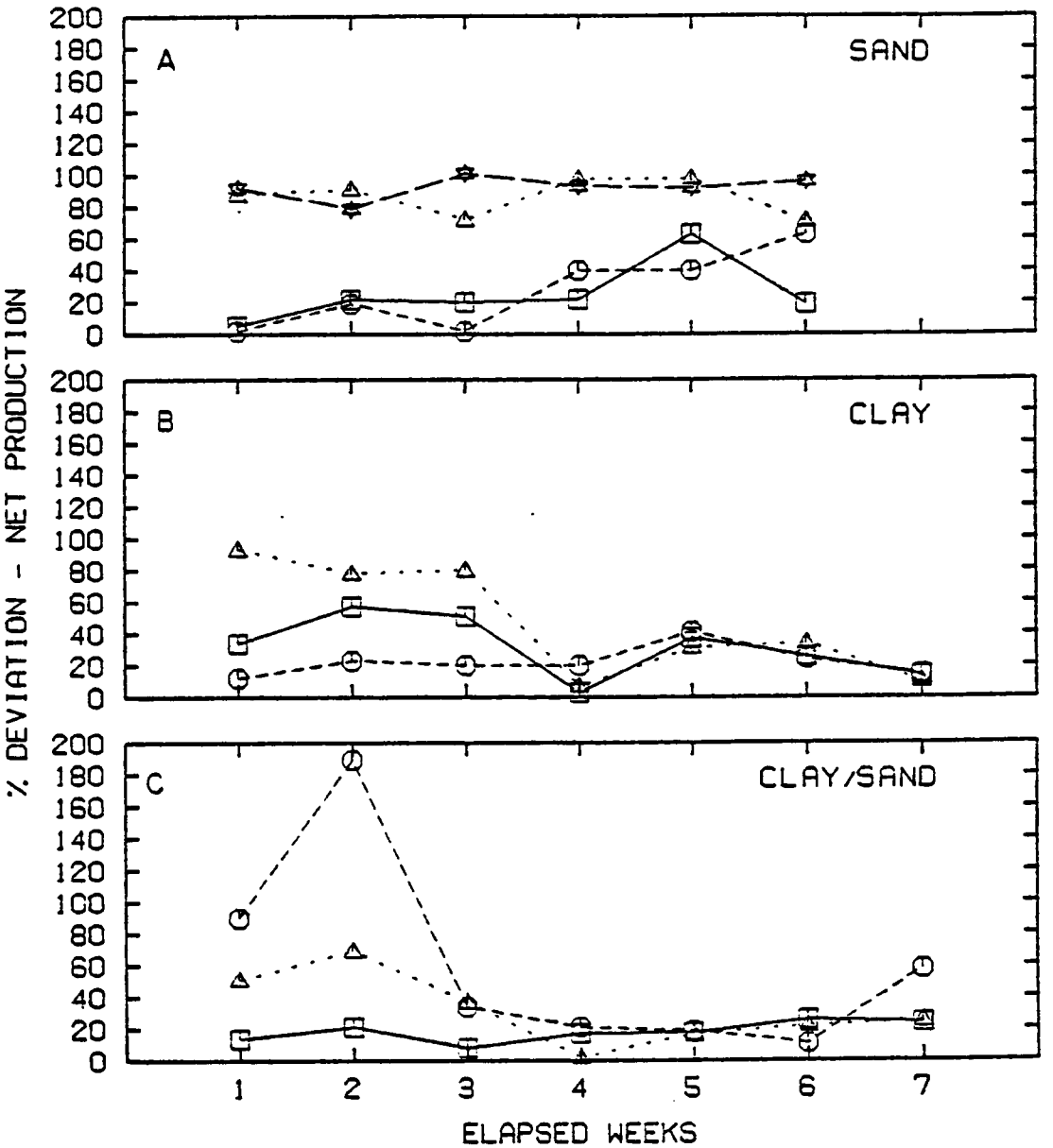

Figure 4. Mean percent deviation from the controls of net production (mg/L-O) in microcosms with quinoline added for each sediment type and week after addition. Level 1 (10 mg/L) =□—□, level 2 (14-25 mg/L) =○-○, level 3 (33-88 mg/L) =△--△, and level 4 (206 mg/L) =✡--✡(n = 3-4).

(Figure 5A, B & C).

The highest deviations in production within the various microcosms were as follows: sand-sediment--naphthoquinone dose, Ø.Ø4 mg/L: 58% on week 1; clay-sediment--naphthoquinone dose, Ø.Ø1 mg/L: 14Ø% on week 4; clay/sand-sediment--naphthoquinone dose, Ø.Ø2 mg/L: 89% on week 7 (Figure 5A, B & C).

The dose-response of naphthoquinone and production was not linear, and the lowest percent deviation was not always found in the lowest dose. This relationship for naphthoquinone and response variables will be discussed in a later section.

Summary. Sediment type did significantly influence net primary production in both the control and treatment microcosms. Higher production was observed in control microcosms containing sand only. TEG and naphthoquinone exhibited the greatest toxicity to net production in the microcosms containing clay only. Quinoline exhibited the greatest toxicity in microcosms containing sand only, while all three chemicals exhibited the lowest toxicity in the microcosms containing both clay and sand.

Effects on Heterotrophic Activity

Controls. Heterotrophic activity in the controls with sand only (mean heterotrophic activity = 18,891

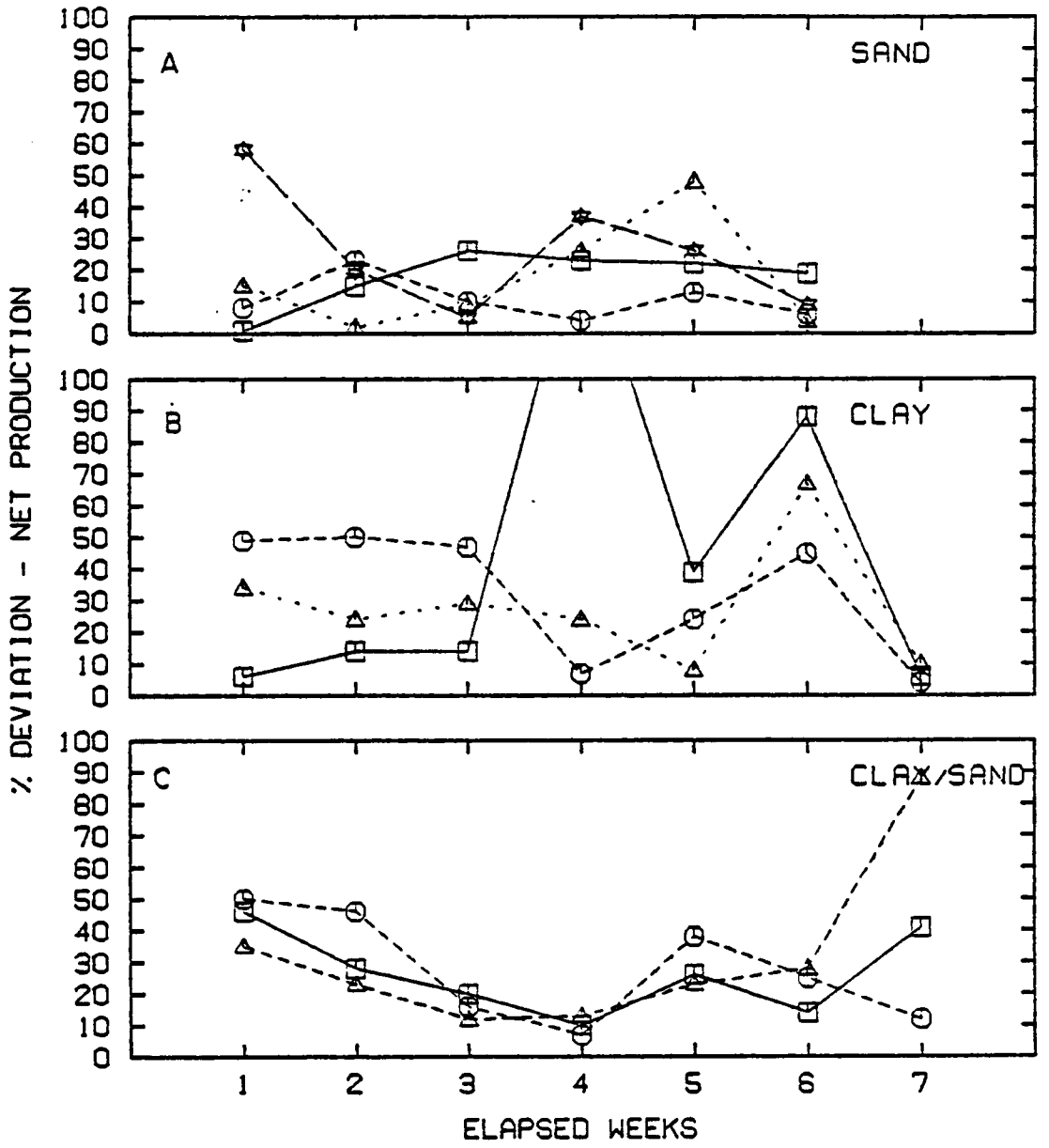

Figure 5. Mean percent deviation from the controls of net production (mg/L-O) in microcosms with naphthoquinone added for each sediment type and week after addition. Level 1 (0.01 mg/L) =□—□, level 2 (0.015-0.02 mg/L) =○—○, level 3 (0.02-0.03 mg/L) =△--△, and level 4 (0.04 mg/L) =✡--✡(n = 3-4).

cpm), and with both clay and sand (mean heterotrophic activity = 17,226 cpm) were similar though some differences were noted (Figure 6A, B & C). For example, heterotrophic activity in the microcosms with sand only was high on week 5 (mean heterotrophic activity = 37,268 cpm)(Figure 5). Heterotrophic activity was significantly lower in the microcosms with clay only (mean heterotrophic activity = 11,367 cpm) than the microcosms containing the other two sediment types.

TEG toxicity. There appeared to be no significant difference in TEG toxicity to heterotrophic activity in microcosms with sand only (mean percent deviation = 27.9%) or clay only (mean percent deviation = 29.0%) (Figure 7A & B). Toxicity was lower in the microcosms with both clay and sand (mean percent deviation = 16.0%) (Figure 7C). The highest heterotrophic activity was in the microcosms with sand only (mean heterotrophic activity = 14,920 cpm), and the microcosms with both clay and sand had similar heterotrophic activity (mean = 14,805 cpm) (Figure 8A & C). The lowest heterotrophic activity was in the microcosms with clay only (mean = 10,405 cpm) (Figure 8B).

Quinoline toxicity. Sediment type appeared to have an effect on toxicity of quinoline to heterotrophic activity. The highest toxicity was in microcosms with clay only (mean percent deviation = 55.3%) and the next

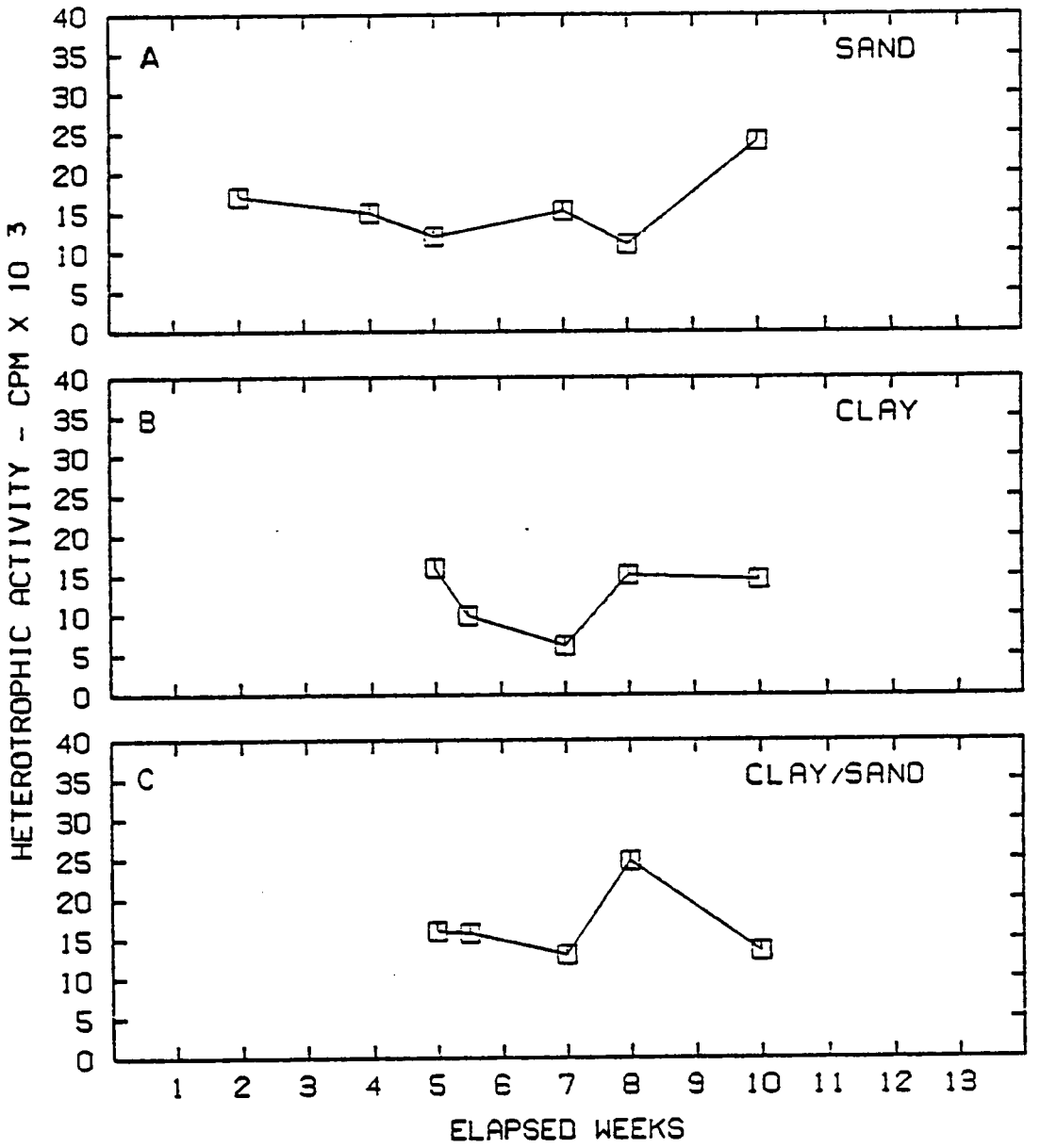

Figure 6. Mean heterotrophic activity in control microcosms for each week sampled during experimental period and each sediment type. Weeks 1-7 are pretreatment weeks (n = 144) and weeks 8 - 13 are posttreatment weeks (n = 4).

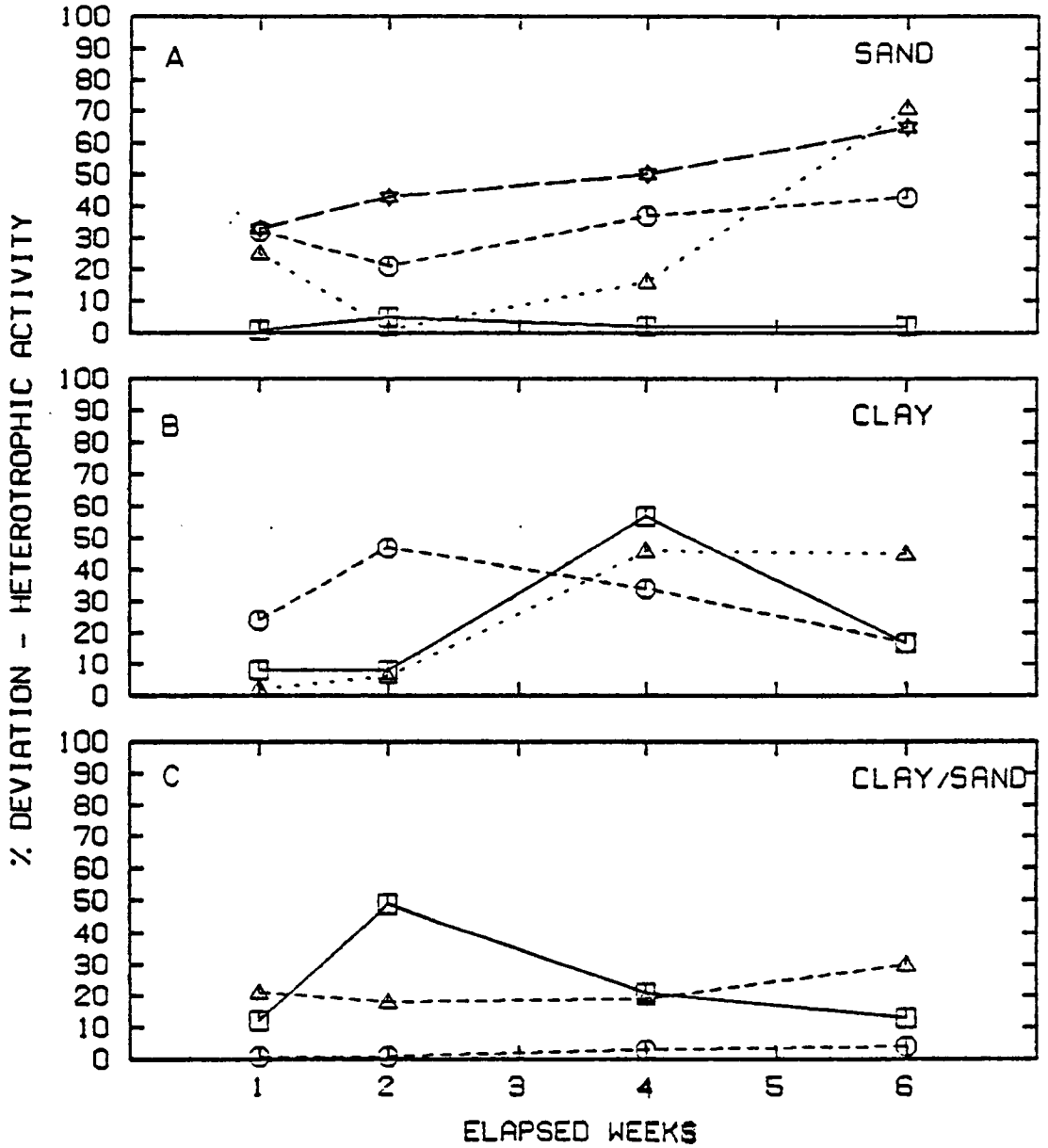

Figure 7. Mean percent deviation from the controls of heterotrophic activity (cpm) in microcosms with TEG added for each sediment type and week after addition. Level 1 (10 mg/L) =□——□, level 2 (14-20 mg/L) =○- -○, level 3 (17-42 mg/L) =△- -△, and level 4 (250 mg/L) =☼— —☼(n = 3-4).

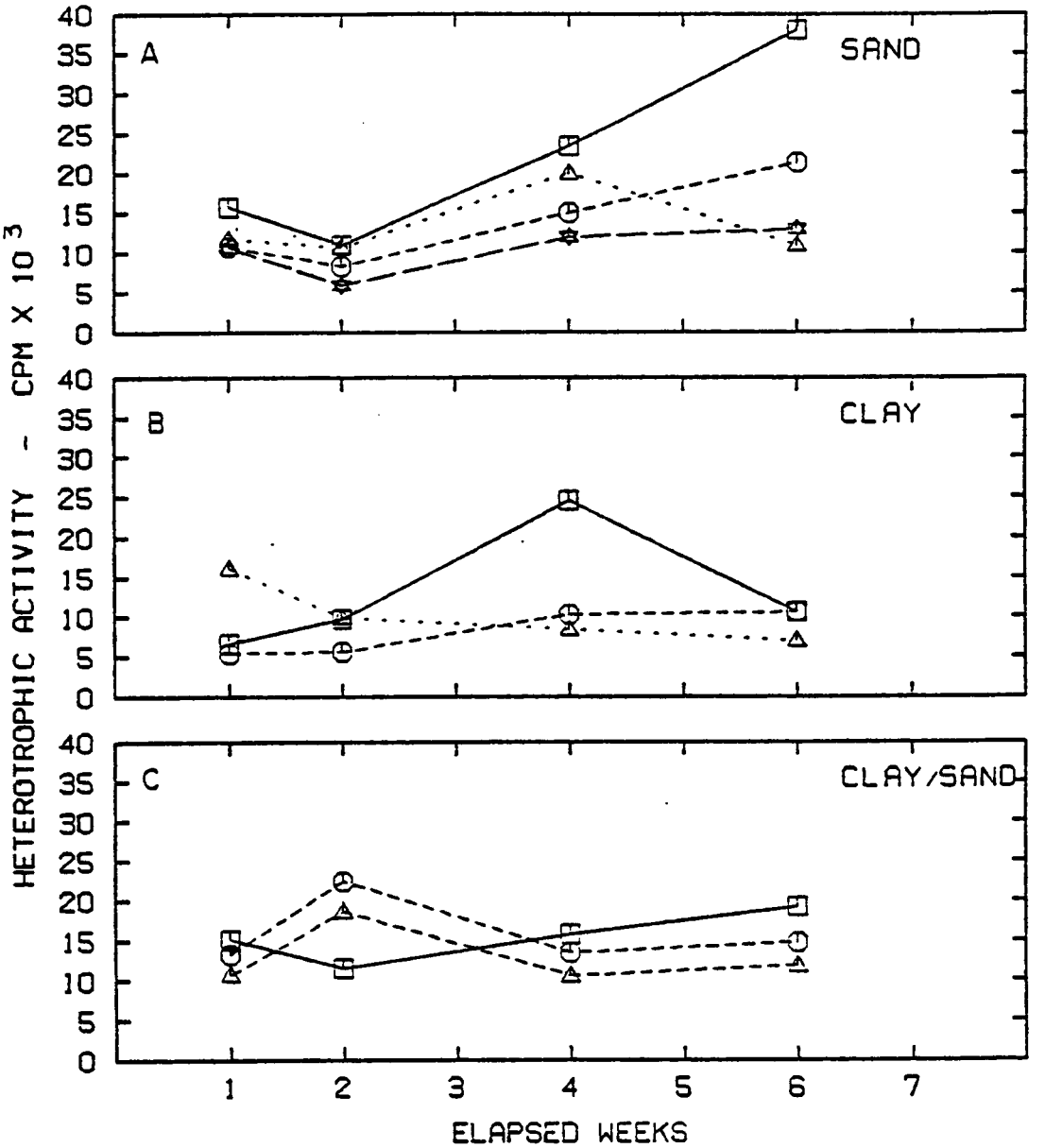

Figure 8. Mean heterotrophic activity of microcosms with TEG added for each sediment type and week after addition. Level 1 (10 mg/L) = □——□, level 2 (14-20 mg/L) = ⊖ - - ⊖, level 3 (17-42 mg/L) = △- - △, and level 4 (250 mg/l) = ✡— —✡ (n = 3-4).

highest toxicity was in the microcosms with both clay and sand (mean percent deviation = 47.5%). The lowest toxicity was in the microcosms with sand only (mean percent deviation = 40.1)(Figure 9A, B & C). The percent deviation of the heterotrophic activity from the controls' was calculated omitting the highest level (200 mg/L) since this high a concentration was not found in the microcosms with the other sediments. In all microcosms regardless of sediment type, as the quinoline concentration increased, the heterotrophic activity decreased for two weeks after the toxicant was added, but by the fourth week recovery had begun in all the microcosms irrespective of the initial dose of quinoline (Figure 10A, B & C). In fact, stimulation of heterotrophic activity to levels greater than the controls occurred in the highest quinoline dosed microcosms with clay only and with both clay and sand (39,433 cpm and 38,451 cpm) (Figure 10B & C). The increase in heterotrophic activity occurred in posttreatment week six.

Naphthoquinone toxicity. Naphthoquinone toxicity was highest in microcosms with clay only (mean percent deviation = 36%) (Figure 11B). The toxicity of naphthoquinone was similar in the microcosms with sand only (percent deviation = 28.5%) and with both clay and sand (percent deviation = 29.3%) (Figure 11A & C). After

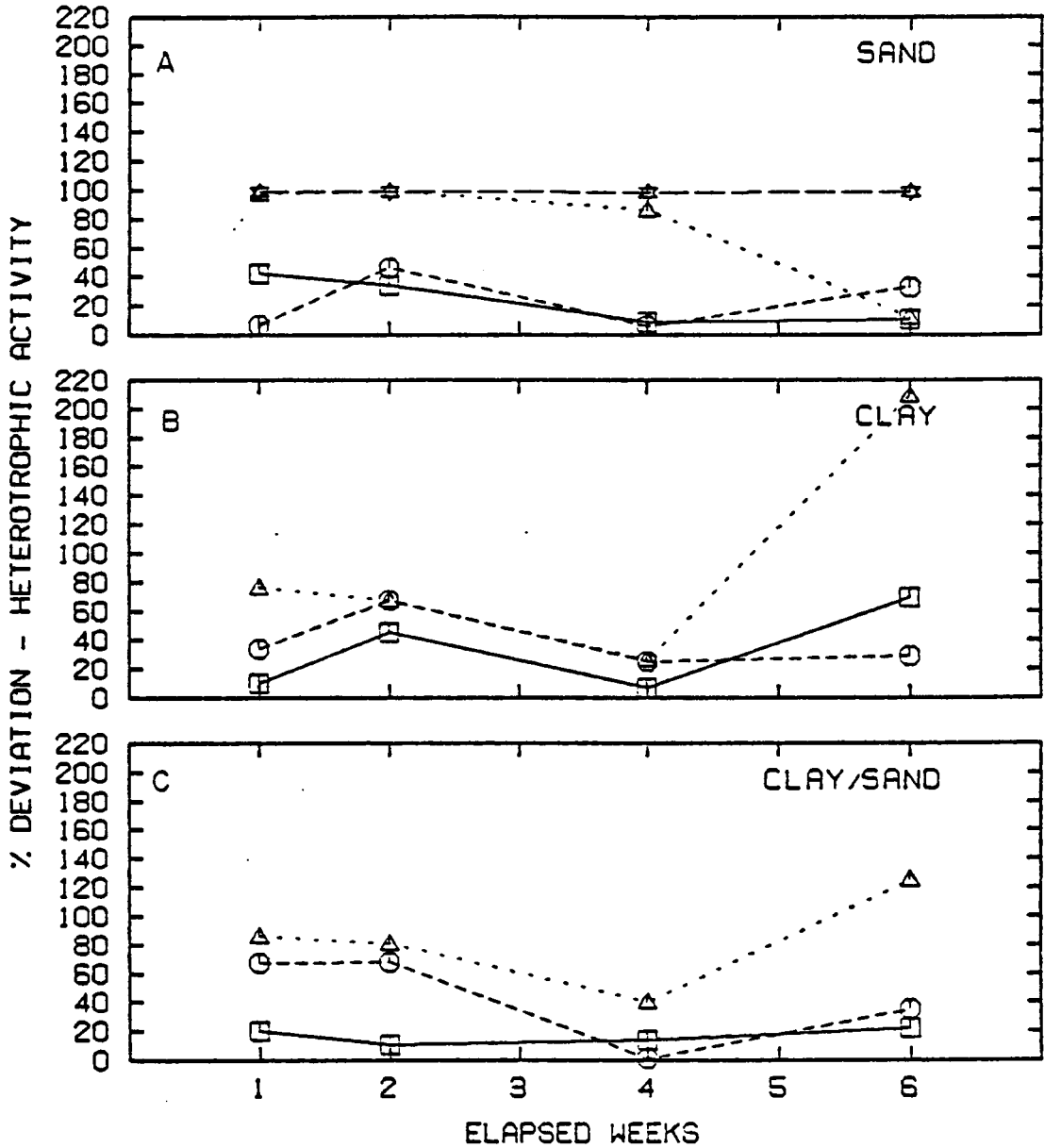

Figure 9. Mean percent deviation from the controls of heterotrophic activity (cpm) in microcosms with quinoline added for each sediment type and after addition. Level 1 (10 mg/L) =□—□, level 2 (14-20 mg/L) =○- - -○, level 3 (33-88 mg/L) =△- - -△, and level 4 (206 mg/L) =✡— —✡(n = 3-4).

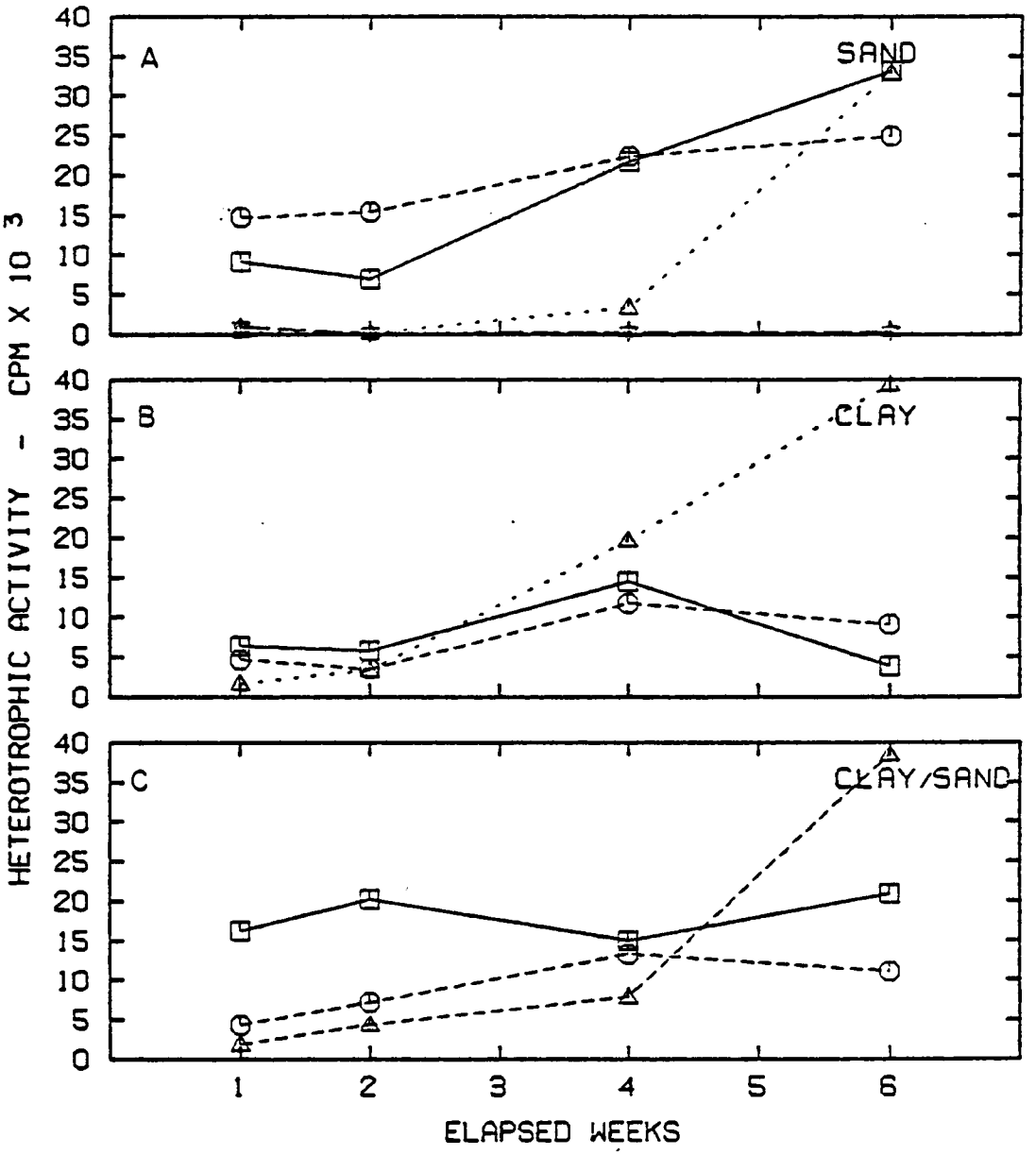

Figure 10. Mean heterotrophic activity of microcosms with quinoline added for each sediment type and week after addition. Level 1 (10 mg/L) =□—□, level 2 (14-20 mg/L) =○- -○, level 3 (33-88 mg/L) =△--△, and level 4 (206 mg/L) =✡—✡(n = 3-4).

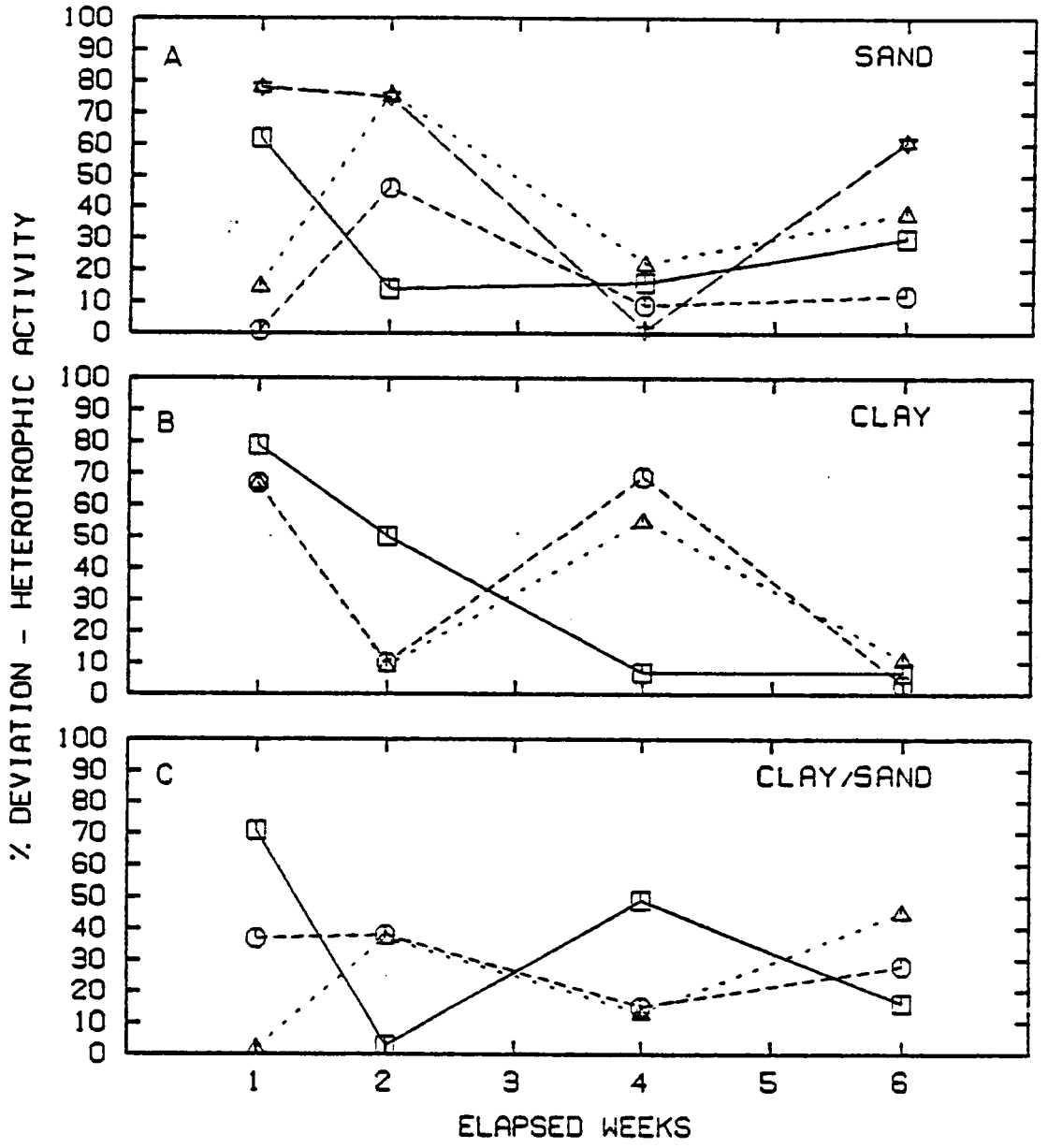

Figure 11. Mean percent deviation from the controls of heterotrophic activity (cpm) in microcosms with naphthoquinone added for each sediment type and week after addition. Level 1 (0.01 mg/L) = □——□, level 2 (0.015-0.02 mg/L) =○---○, level 3 (0.02-0.03 mg/L) =△---△, and level 4 (0.04 mg/L) =☆---☆(n = 3-4).

the first week heterotrophic activity in microcosms, irregardless of sediment type, exhibited no correlation with naphthoquinone concentration. By the fourth week, heterotrophic activity was stimulated in the microcosms containing sand only (Figure 12A, B & C).

Summary. Sediment type appeared to have had an effect on the heterotrophic activity in the control microcosms and affected the toxicity of the three toxicants. Heterotrophic activity was lower in those microcosms with clay only, and was not different from each other in the other two sediment types.

Effects on Alkalinity, pH and Carbon Limitation

The pH began to decrease in all three sediments three to four weeks after TEG was added (Figure 13A, B & C). The largest decline (3.2 pH units) in the pH was from 7.5 to 4.3 in the microcosm containing 17 mg/L TEG and both clay and sand (Figure 13C). The smallest pH decline was from 7.Ø to 5.5 in the microcosms containing 2Ø mg/L and sand only (Figure 13A). The pH declined from 7.4 to 4.5 in the microcosms with 17 mg/L TEG and clay sediment only (Figure 13B). The pH fluctuated at a lower level in the controls during the posttreatment weeks than during the pretreatment weeks, however not at the low pH found in the microcosms with TEG (Figure 14A & B). No

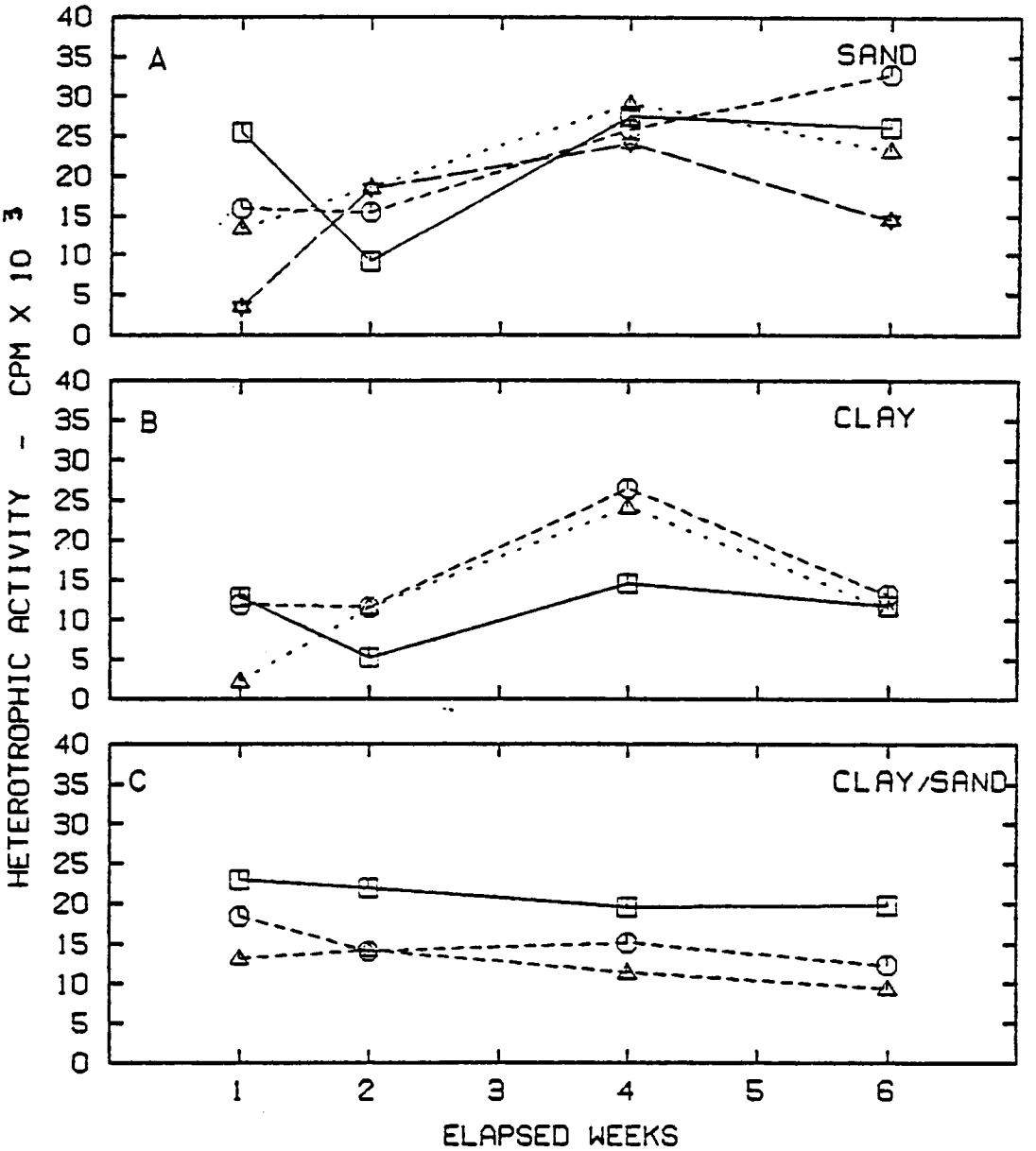

Figure 12. Mean heterotrophic activity of microcosms with naphthoquinone added for each sediment type and week after addition. Level 1 (10 mg/L) =□——□, level 2 (0.015-0.02 mg/L) =○- - -○, level 3 (0.02-0.03 mg/L) =△- - -△, and level 4 (0.04 mg/L) = ✡— —✡(n = 3-4).

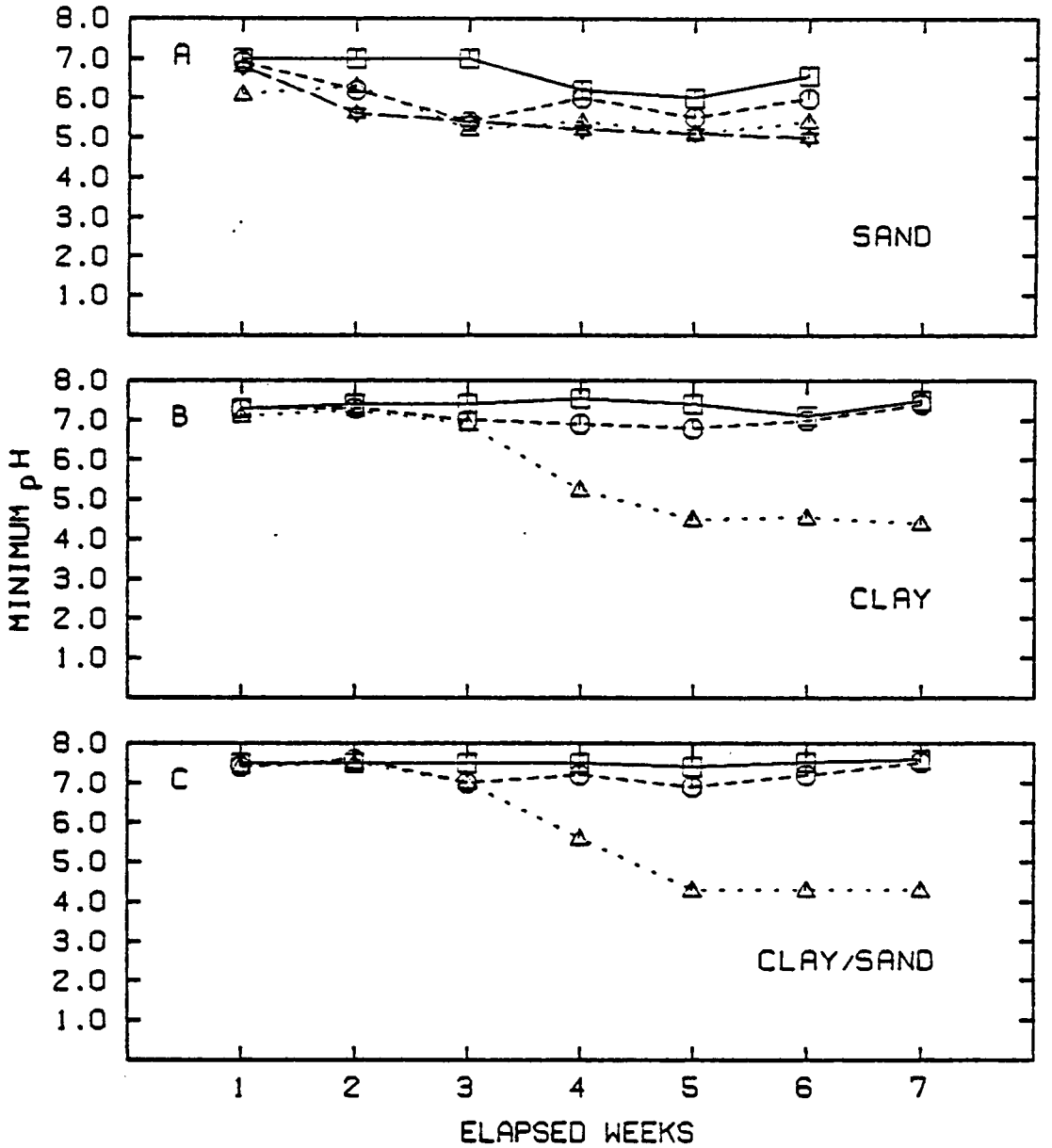

Figure 13. Mean minimum pH of microcosms with TEG added for each sediment and week after addition. Level 1 (10 mg/L) = □——□, level 2 (14-20 mg/L) = ○- - -○, level 3 (17-42 mg/l) = △- - △, and level 4 (250 mg/L) = ✡— —✡ (n = 3-4).

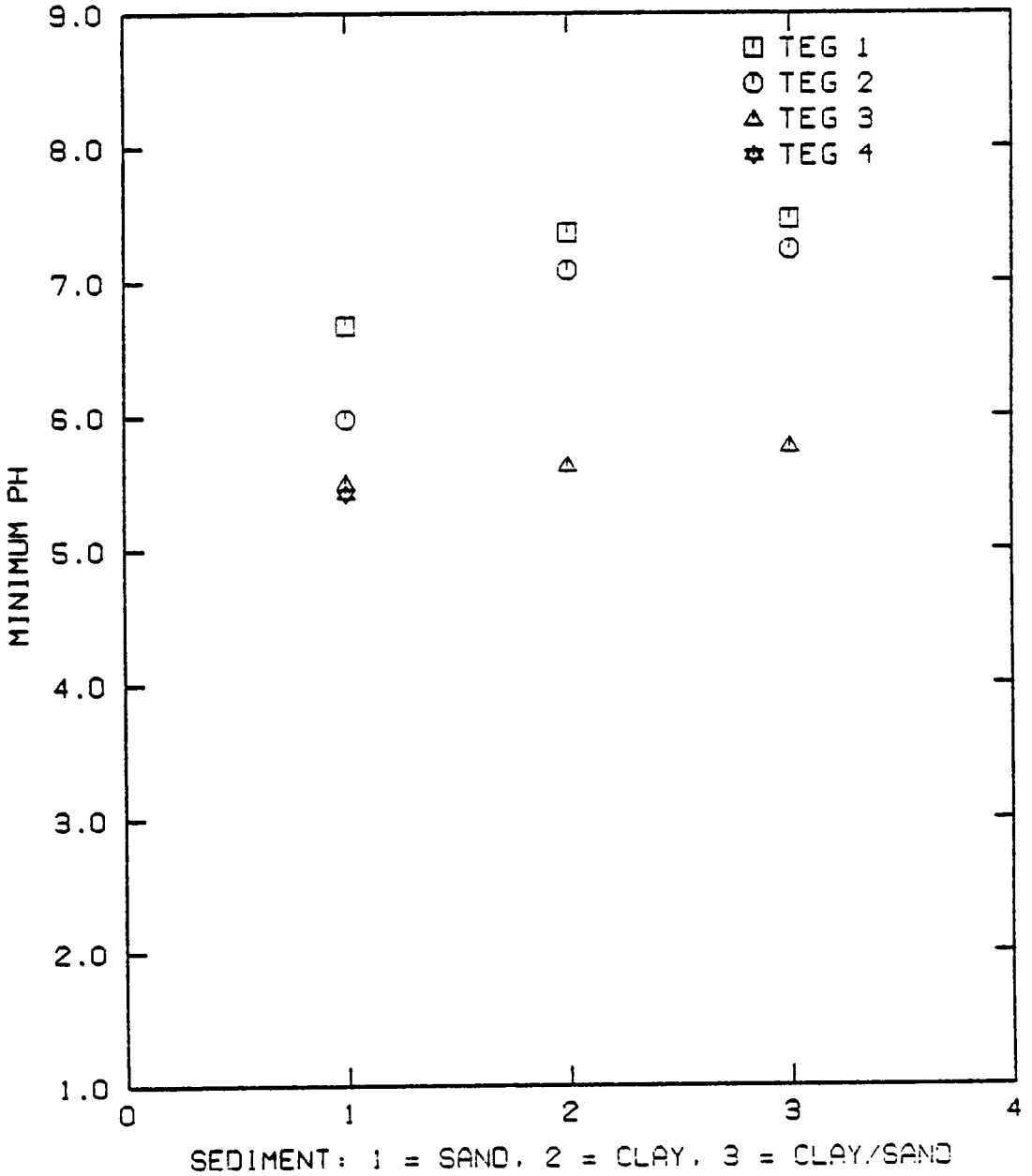

Figure 14. Mean minimum pH of microcosms with TEG added for each sediment type pooled for all weeks (n = 52 for sand and n = 39 for clay and clay/sand). Level 1 was 10 mg/L, level 2 was 14-20 mg/L, level 3 was 17-42 mg/L and level 4 was 250 mg/L.

significant changes in average daily minimum pH with time were observed in the microcosms dosed with quinoline or naphthoquinone. The pH was approximately the same in all microcosms immediately after the toxicant was added (pH 7.5) and it fluctuated daily between a pH of 6.Ø to 8.Ø because of primary production.

Alkalinity four weeks after toxicant addition was higher both in the control microcosms and treatment microcosms containing both clay and sand than those with clay only (regardless of toxicant concentrations) (Figure 15B & C). The sand-containing microcosms (phase 1) contained less alkalinity than those containing the other sediments (phase 2) because less was added to begin with (Ø.6 mequiv/L during phase 1 and 1.3 mequiv/L during phase 2) (Figure 15A, B & C). In all microcosms irrespective of the type of sediment they contained alkalinity at six weeks after TEG addition decreased significantly as the TEG concentration was increased (Figure 16). The reduction amounted to as much as one-third of the original alkalinity (Figure 16A, B & C).

Alkalinity was higher four weeks after the toxicant addition in microcosms with the greatest concentration quinoline regardless of sediment type (Figure 17A, B & C). The alkalinity in these microcosms was from two to ten times higher than the beginning alkalinity (highest mean alkalinity in sand microcosms = 6.3 mequiv/L;

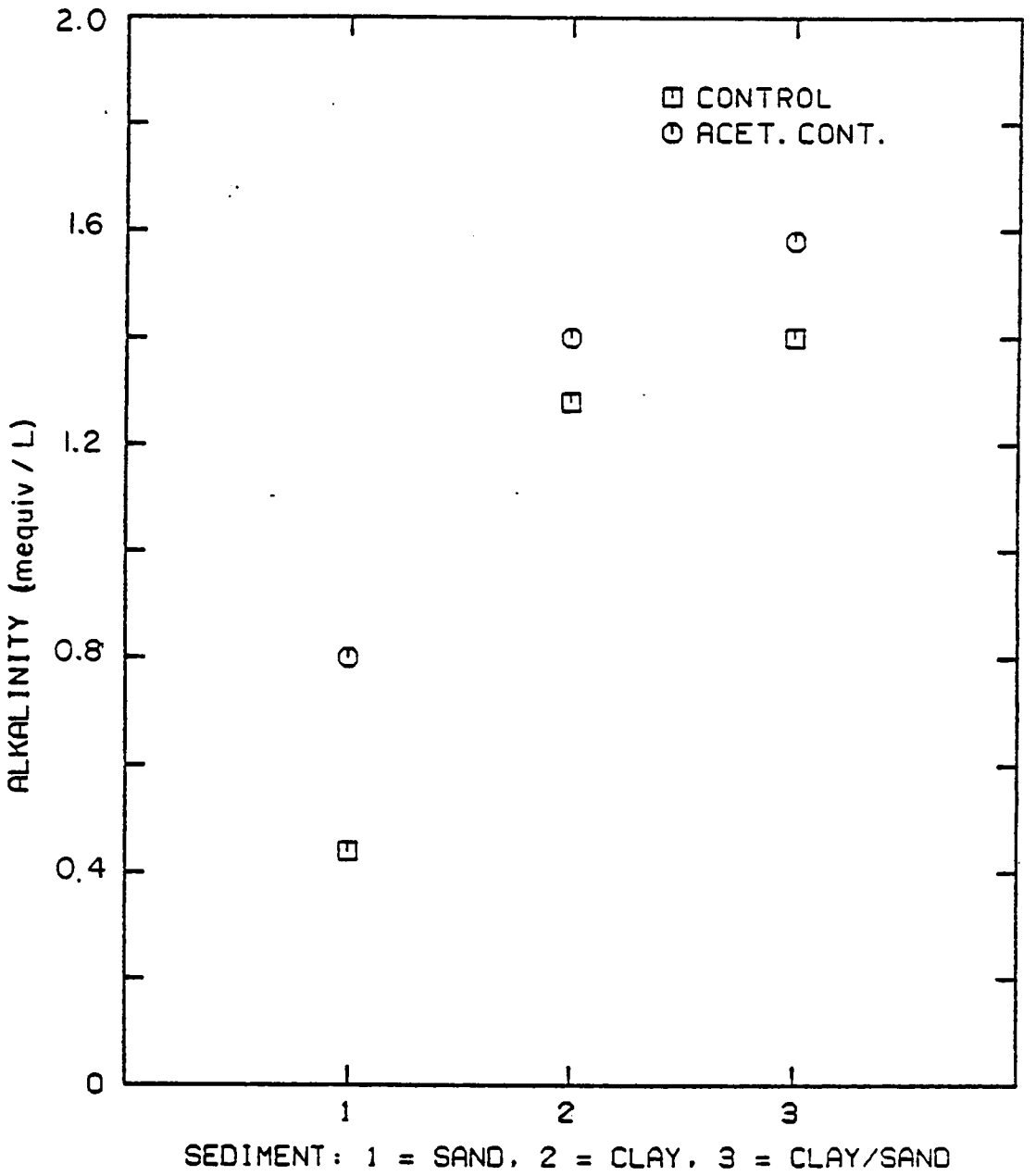

Figure 15. Mean alkalinity of control microcosms and acetone control (carrier) microcosms for each sediment type on the sixth post-treatment week (n = 52 for sand and n = 39 for clay and clay/sand).

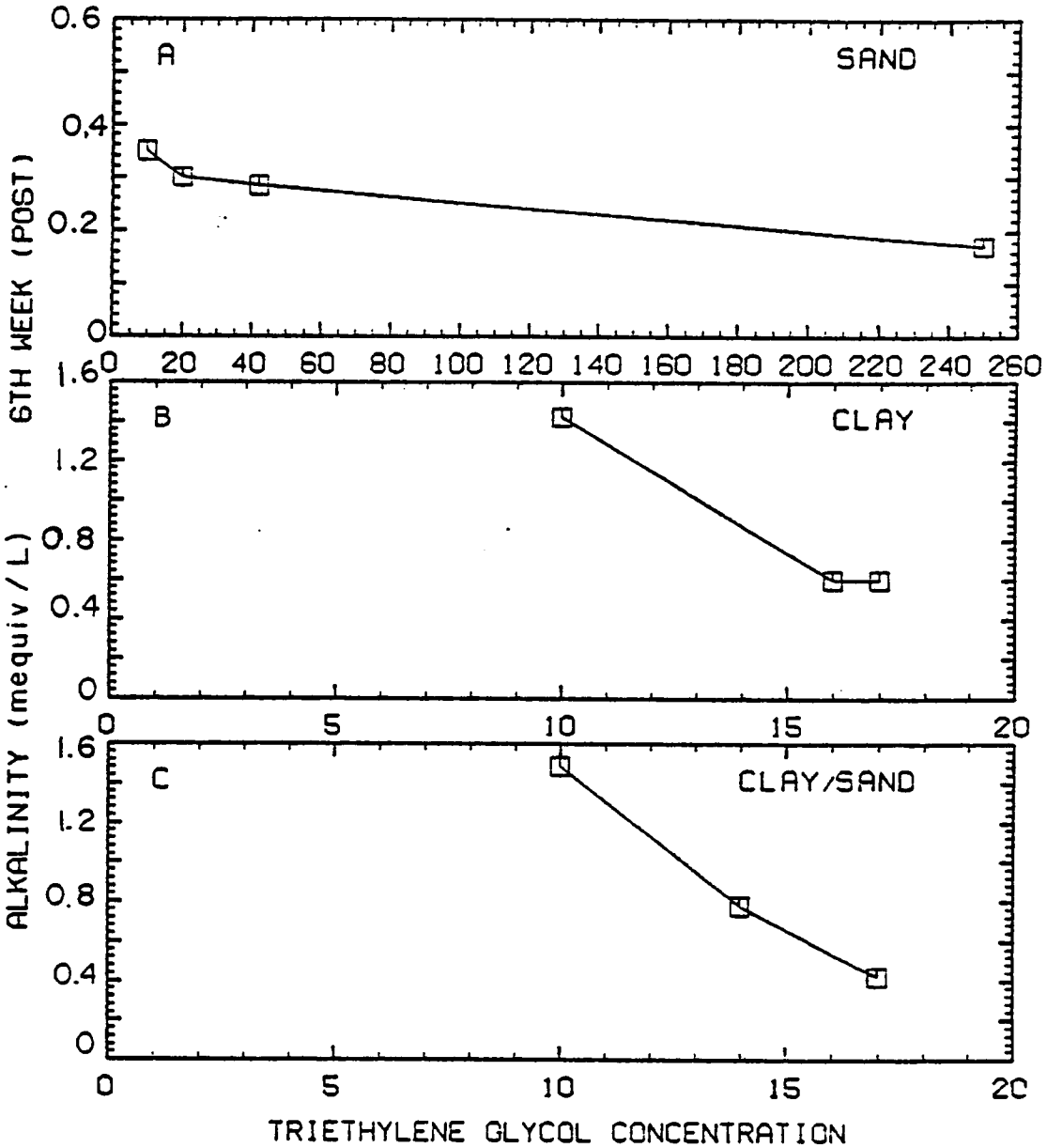

Figure 16. Mean alkalinity of microcosms with TEG added for each sediment type and each concentration of TEG, six weeks after TEG addition (n = 3-4).

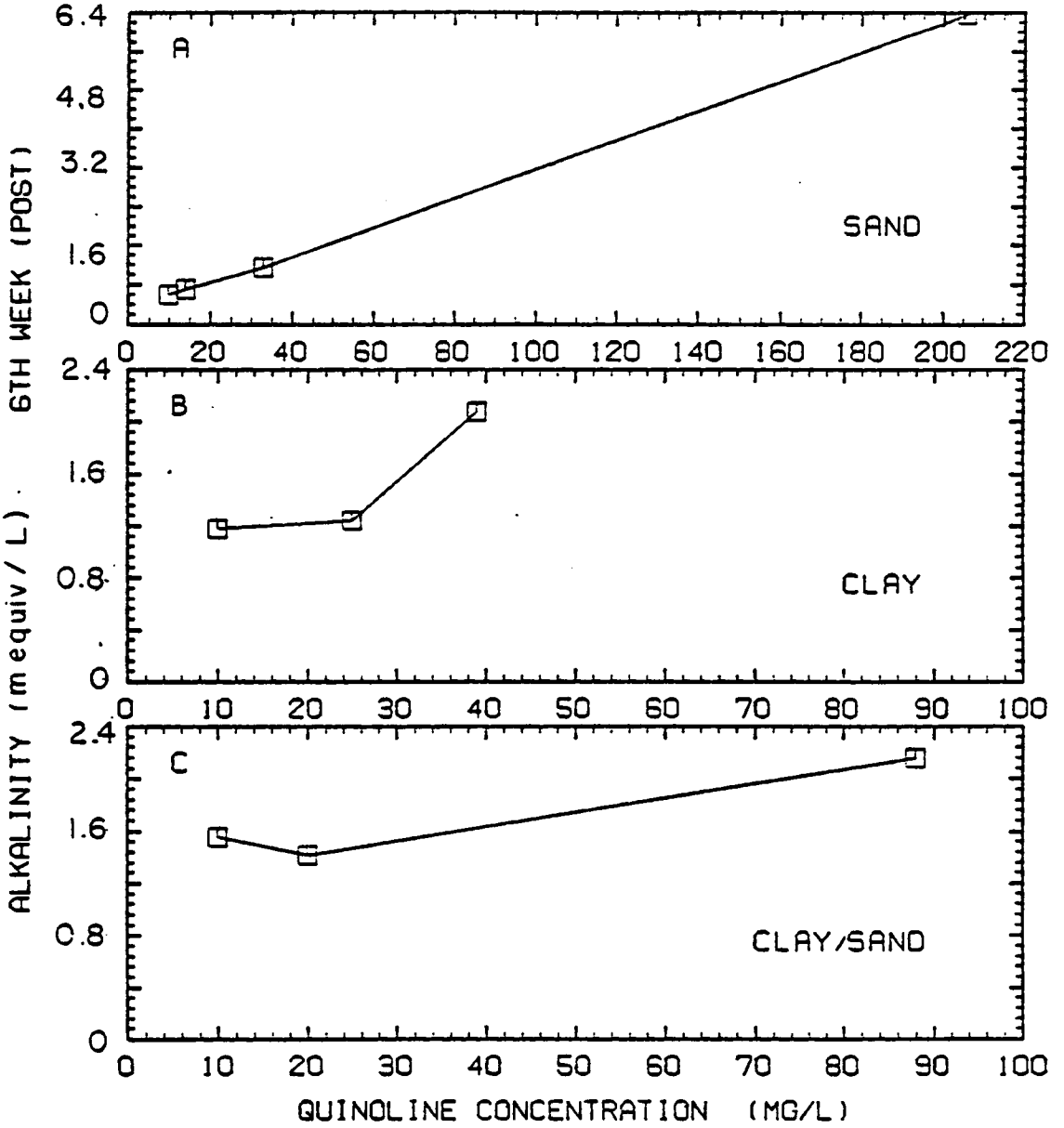

Figure 17. Mean alkalinity of microcosms with quinoline added for each sediment type and each concentration of quinoline, six weeks after quinoline addition (n = 3-4).

highest mean alkalinity in clay only and both clay and sand microcosms = 2.2 mequiv/L) although the mean pH of the microcosms did not increase. The initial mean alkalinity of all microcosms with sand only was Ø.6 mequiv/L and 1.3 mequiv/L in the clay only and both the clay and sand sediments.

Alkalinity in microcosms with naphthoquinone added was not significantly different six weeks after the addition than the initial alkalinity of the microcosms; this was true for all three sediment types (Figure 18A, B & C).

The microcosms with the higher dosages of TEG and in which the pH and alkalinity were lower contained the only blue green algae observed (an indicator of carbon limitation; Moss, 1982 & 1983, King and Novak, 1974).

In summary the pH decreased the most in microcosms with TEG with both clay and sand and the alkalinity decreased the most in microcosms containing clay only.

Effects on Community Composition

Sediment type did appear to have an effect on community composition in the control microcosms. Attached algae were found in all control microcosms with sand only. The crustacean (grazer) levels were low in these microcosms, a fact that could account for the high

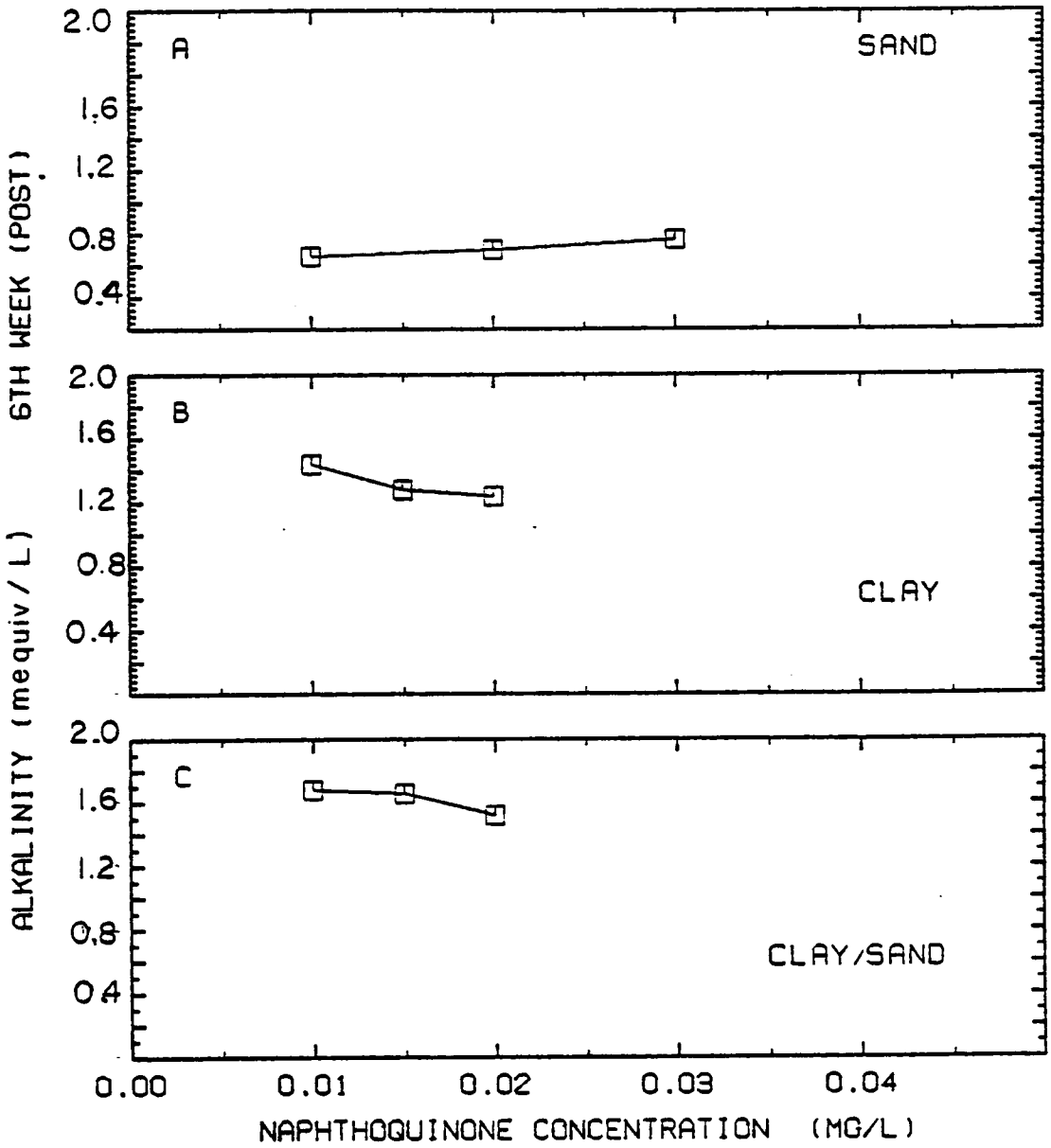

Figure 18. Mean alkalinity of microcosms with naphthoquinone added for each sediment type and each concentration of naphthoquinone, six weeks after naphthoquinone addition (n = 3-4).

levels of attached algae. Microcosms with clay only or both the clay and sand contained no attached algae, but they did contain large populations of cladocerans and ostracods. There were no obvious differences in populations in the microcosms containing clay only or the clay and sand mixture (see Appendix 3).

Summary of Sediment Effects on Microcosm Attributes

Sediment type significantly affected microcosm attributes such as net production, heterotrophic activity, pH and alkalinity, and affected the toxicity of the three toxicants with respect to these attributes. Lower net production and lower heterotrophic activity was found in control microcosms containing clay only. Toxicity to net production was greater in microcosms with clay only and dosed with TEG or naphthoquinone, than in the other two sediment types. Toxicity to heterotrophic activity was greatest in microcosms with clay only, irregardless of the toxicant added, although the ostracod populations were not lower (detritivores).

Alkalinity, pH and community composition indicated no significant effect due to sediment type except in a few cases noted above.

Effects of Toxicants on Microcosm Attributes

Phenol Effects

Introduction. Phenol was added at four concentrations in phase one of this study (sand sediment only) but was not added to any microcosms during phase 2 (clay only and clay and sand mixture sediments) because of time and space considerations described in "Materials and Methods" section. The actual concentrations of phenol at each of the our treatment levels (as determined by gas chromatography) were: 10 mg/L, 18 mg/L, 108 mg/L, and 501 mg/L. After approximately three weeks (25 days) after addition, the phenol concentration had decreased by an average of 75%, and phenol concentration was even lower when measurements were made six weeks after addition (Figure 19).

Effects on net production. Increasing the phenol concentration significantly affected net production values. The second level (19 mg/L) appeared to affect it significantly more than the lowest level (10 mg/L) and the highest doses (108 mg/L and 501 mg/L) reduced production to levels that were significantly lower than either of the lower concentrations of phenol. In spite of the fact that 18 mg/L phenol seemed to stimulate net production (Figure 20A & B) there was a significant, inverse, overall relationship between production and

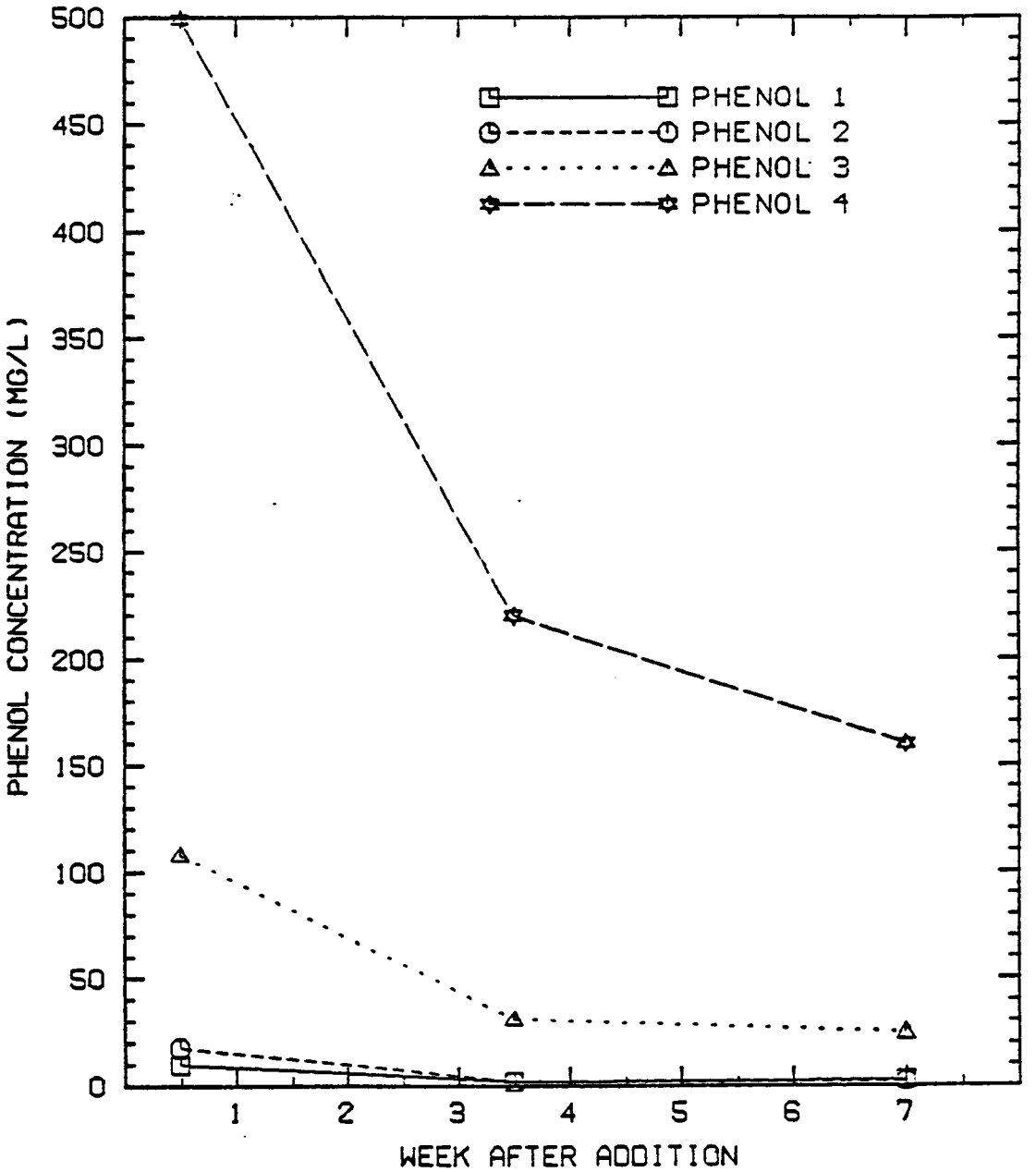

Figure 19. Mean concentration of phenol measured in microcosms for the weeks after addition of phenol (n = 4).

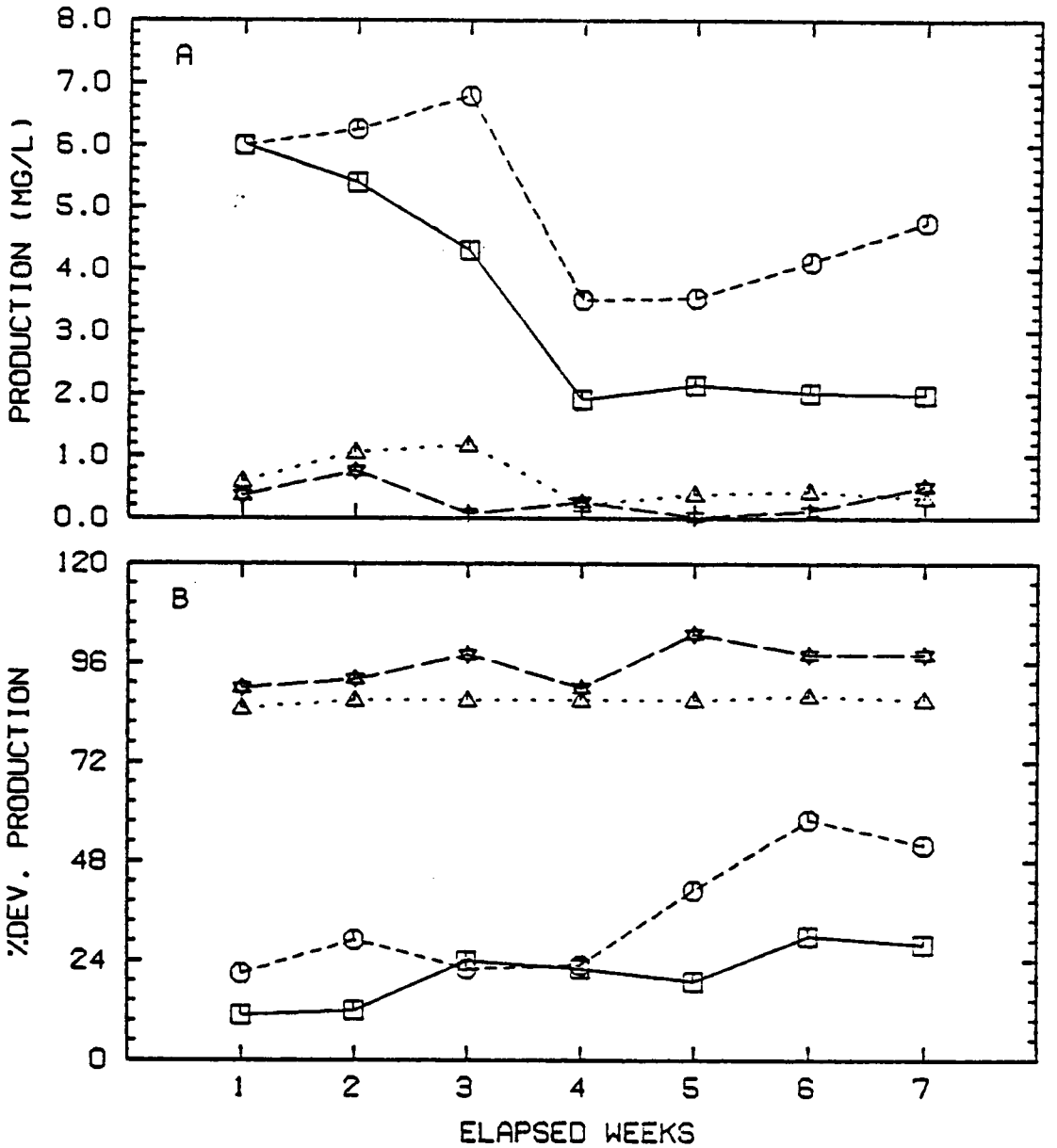

Figure 20. (A) Mean net production in microcosms with phenol added for each week and (B) mean percent deviation from controls for net production in microcosms with phenol added for each week. Level 1 (10 mg/L) = ⊡——⊡, level 2 (18 mg/L) = ⊖ - - ⊖, level 3 (108 mg/L) = △ - - - △, and level 4 (501 mg/L) = ✡ _ _ ✡ (n = 3-4).

phenol concentration (n=96, r=Ø.76) (Figure 21B). The highest concentration of phenol (5Ø1 mg/L) reduced the average production throughout the experimental period to Ø.244 mg/L of oxygen (from 6.3 mg/L in controls), and the next highest concentration (1Ø8 mg/L) reduced the average production through out the experimental period to Ø.571 mg/L of oxygen. The microcosms dosed with 18 mg/L phenol had higher average production throughout the experimental period than the microcosms with the lowest concentration of phenol (1Ø mg/L) (Figure 2ØB). All concentrations of phenol produced significantly lower production levels than was observed in the control microcosms. The average production in the control microcosms was 6.3Ø5 mg/L oxygen.

Average production in the microcosms treated with 1Ø mg/L phenol had deviated from production in the controls by 19.7% throughout the experimental period. The next level of phenol (18 mg/L) produced an average percent deviation from the controls of 31.69%, the third level (1Ø8 mg/l), 84.9%, and the highest level (5Ø1 mg/l), 95.6% (Figure 2ØB).

No recovery of primary producers was evident six weeks after the toxicant was added. There was a significant relationship between phenol concentration and the percent deviation from the controls in net production in the sixth week after the microcosms were dosed.

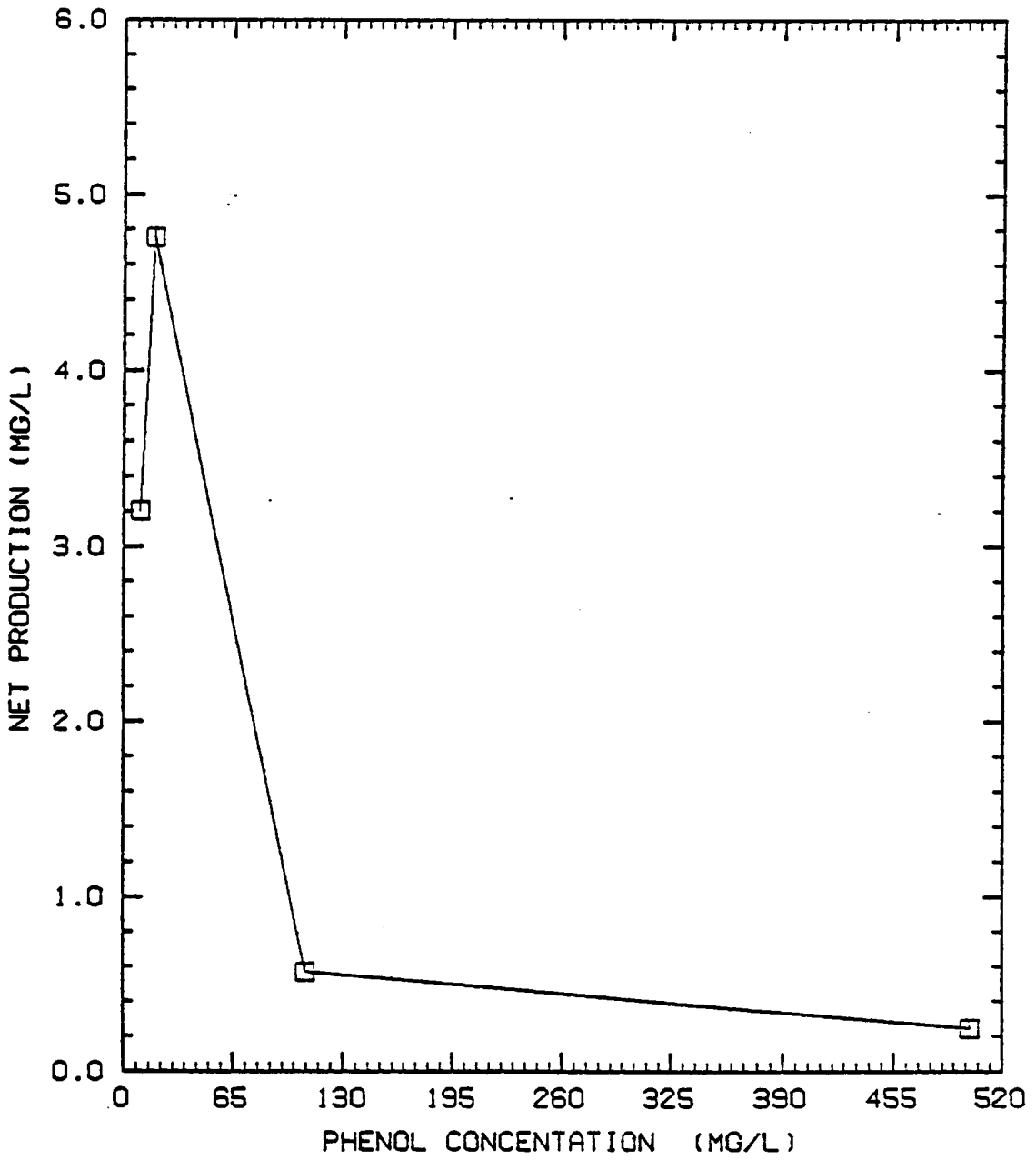

Figure 21. Mean net production in microcosms with phenol added for each concentration pooled over all weeks (n = 24).

Phenol addition significantly decreased production, and at the three higher levels (18, 108 & 501 mg/L), production values on the average, deviated more than 20% deviant from the controls. Six weeks after phenol was added the percent deviation from control was still significantly correlated with increasing phenol concentration (m=5.87, r=0.78, n=16). The EC20 for phenol, summed over all weeks (chronic) was 28 mg/L. The acute EC20 was 18 mg/L.

Effects on heterotrophic activity. Phenol had a significant effect on heterotrophic activity. As phenol concentration increased, heterotrophic activity decreased (n=16, r=-0.642), except at a phenol dose of 18 mg/L. The microcosms with 10 & 18 mg/L phenol recovered by the fourth week after the addition of the toxicant to a level significantly higher than the controls (Figure 22A & B). Microcosms with 108 mg/L phenol began to recover the fourth week but never recovered to the heterotrophic activity level of the control microcosms. The microcosms with 501 mg/L phenol added did not recover throughout the experimental period (6 weeks). Heterotrophic activity in them remained significantly lower by a wide margin than the controls. Microcosms with 501 mg/L of phenol added had a mean counts per minute (cpm) of 260, while the average cpm measured in the controls microcosms was 21,875, a hundred fold difference (Figure 22A).

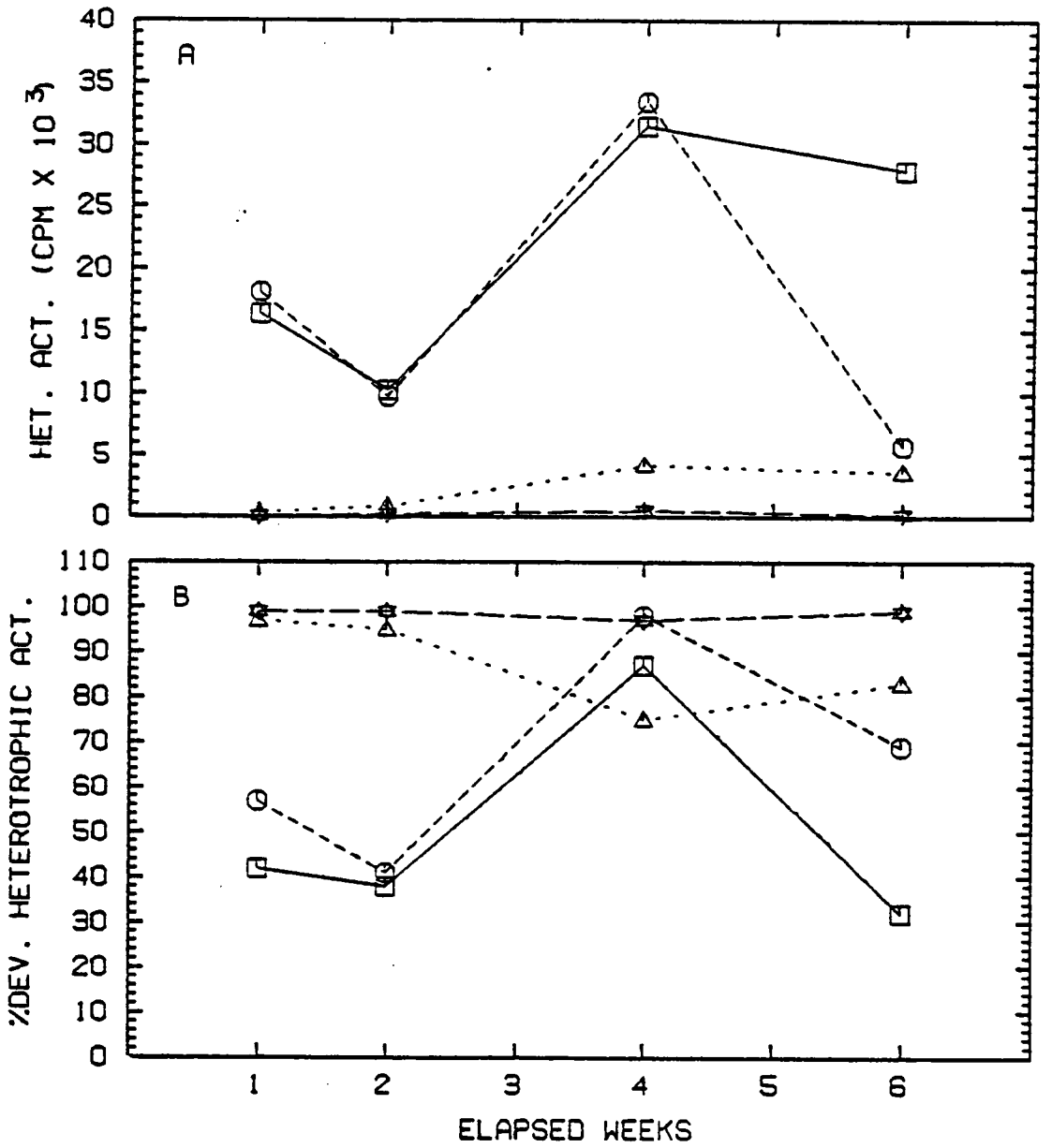

Figure 22. (A) Mean heterotrophic activity in microcosms with phenol added for each week and (B) mena percent deviation from controls for heterotrophic activity in microcosms with phenol added for each week. Level 1 (10 mg/L) =□——□, level 2 (18 mg/L) =○—○, level 3 (108 mg/L) =△ _ _△, and level 4 (501 mg/L) =✡ _ _✡ (n = 3-4).

The percent deviations from the control microcosms, recorded in the microcosms with 501 mg/L phenol were between 83% and 99%, throughout the experimental period (Figure 22B). The percent deviations recorded in the microcosms with 10 mg/L and 18 mg/L were less than 20% at all sample ties except the sample taken four weeks after phenol was added and then the heterotrophic activity was 32-40% higher than the controls. There was a significant correlation between phenol concentration and percent deviation from controls for heterotrophic activity (n=16, m=3.704, r=0.747) with a chronic EC20 value calculated of 45 mg/L phenol, and an acute EC20 of 52 mg/L.

Phenol addition significantly decreased heterotrophic activity at the two highest levels (108 mg/L and 501 mg/L)(Figure 23), both of which produced a mean percent deviation in heterotrophic activity greater than 20% from that in the control microcosms.

Effects on alkalinity, pH and carbon limitation. There was no significant change in the minimum pH in microcosms treated with phenol throughout the experimental period, and the minimum pH in treated microcosms did not vary significantly from that in the control microcosms (Figure 24A & B).

Variations in the alkalinity concentration as a function of phenol concentration showed no distinct trend (Figure 24C).

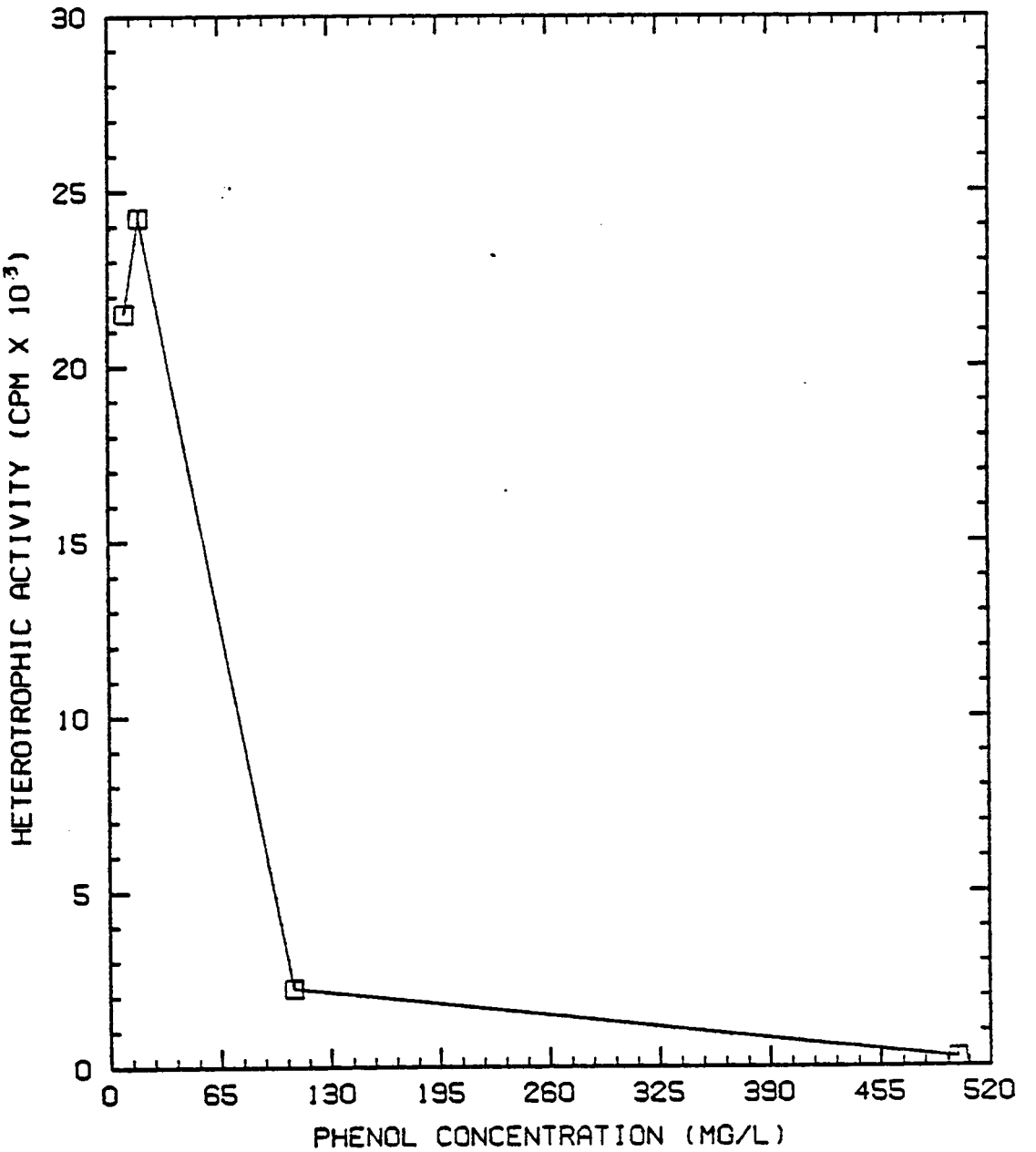

Figure 23. Mean heterotrophic activity in microcosms with phenol added for each concentration pooled over all weeks (n = 24).

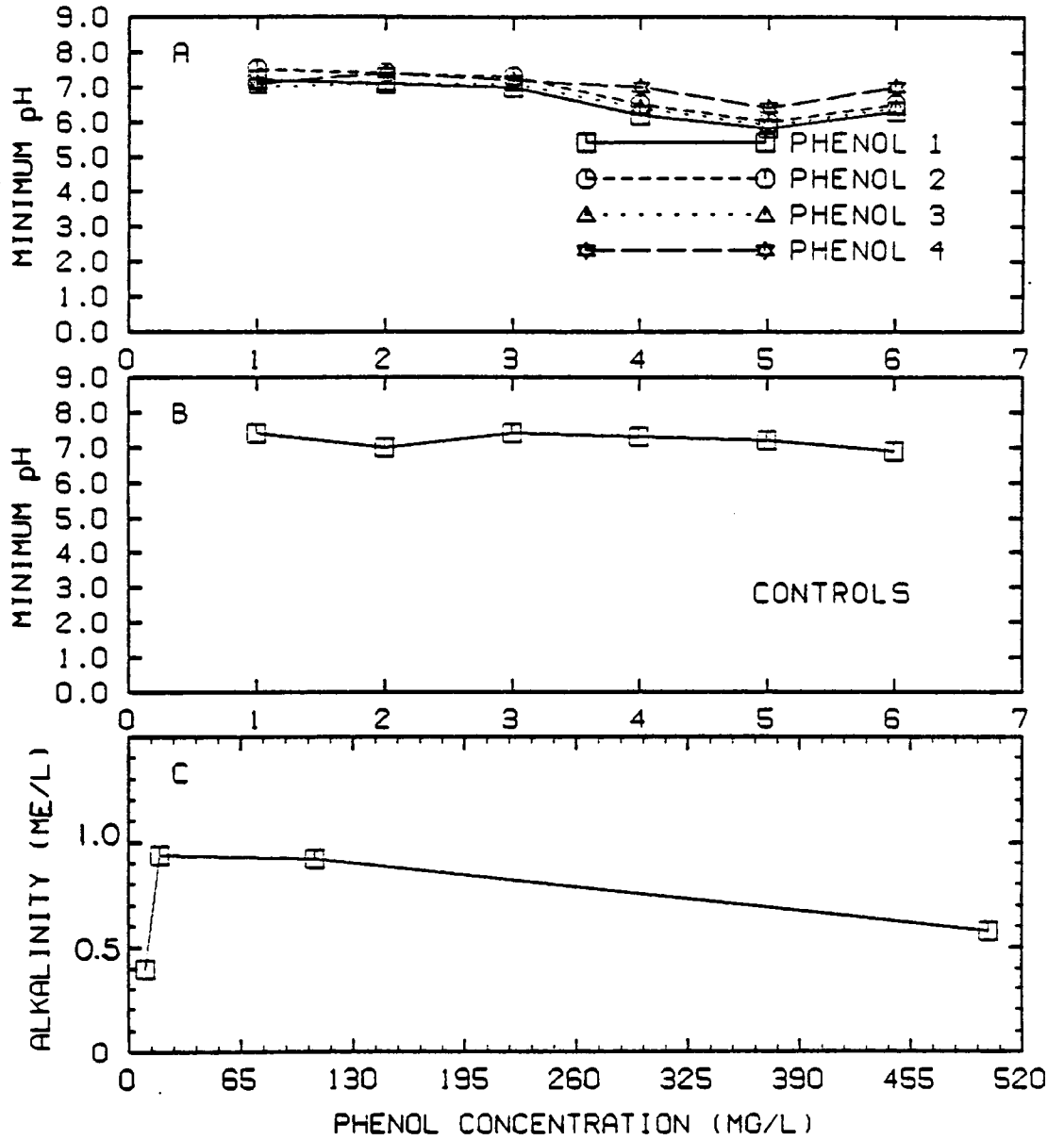

Figure 24. (A) Mean minimum pH in microcosms with phenol added for each week. Level 1 (10 mg/L) = ⊡——⊡, level 2 (18 mg/L) = ⊙ - - ⊙, level 3 (108 mg/L) = △ - - △, and level 4 (501 mg/L) = ✡ — ✡(n = 4). (B) Mean minimum pH in control microcosms for each week, (Week 1-6 = Pretreatment and 7-13 = Posttreatment). (C) Mean alkalinity in microcosms with phenol added measured in the 6 th week after treatment for each concentration (n = 4).

Because the minimum pH and the alkalinity are influential factors in predicting carbon dioxide limitations and because they were not different, carbon dioxide limitation was not an effect of phenol addition to the microcosms.

Effects on community composition. Microcosms with the two highest levels of phenol (108 mg/L and 501 mg/L) appeared to have no crustaceans (cladocera, amphipods, ostracods and copepods). Only half of the microcosms dosed with 18 mg/L phenol contained crustaceans while all those dosed at level 1 (10 mg/L) contained crustaceans.

Microcosms dosed with 108 mg/L or 501 mg/L phenol contained no algal populations, either attached or planktonic. All microcosms dosed with 10 mg/L or 18 mg/L phenol contained attached algae, whereas a few contained planktonic algae (Appendix 1). The water in microcosms dosed at the two highest levels became brown, probably because of the changes in phenol, when exposed to light, and light likely was attenuated.

In summary, phenol had a significant effect on the algal and crustacean populations. The extent of this effect was correlated with increasing phenol concentration.

Summary. The effects of phenol on primary producers and heterotrophs were similar. All levels of phenol caused a reduction in net primary production over that

observed in the controls. There was no evidence of effects on pH or alkalinity

The two highest phenol concentrations (108 and 501 mg/L) caused approximately a 100% decrease in both net production and heterotrophic activity over that observed in the control microcosms.

The EC20 value for phenol effects in primary producers was between 18 and 28 mg/L, and the EC20 value for phenol effects on heterotrophic activity was 45 to 52 mg/L.

Phenol additions either reduced the population density or killed all algae and crustaceans during the first week after addition. By the sixth week, the algal and crustacean populations had recovered in the microcosms with 10 mg/L and 18 mg/L phenol. The populations had not recovered in the microcosms with 108 mg/L and 501 mg/L.

Triethylene Glycol Effects

Introduction. Triethylene glycol (TEG) was added at four concentrations to the microcosms containing sand sediment (Phase I) and at three concentrations in those containing clay and clay/sand sediments (Phase 2). The actual concentrations of TEG in the sand containing microcosms as determined by gas chromatographic analyses,

were: level 1 = 10 mg/L, level 2 = 20 mg/L, level 3 = 42 mg/L, and level 4 = 250 mg/L (Figure 25A). In the clay sediment microcosms the concentrations of TEG were: level 1 = 10 mg/L, level 2 = 16.2 mg/L, and level 3 = 17.0 mg/L (Figure 25B). The concentrations of TEG in the microcosms containing clay/sand sediment were: level 1 = 10 mg/L, level 2 = 14.7 mg/L and level 3 = 17.3 mg/L (Figure 25C). After three to six weeks the TEG concentrations had decreased by 30-60% of the original concentration in all three sediments (Figure 25A, B & C). The gas chromatographic analytical precision of TEG was highly variable.

Effects on Production. Net production was lower in TEG microcosms than the control microcosms. The mean production was 2.1 mg/L oxygen for all TEG microcosms for all weeks and sediments, significantly lower than the mean for the control microcosms which was 4.2 mg/L oxygen (Figure 26A & B).

The net production was lower in microcosms with TEG than the controls; however, the decrease in net production could not be correlated with increasing TEG concentrations (Figure 27). Net production values were greater than 20% deviant in the microcosms with 17 to 42 mg/L from the controls net production for the entire experimental period (Figure 26B), and the deviation from controls increased significantly to 50% four weeks after

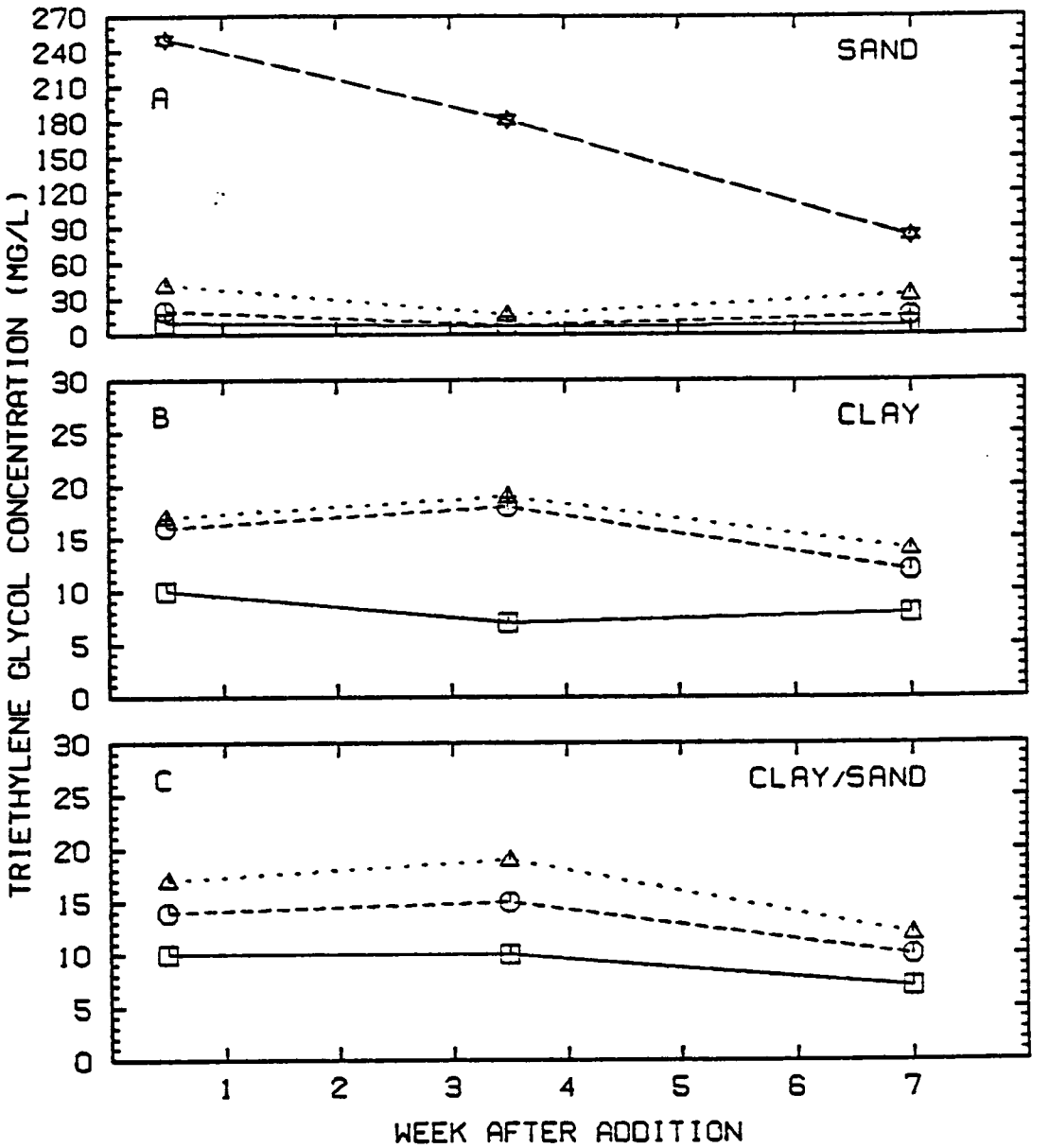

Figure 25. Mean concentration of TEG measured in microcosms for the weeks after addition of TEG for each sediment type (n = 3-4). Level 1 = □—□, level 2 = ○ - - ○, level 3 = △ - - △, and level 4 = ✡ - - ✡.

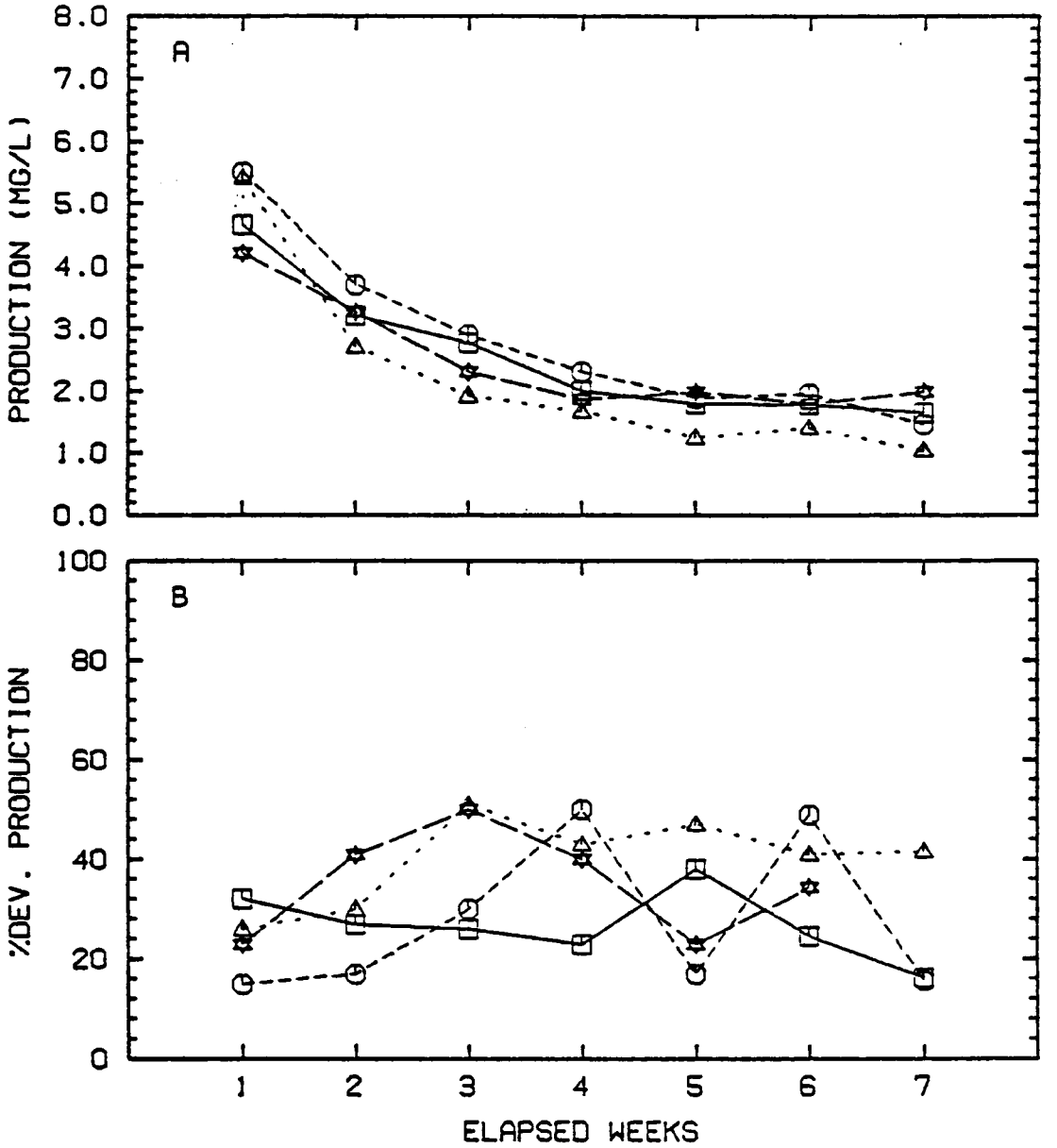

Figure 26. (A) Mean net production in microcosms with TEG added for each week and (B) mean percent deviation from controls for net production in microcosms with TEG added for each week. Level 1 (10 mg/L) = □——□, level 2 (14-20 mg/L) = ⊖— — ⊖, level 3 (17-42 mg/L) = △ - - △, and level 4 (250 mg/L) = ✡ - ✡ (n = 3-4).

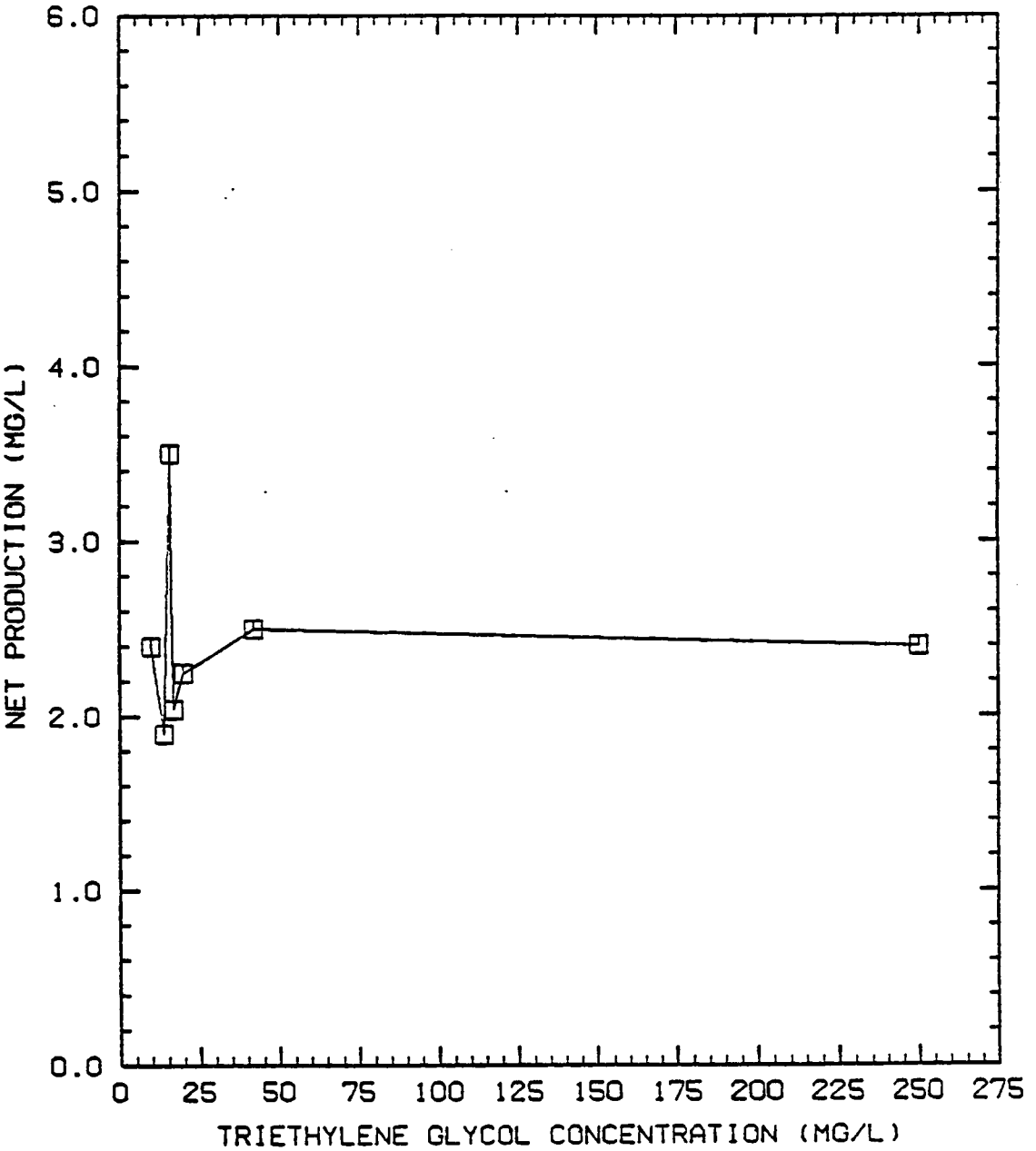

Figure 27. Mean net production in microcosms with TEG added for each concentration pooled over all weeks (n = 24).

the TEG addition. The average percent deviation from controls increased significantly in microcosms treated with all levels of TEG after week 4 (Figure 26B).

The chronic EC20 values for triethylene glycol's effect on net production was 35.0 mg/L. Net production in microcosms with triethylene glycol in the first two weeks of the experimental period did not exhibit a significant relationship with TEG concentration, but the EC20 calculated in the last week of the experimental period was 26 mg/L.

Effects on Heterotrophic Activity

Effects on heterotrophic activity. Triethylene glycol had a significant effect on heterotrophic activity by decreasing the heterotrophic activity in the microcosms dosed with the three highest concentration of TEG (17, 42, and 250 mg/L). The decrease in heterotrophic activity in microcosms exposed to the higher levels was greater in the later weeks of the experimental period (week 4 and 15) than in the first weeks (Figure 28A & B; Figure 29). The average percent deviation from the controls in the first week after the toxicant was added was 22.5% for the higher levels of TEG, but the average at the sixth week was 45.1% in microcosms dosed with the three highest concentrations of

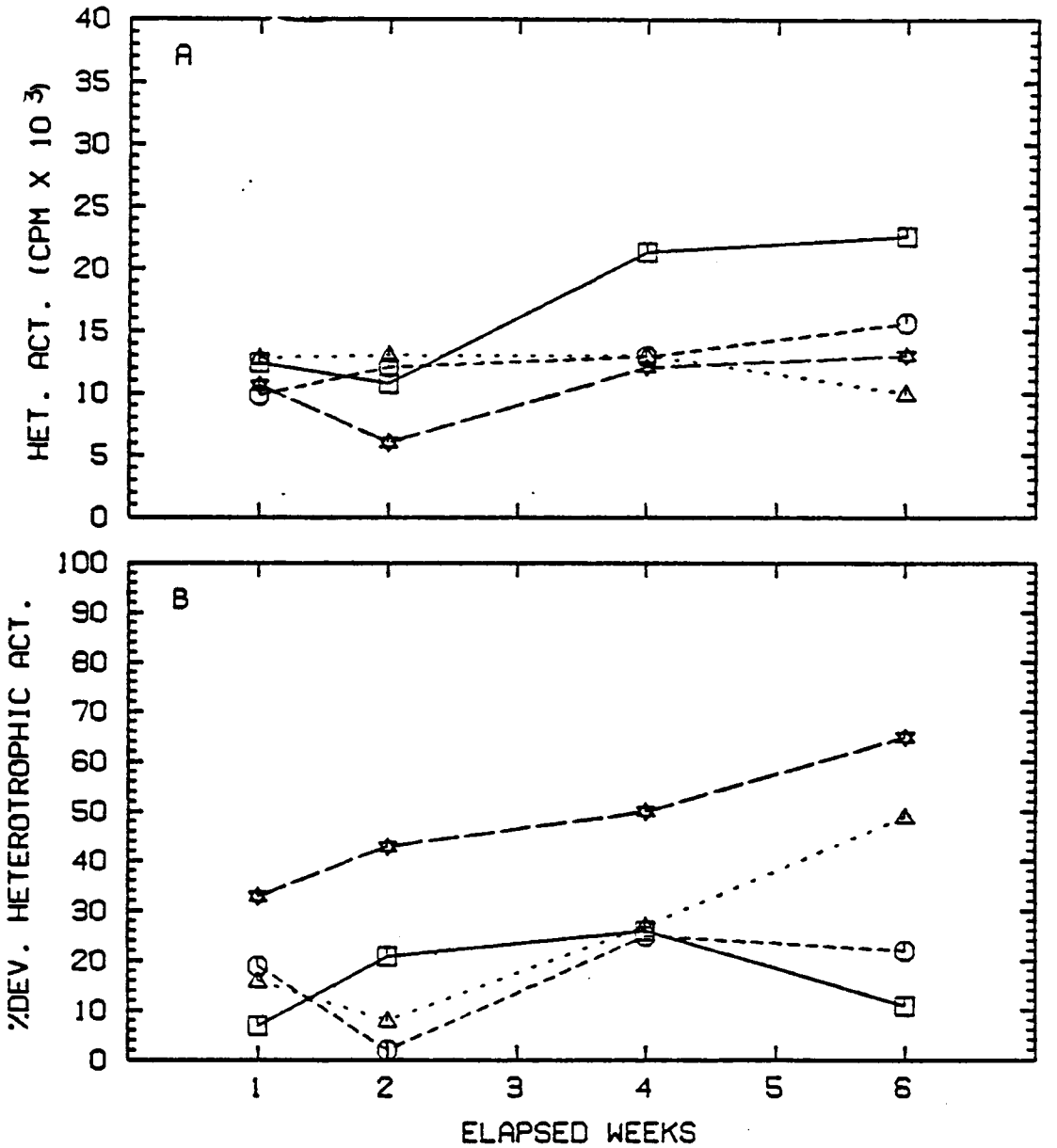

Figure 28. (A) Mean heterotrophic activity in microcosms with TEG added for each week and (B) mean percent deviation from controls for heterotrophic activity in microcosms with TEG added for each week. Level 1 (10 mg/L) = ⊡——⊡, level 2 (14-20 mg/L) = ⊖ - - ⊖, level 3 (17-42 mg/L) = △ - - △, and level 4 (250 mg/L) = ✡ — — ✡ (n = 3-4).

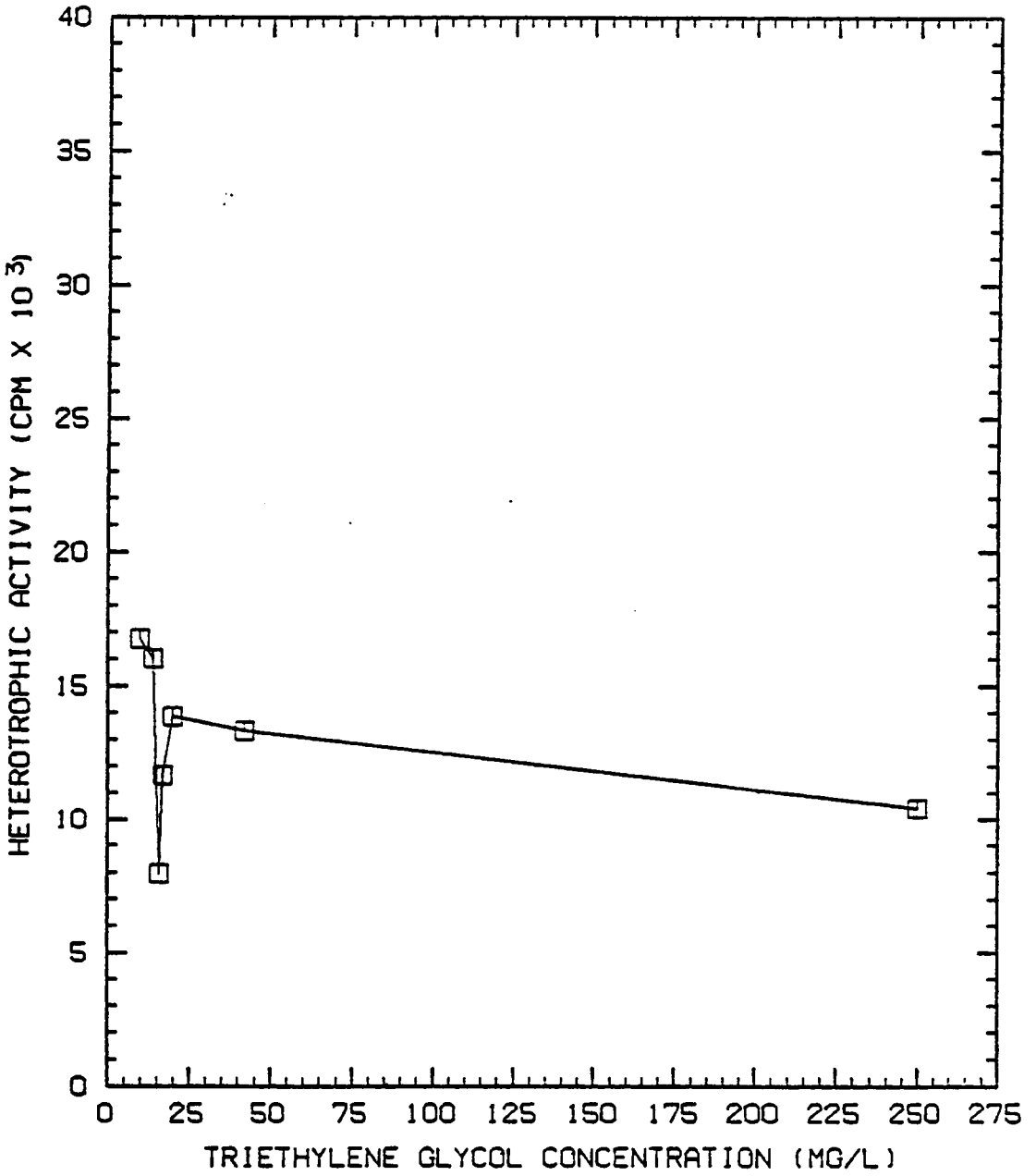

Figure 29. Mean heterotrophic activity in microcosms with TEG added for each concentration over all weeks (n = 18-24).

TEG (Figure 28B).

The chronic EC20 value for heterotrophic activity when TEG was the toxicant was 15.5 mg/L (least squares statistics: n = 54, m = 4.67, r = 0.737) throughout the experimental period. The acute EC20 value was 12.6 mg/L (n=16, m=9.57, r=0.90).

Effects on alkalinity, pH and carbon limitation.

There was a significant decrease in mean minimum pH in microcosms with triethylene glycol added beginning four weeks after the toxicant was added. The decrease in pH was observed in all three sediment types although it was more gradual in microcosms with the sand sediment than in microcosms with the other two sediment types (Figure 13A, B & C). The pH decreased to 4.0 in the microcosms dosed with 17 mg/L TEG (clay and the clay/sand sediments) during the last week of the experimental period (Figure 13B).

The alkalinity was significantly lower in all microcosms containing triethylene glycol. Alkalinity was less in sand-sediment microcosms to begin with, so the decrease was not as great (Figure 16A), but the alkalinity in the clay and clay-sand sediment microcosms was 0.6 mequiv/L and 0.5 mequiv/L respectively, by week 6 (Figure 16B & C). The average alkalinity of the control microcosms was 1.4 mequiv/L in week 6, and changed very little throughout the experimental period. The greater

the TEG concentration the lower was the alkalinity (n=12, m=Ø.Ø4, r=Ø.6Ø2) (Figure 3Ø).

Alkalinity and pH determine the carbon dioxide concentration in an aquatic system as well as the relative concentrations of other inorganic carbon forms, and when both are low, the available inorganic carbon is low. Therefore a CO_2 limitation may have occurred in the later weeks of the experimental period. Shifts in community composition support this possibility because the microcosms with the higher levels of TEG (also the lowest pH and alkalinity) were the only microcosms to have a large population of blue green algae.

Effects on community composition. Algal growth was observed in all microcosms with TEG however the microcosms with 17 mg/L, 42 mg/L or 25Ø mg/L had mostly blue green algae by the end of the experimental period. Ostracods were found in almost all of the triethylene glycol microcosms, but only the microcosms dosed with 1Ø mg/L had any other crustaceans. Ostracods are usually the most tolerant crustaceans in the microcosms, as indicated by these observations.

Summary. Triethylene glycol affected the heterotrophs more than the primary producers, but TEG did decrease both populations. The decrease in primary production was not correlated with TEG concentration but the heterotrophic activity was correlated significantly.

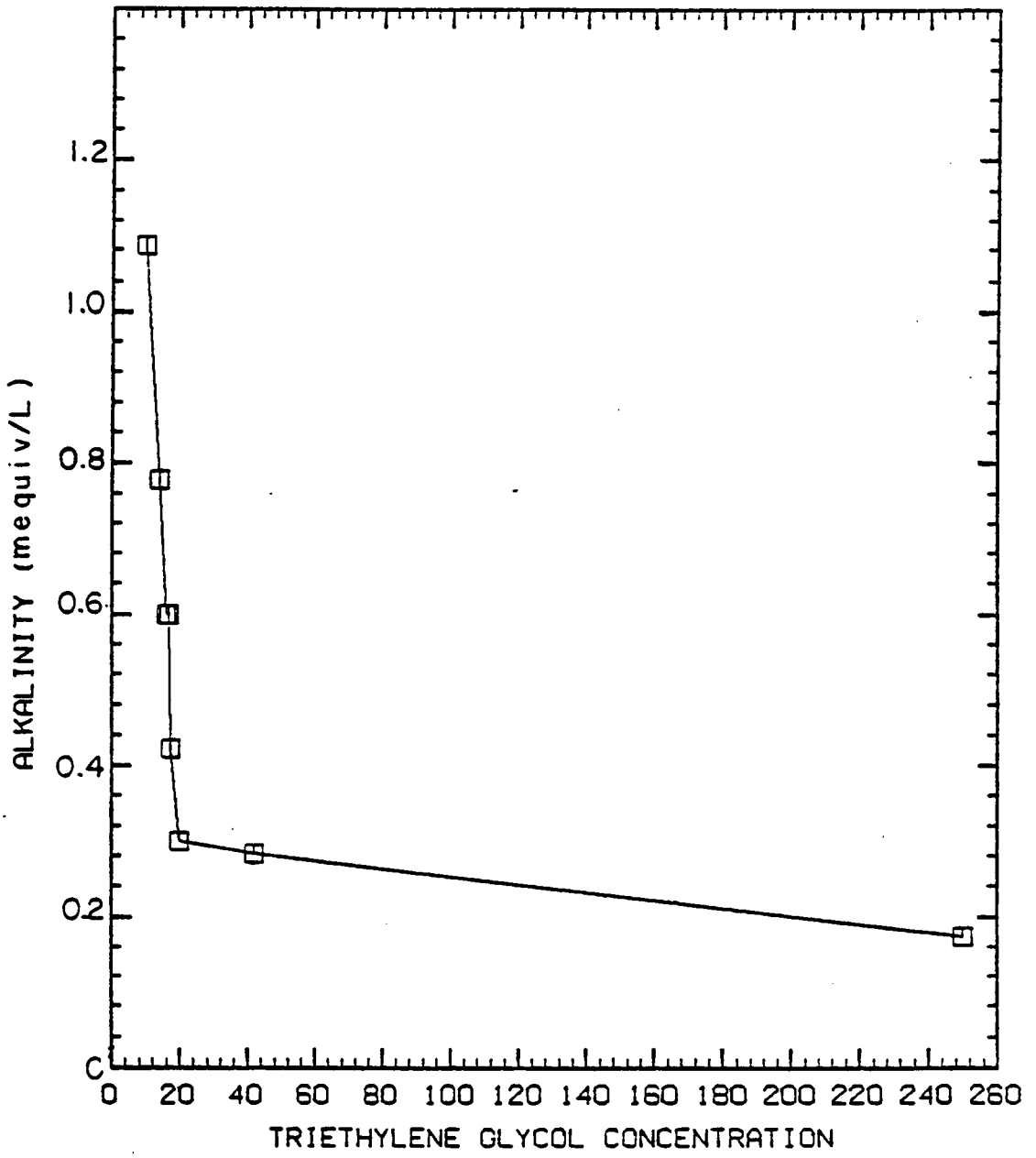

Figure 30. Mean alkalinity in microcosms with TEG added for each concentration pooled over all sediments measured on the 6th week after addition (n = 12-16).

The chronic EC 20 value for the net production was 35.0 mg/L and the chronic EC 20 value for heterotrophic activity was 15.5 mg/L.

Triethylene glycol additions significantly decreased the minimum pH and the alkalinity thereby encouraging blue green algal growth. In addition it significantly reduced the levels of crustaceans other than ostracods.

Quinoline Effects

Introduction. Quinoline was added in four concentrations to the microcosms containing sand-sediment (Phase I) and at three concentrations in those containing the clay and the clay and sand sediments (Phase II). The actual concentrations of quinoline in the microcosms containing sand as determined by gas chromatographic analyses were: level 1= 10 mg/L, level 2 = 14 mg/L, level 3 = 33 mg/L, and level 4 = 206 mg/L. The concentrations of quinoline in the microcosms containing clay were: level 1 = 10 mg/L, level 2 = 25.3 mg/L and level 3 = 38.8 mg/L (Figure 31B). The concentrations of quinoline in the microcosms containing clay/sand sediment were: level 1 = 10 mg/L, level 2 = 20.4 mg/L and level 3 = 88.2 mg/L (Figure 31C). Three weeks after the addition of quinoline, the concentrations had not decreased measureably in any of the microcosms, and in many of them the concentration had increased, especially in those

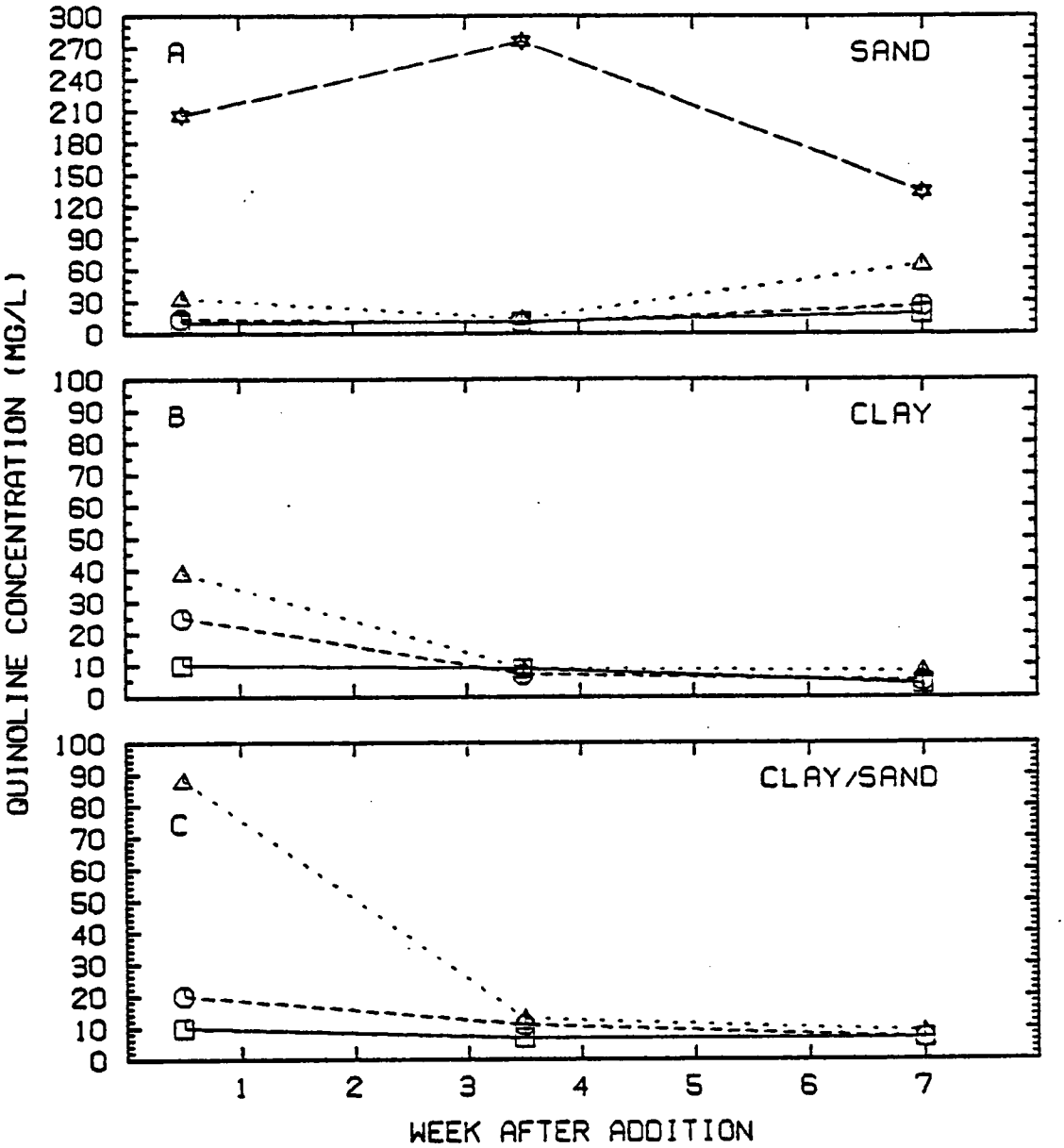

Figure 31. Mean concentration of quinoline measured in microcosms for the weeks after addition of quinoline for each sediment type (n = 3-4). Level 1 = □—□, level 2 = ⊖ - -○, level 3 = △ - - △, and level 4 = ✡ - ✡.

dosed with the highest level. After six weeks there was no measureable decreases in quinoline and again quinoline levels were greater than those measured initially in several of the samples.

Effects on production. Quinoline had a significant effect on primary production values. As quinoline concentration increased, net production decreased except in the microcosms containing 14-25 mg/L (Figure 32A & B; Figure 33). The mean production in all microcosms treated with quinoline was lower than in the controls, except in those microcosms dosed with 14-25 mg/L. The toxic effect of quinoline appeared to decrease in all microcosms with quinoline added between two and four weeks after the toxicant was added (Figure 32B). This decrease in toxicity would be an indication of recovery and acclimatization of the primary producers to the quinoline levels.

Net production in microcosms with level one (10 mg/L) of quinoline was not significantly different from the control net production throughout the experimental period and net production in the level two (14-25 mg/L) microcosms was significantly higher than the control microcosms' net production throughout the experimental period. A net production significantly lower than the control microcosms production was observed in microcosms

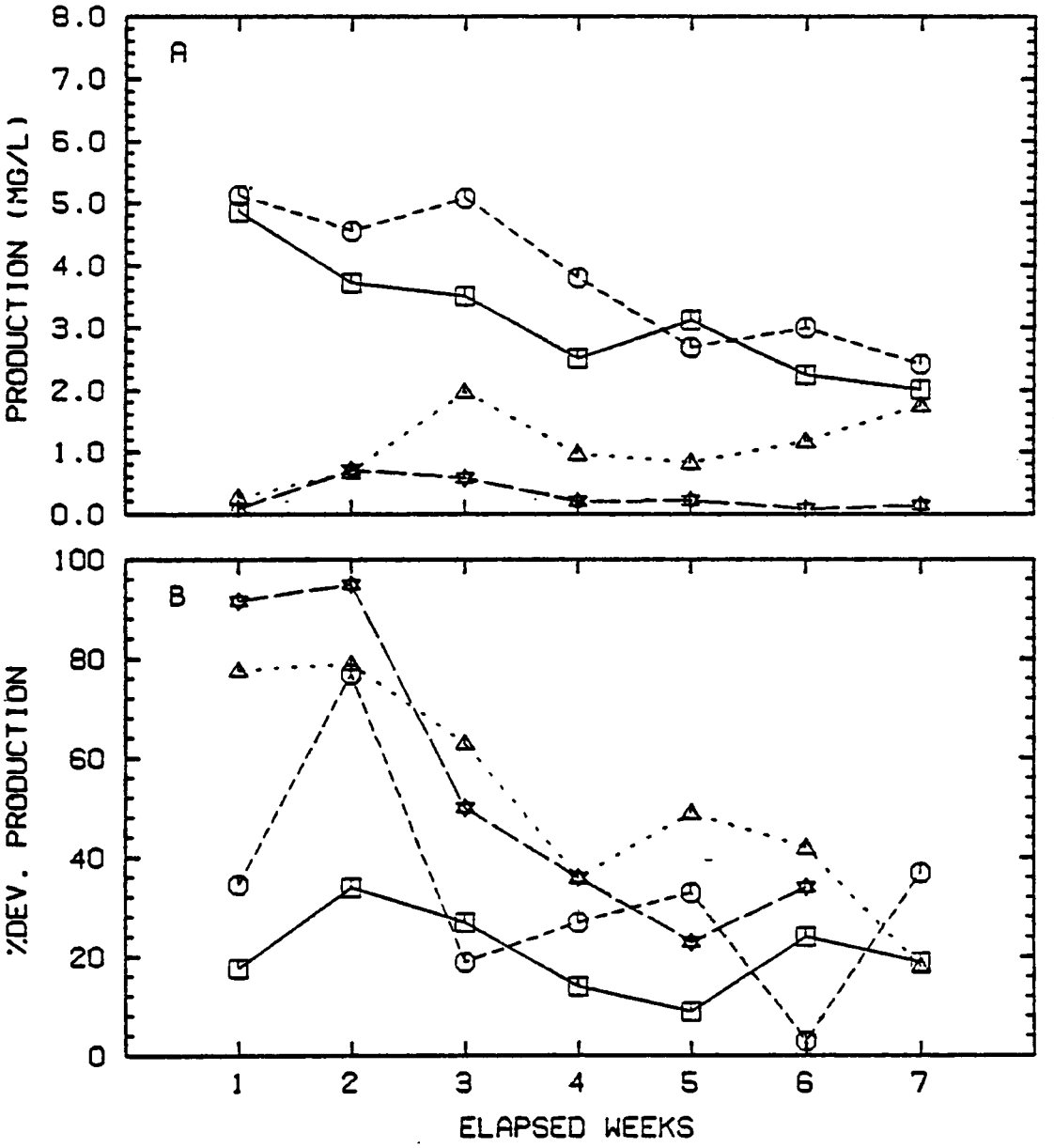

Figure 32. (A) Mean net production in microcosms with quinoline added for each week and (B) mean percent deviation from controls for net production in microcosms with quinoline added for each week. Level 1 (10 mg/L) = □——□, level 2 (14-20 mg/L) = ○ – ○, level 3 (33-88 mg/l) = △ – –△, and level 4 (206 mg/L) = ✡ — ✡ (n = 3-4).

dosed with levels three (33-88 mg/L) and four (206 mg/L) until four or five weeks after the quinoline was added. At that time production in microcosms with level three of quinoline was recovered to the level of net production in the control microcosms. Level four microcosms never recovered to the level of net production in the control microcosms. Microcosms with quinoline concentration greater than 10 mg/L deviated from that in the control microcosms by more than 20% until two weeks after the addition of the quinoline. Level three (33-88 mg/L) net production values were still 65% deviant from the controls at this time, but the deviation from the controls was decreasing each week. By week six the production was not significantly different in the microcosms dosed at level three than in the control microcosms'. Net production in microcosms dosed with level four (206 mg/L) was greater than 90% deviant from the controls throughout the experimental period (Figure 33).

Quinoline concentration was significantly correlated with reductions in net production in the aquatic microcosms ($n = 4$, $= 2.975$, $r = 0.983$). The chronic EC20 for quinoline ($n = 8$, $m = 2.05$, $r = 0.712$) was 21 mg/L and the acute EC20 was 32 mg/L.

Effects on heterotrophic activity. Quinoline had a significant effect on heterotrophic activity. A lower

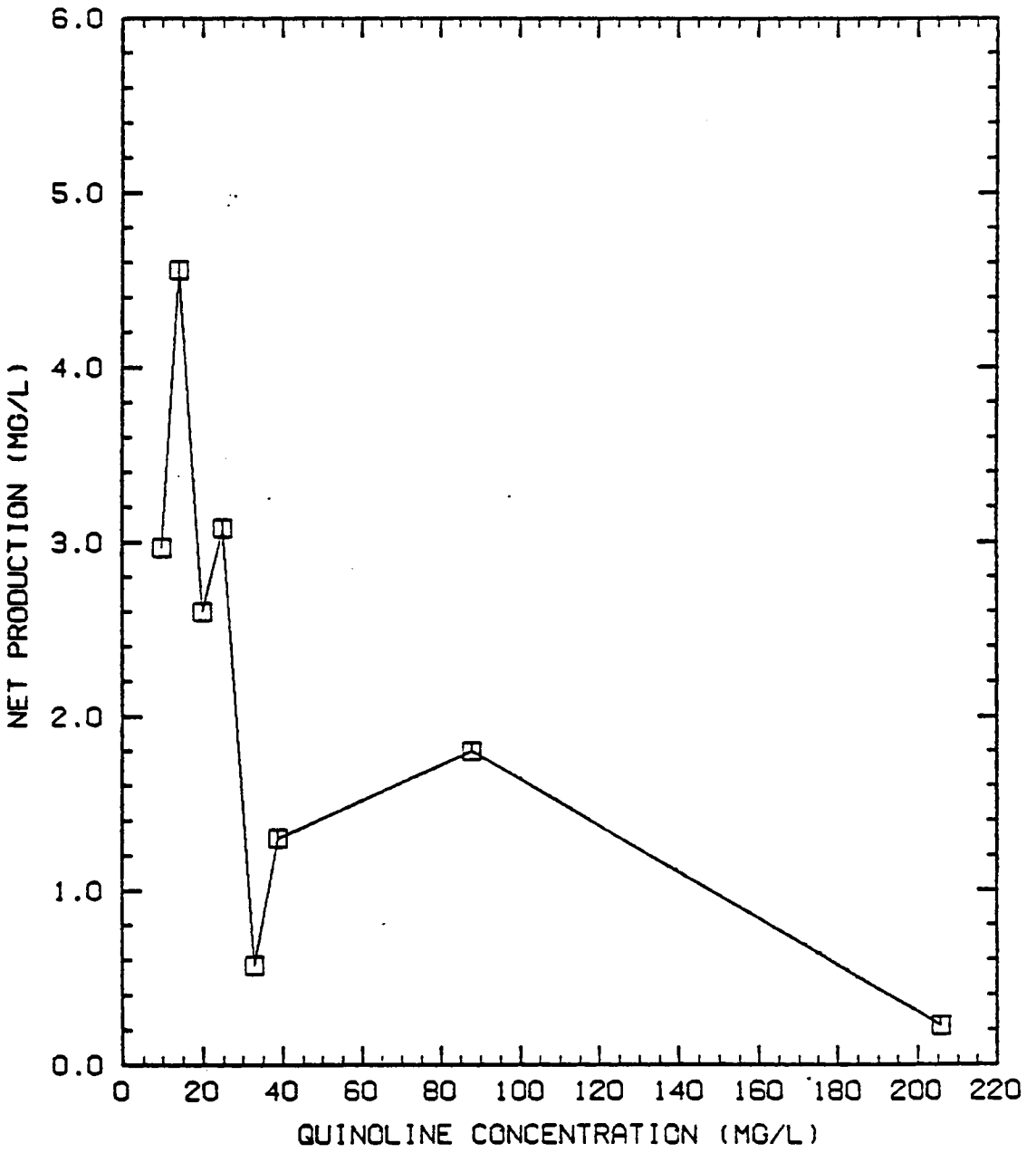

Figure 33. Mean net production in microcosms with quinoline added for each concentration pooled over all weeks (n = 18-24).

mean heterotrophic activity was observed in all microcosms with quinoline than that observed in the controls except for quinoline level three in the six week. The higher the quinoline concentration was the lower the heterotrophic activity measured (Figure 34A & B; Figure 35). The mean control heterotrophic activity was 16,535 cpm (10 mg/L). A heterotrophic activity of 14,497 cpm was measured in level 1 microcosms, 11,876 cpm in level 2, 16,042 cpm in level 3 and 241 cpm in level 4 (Figure 34A).

Heterotrophic activity in microcosms with quinoline level 1 (10 mg/L) was never significantly different from the controls, but significantly lower heterotrophic activity was observed in level 2 (14-25 mg/L) than the controls for two weeks after quinoline was added (Figure 34B). The fourth week after the toxicant addition, the heterotrophic activity in the microcosms appeared to have recovered to levels similar to that in the controls. In microcosms with levels 3 and 4 (33-88 mg/L and 206 mg/L) of quinoline, the heterotrophic activity remained significantly lower than in the controls throughout the experimental period, except for level 3 on week 6 when stimulation of heterotrophic activity was observed. Even though the heterotrophic activity in the microcosms with higher concentrations of quinoline remained significantly lower than that in the control, heterotrophic activity

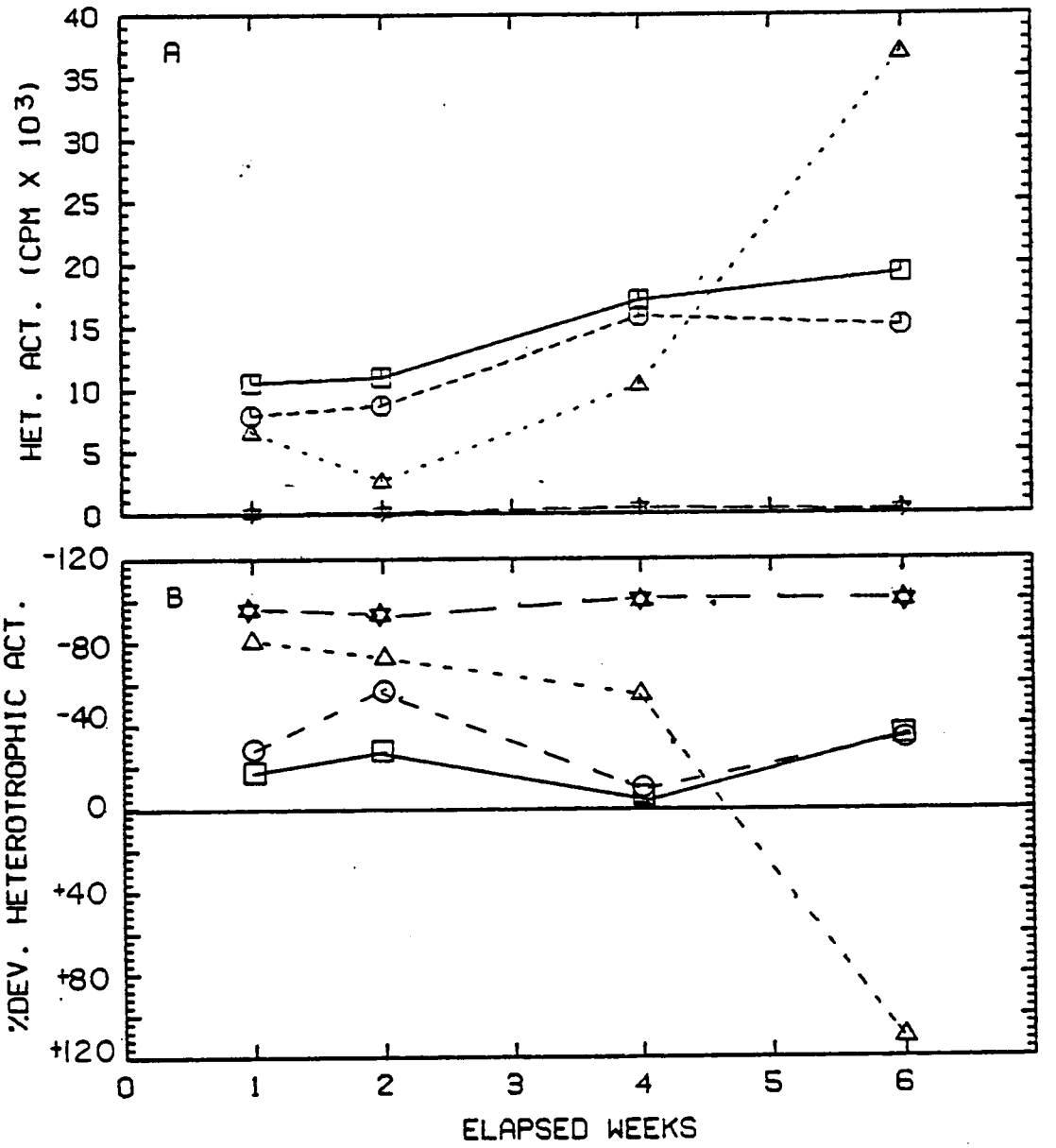

Figure 34. (A) Mean heterotrophic activity in microcosms with quinoline added for each week and (B) mean percent deviation from controls for heterotrophic activity in microcosms with quinoline added for each week. Level 1 (10 mg/L) =□——□, level 2 (14-20 mg/L) =○- - -○, level 3 (33-88 mg/L) =△- - -△, and level 4 (206 mg/l) =✡- - -✡ (n = 3-4).

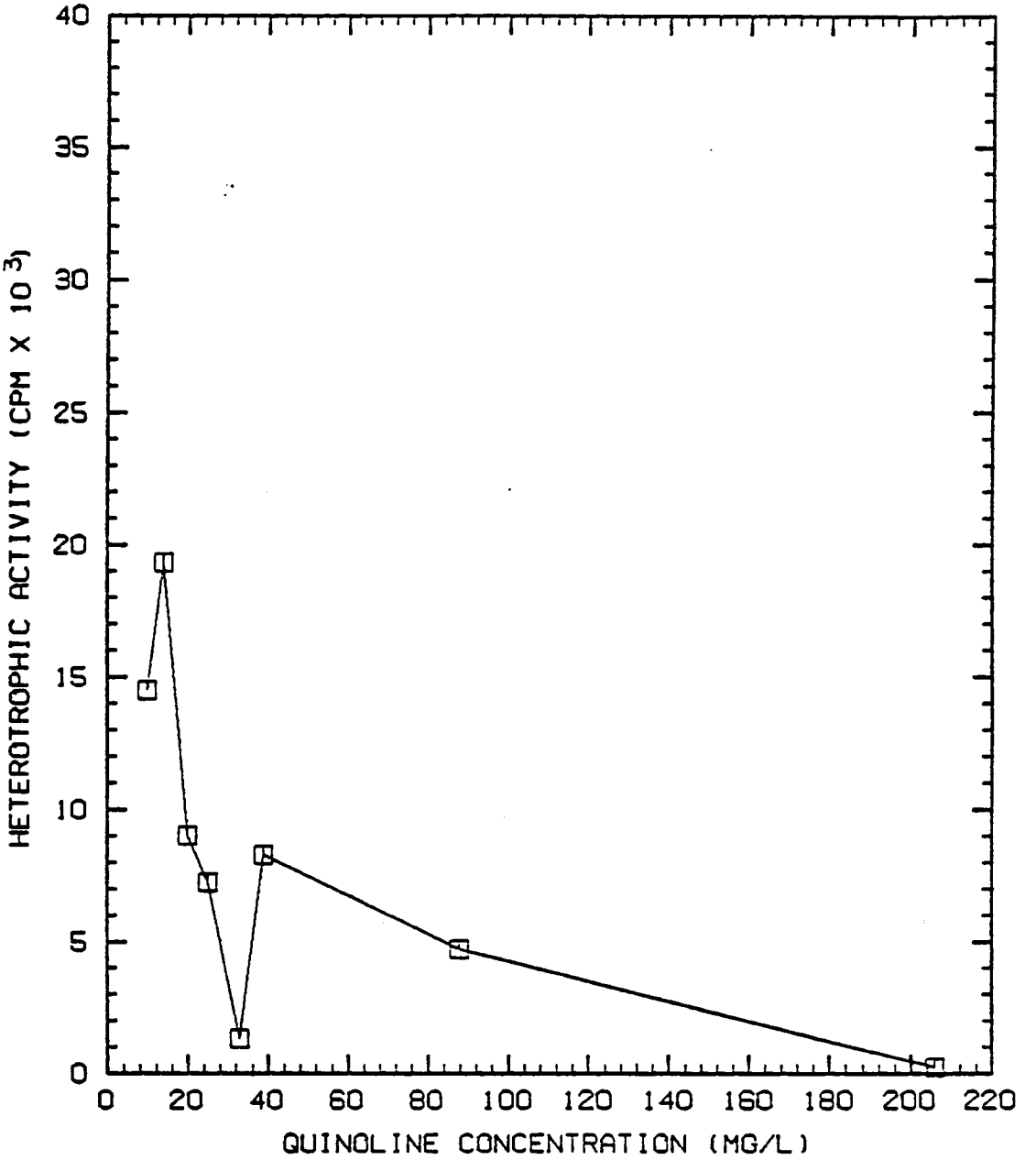

Figure 35. Mean heterotrophic activity in microcosms with quinoline added for each concentration pooled over all weeks (n = 18-24).

did begin to recover to control levels at least by four weeks after the toxicant had been added (Figure 34B).

The mean percent deviations in heterotrophic activity from the control microcosms were greater than 20% in microcosms at all four levels. Heterotrophic activity in microcosms dosed at 10 mg/L were 24.6% deviant, level 2 (14-25 mg/L) was 34.8% deviant, level 3 (33-88 mg/L) was 83.6% deviant and level 4 (206 mg/L) was 98.6% deviant. There was a significant relationship between quinoline concentration and percent deviation from the controls ($n = 16$, $m = 1.696$, $r = 0.750$).

Four weeks after quinoline was added to microcosms at levels 1 and 2 the heterotrophic activities were only approximately 10% lower than in the control, and the percent deviation in heterotrophic activity in level 3 microcosms was decreasing until week six when stimulation apparently occurred. Heterotrophic activity in microcosms at level 4 was greater than a 98% deviations from the controls throughout the experimental period (Figure 34B).

Quinoline did significantly decrease heterotrophic activity, but the microcosms treated at the lower levels did appear to begin recovery or acclimatization by four weeks after quinoline had been added. The chronic EC20 value predicted for quinoline by suppression or stimulation of heterotrophic activity was 30 mg/L. The

acute EC 20 for quinoline as predicted by suppression of heterotrophic activity, was 36 mg/L (n = 8, m = 2.18, r = 0.805).

Effects on alkalinity, pH and carbon limitation. There was a significant increase in alkalinity correlated with an increase in quinoline concentration (n = 4, m = 0.071, r = 0.996) (Figure 36). The mean alkalinity in microcosms with level 4 of quinoline (206 mg/L) was 6.4 mequiv/L which is six times greater than the control microcosm's mean alkalinity of 1.04 mequiv/L. The alkalinity in microcosms with all three sediments showed similar increases in alkalinity with increase in quinoline concentration.

There was no significant difference in maximum or minimum pH correlated with quinoline concentration. The high alkalinity and the approximately neutral pH would both indicate relatively normal to high levels of inorganic carbon and carbon dioxide limitation would not be a factor.

Effects on community composition. Quinoline appeared to be toxic to crustaceans, i.e. cladocerans, ostracods and amphipods. Cladocerans, ostracods and amphipods were found in quinoline levels 2, 3, and 4 microcosms and reduced levels of cladocerans and ostracods were found in microcosms dosed at level 1.

Attached algae and some planktonic algae were found

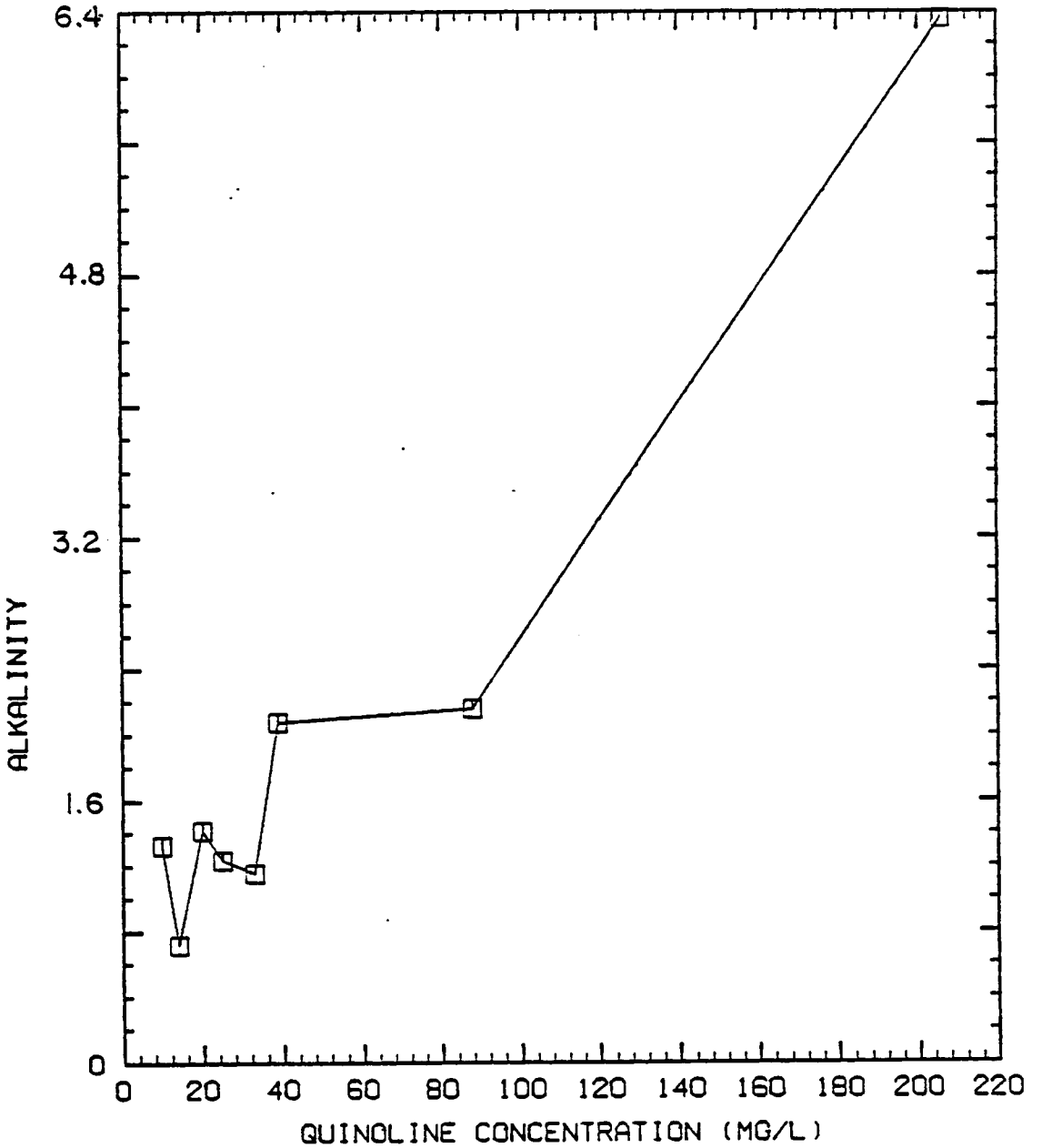

Figure 36. Mean alkalinity in microcosms with quinoline added for each concentration pooled over all sediments measured on the 6th week after addition (n = 12-16).

in quinoline levels 1 and 2 but no obvious algal populations were found in level 3. Water in microcosms with level 3 and 4 was dark brown due to the effect of light on quinoline and this darkly colored water may have filtered out light, thus reducing algal populations.

Summary. Quinoline concentrations in the water column did not decrease with time in the microcosms containing sand sediment but were actually higher in the samples taken three and six weeks later. Quinoline significantly reduced primary production in all microcosms except in the one dosed at level 2; that dose stimulated primary production. The chronic EC20 for production was 21 mg/L and the acute EC20 was 32 mg/L. Quinoline also significantly decreased heterotrophic activity. Microcosms dosed with levels 1 and 2 recovered by week four, level 3 recovered by week six but level 4 did not recover. The chronic EC20 for heterotrophic activity was 30 mg/L.

Quinoline level was significantly correlated with an increase in alkalinity but because there was no change in pH, carbon dioxide limitation was not a factor.

There was a significant decrease in the populations of both consumers and primary producers with quinoline dose.

Naphthoquinone Effects

Introduction. Naphthoquinone was added to microcosms at four concentrations during phase 1: level 1 = Ø.Ø1 mg/L, level 2 = Ø.Ø2 mg/L, level 3 = Ø.Ø3 mg/L, and level 4 = Ø.Ø4 mg/L. During phase 2 it was added to microcosms containing the clay sediment and the clay/sand sediment, at three levels: level 1 = Ø.Ø1 mg/L, level 2 = Ø.Ø15 mg/L, and level 3 = Ø.Ø2 mg/L. Concentrations of naphthoquinone decreased with time (Figure 37A, B & C). The greatest decrease was in the microcosms with the higher levels of naphthoquinone addition (Ø.Ø3 and Ø.Ø4 mg/L). In these microcosms with sand sediment the concentration decreased by 85% to 95% after 25 days (Figure 37A). The concentrations of naphthoquinone decreased slightly during the first three weeks. In all cases but one, the naphthoquinone concentration was lower six weeks after addition than it was at the time of the previous analyses at three and one-half weeks after dosing (Figure 37A, B & C).

Effects on production. Primary production in the microcosms with naphthoquinone were significantly lower than the control microcosms but not significantly lower than the acetone control microcosms. The mean net-production values in all microcosms with naphthoquinone fluctuated above and below the control production values throughout the experimental period and

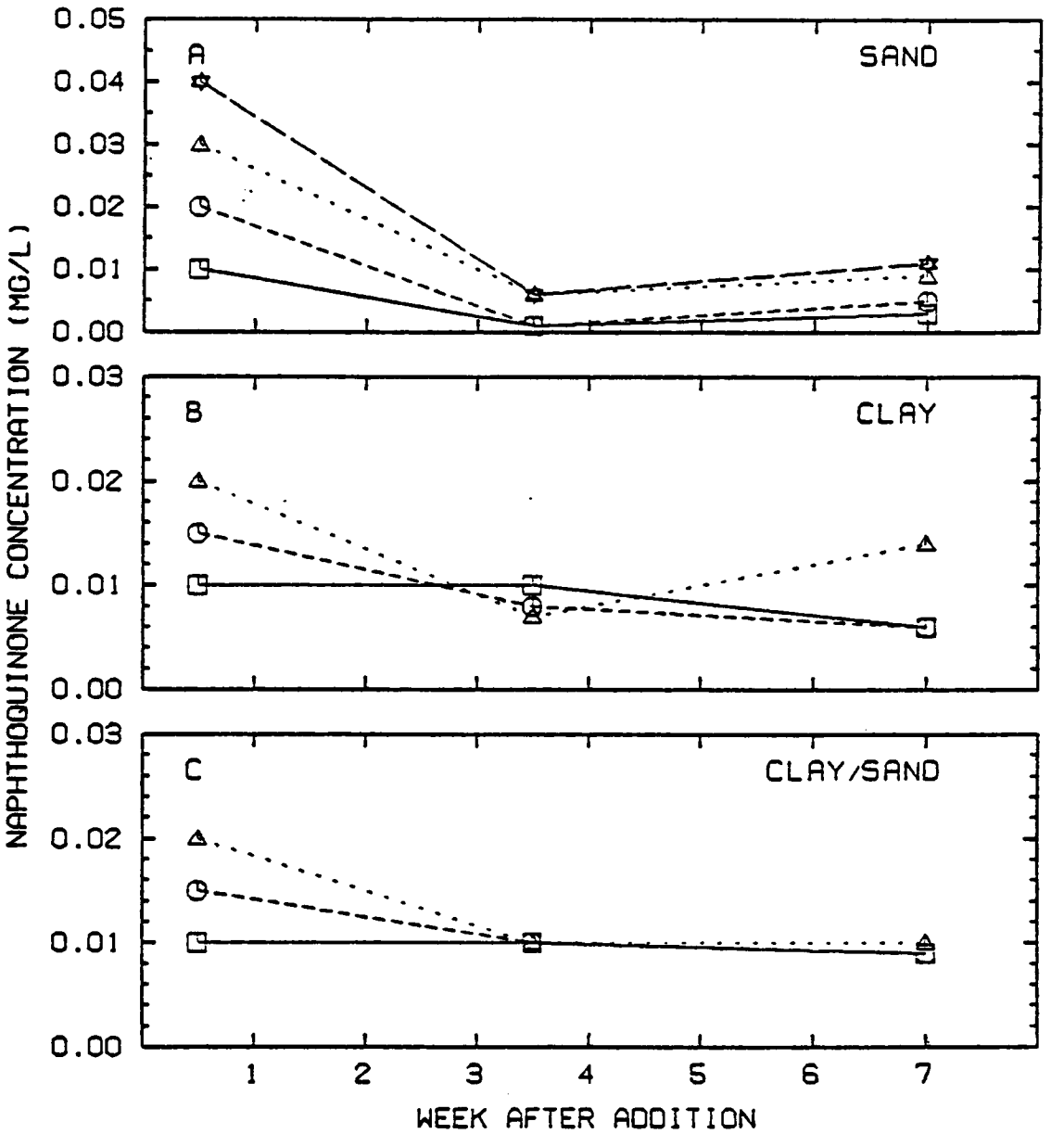

Figure 37. Mean concentration of naphthoquinone measured in microcosms for the weeks after addition of naphthoquinone for each sediment type (n = 3-4). Level 1 = □——□, level 2 = ○– –○, level 3 = △– –△, and level 4 = ☼– –☼

the net production values were not significantly correlated with naphthoquinone concentration (Figure 38A & B; Figure 39). The mean net production values measured in microcosms at each level of naphthoquinone were: level 1 (0.01 mg/L) was 3.4 mg/L oxygen, level 2 (0.015-0.02 mg/L) was 2.1 mg/L oxygen, level 3 (0.03 mg/L) was 3.0 mg/L oxygen, and level 4 (0.04 mg/L) was 2.8 mg/L oxygen (Figure 39B).

Net production in microcosms with naphthoquinone fluctuated around the 20% deviation from the control microcosms with no net production values staying greater than 20% deviant from the controls for long (Figure 38B). Production in microcosms containing the three highest levels was greater than 20% deviant from the controls the first week after addition of the toxicant (level 2 = 35.5%, level 3 = 27.8%, and level 4 = 57.9%), but after the first week, the percent deviation was not correlated with the concentration of naphthoquinone.

Sediment type appeared to exhibit significant influence on production in the naphthoquinone microcosms. The lowest production was found in the clay and sand sediment microcosms, but production in the other two sediment types was not significantly different from each other.

In summary, naphthoquinone depressed production but not significantly and this percent depression was not

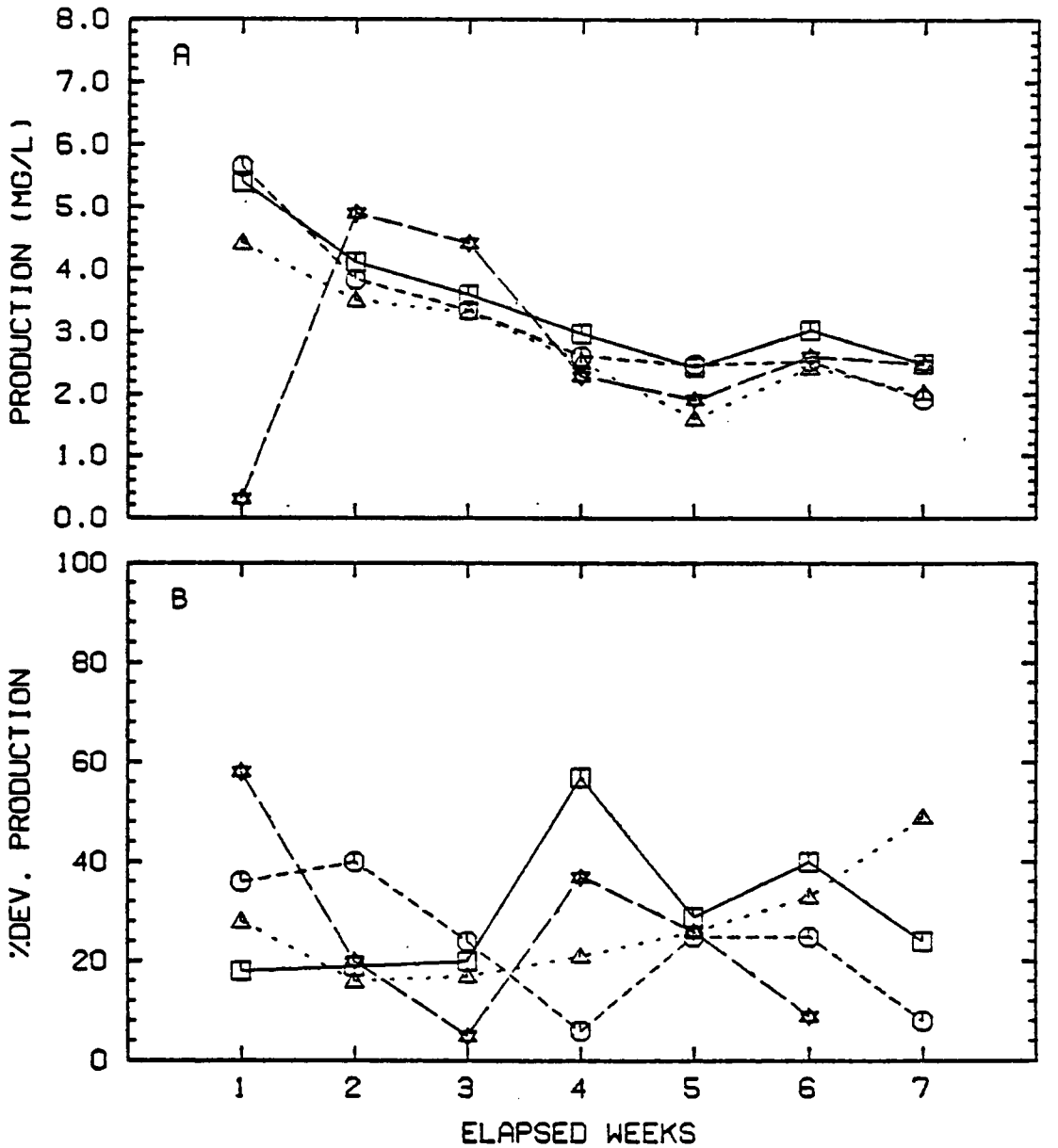

Figure 38. (A) Mean net production in microcosms with naphthoquinone added for each week and (B) mean percent deviation from controls for net production in microcosms with naphthoquinone added for each week. Level 1 (0.01 mg/L) = □——□, level 2 (0.015-0.02 mg/L) = ○ - - ○, level 3 (0.02-0.03 mg/L) = △- - -△, and level 4 (0.04 mg/L) = ☆ – – ☆ (n = 3-4).

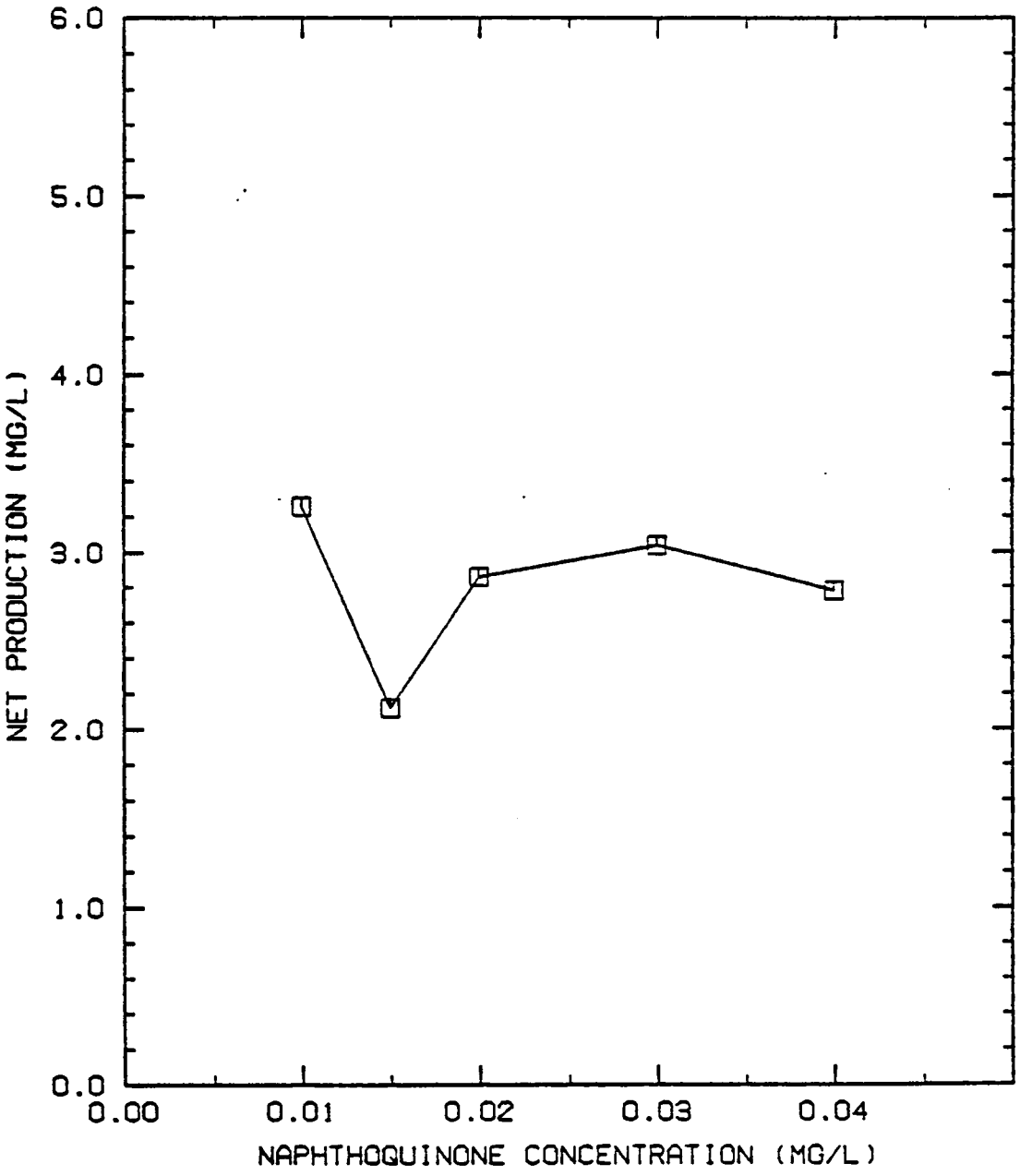

Figure 39. Mean net production in microcosms with naphthoquinone added for each concentration pooled over all weeks (n = 18-24).

correlated with naphthoquinone concentration.

Effects on heterotrophic activity. The effect of naphthoquinone on the heterotrophic activity in the microcosms was correlated slightly (n = 16; r = Ø.33) with the addition of naphthoquinone. Heterotrophic activity was higher than the controls in microcosms dosed at the lower levels and heterotrophic activity was lower than the controls in the higher levels of naphthoquinone addition (Figure 4ØA & B; Figure 41). Heterotrophic activity increased in the fourth week in all microcosms to levels greater than those of the control microcosms (Figure 4ØA).

The mean heterotrophic activity values deviated more than 2Ø% from the control microcosms in all microcosms with naphthoquinone, with the highest deviation (53.6%) exhibited by microcosms with level 4 of naphthoquinone (Figure 4ØB). The greatest deviation from the controls was exhibited at all levels in the first two weeks after naphthoquinone was added and the percent deviation from the controls decreased significantly by the fourth week (Figure 4ØB).

In summary, heterotrophic activity was depressed at all concentrations of naphthoquinone the first two weeks after its addition but it recovered to levels greater than the controls by the fourth week. The initial depression deviated greater than 2Ø% from the controls.

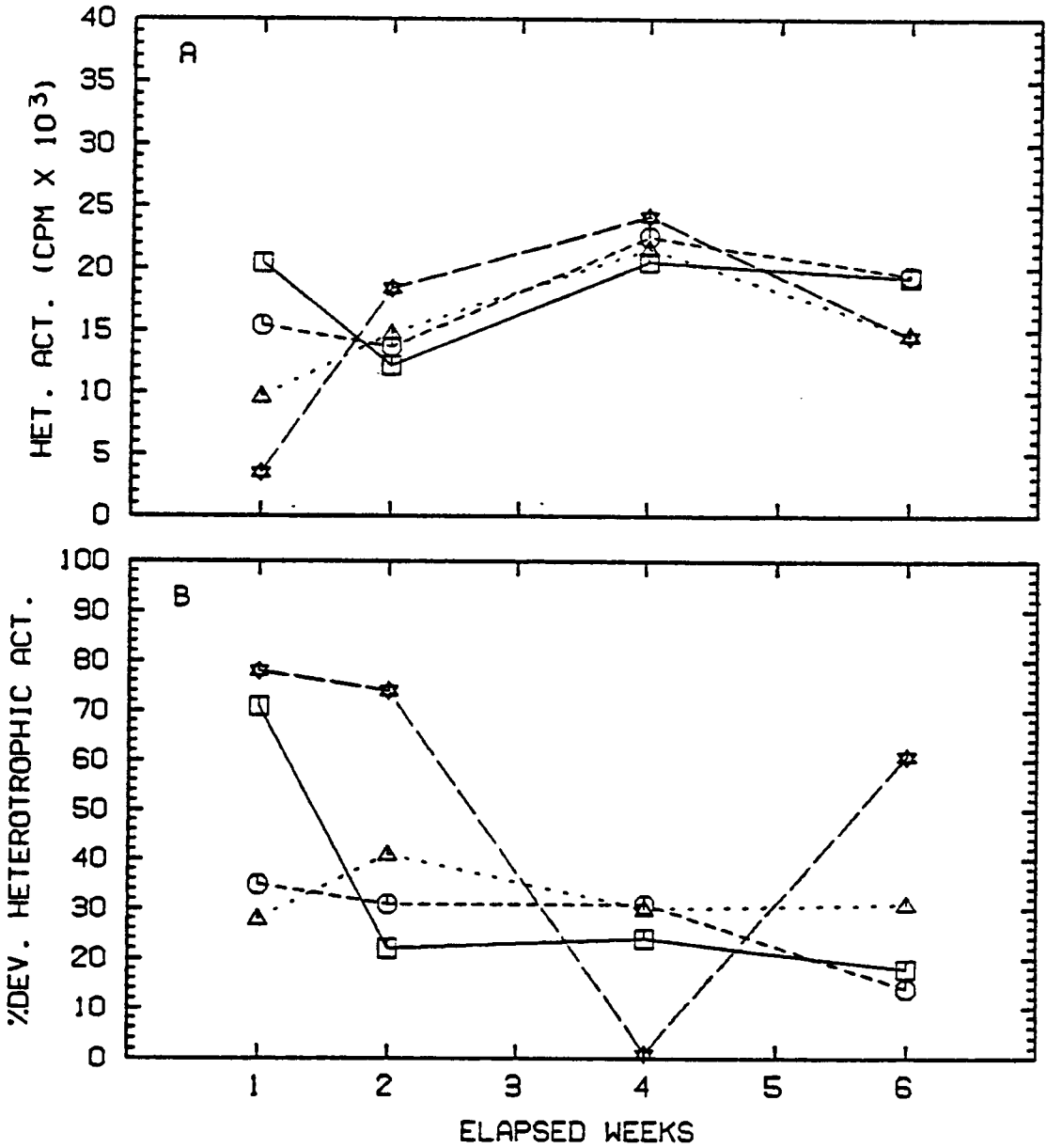

Figure 40. (A) Mean heterotrophic activity in microcosms with naphthoquinone added for each week and (B) mean percent deviation from controls for heterotrophic activity in microcosms with naphthoquinone added for each week. Level 1 (0.01 mg/l) =□—□, level 2 (0.015-0.02 mg/L) =⊖ - -○, level 3 (0.02 mg/L) =△ - △, and level 4 (0.04 mg/L) =✡ - -✡(n = 3-4).

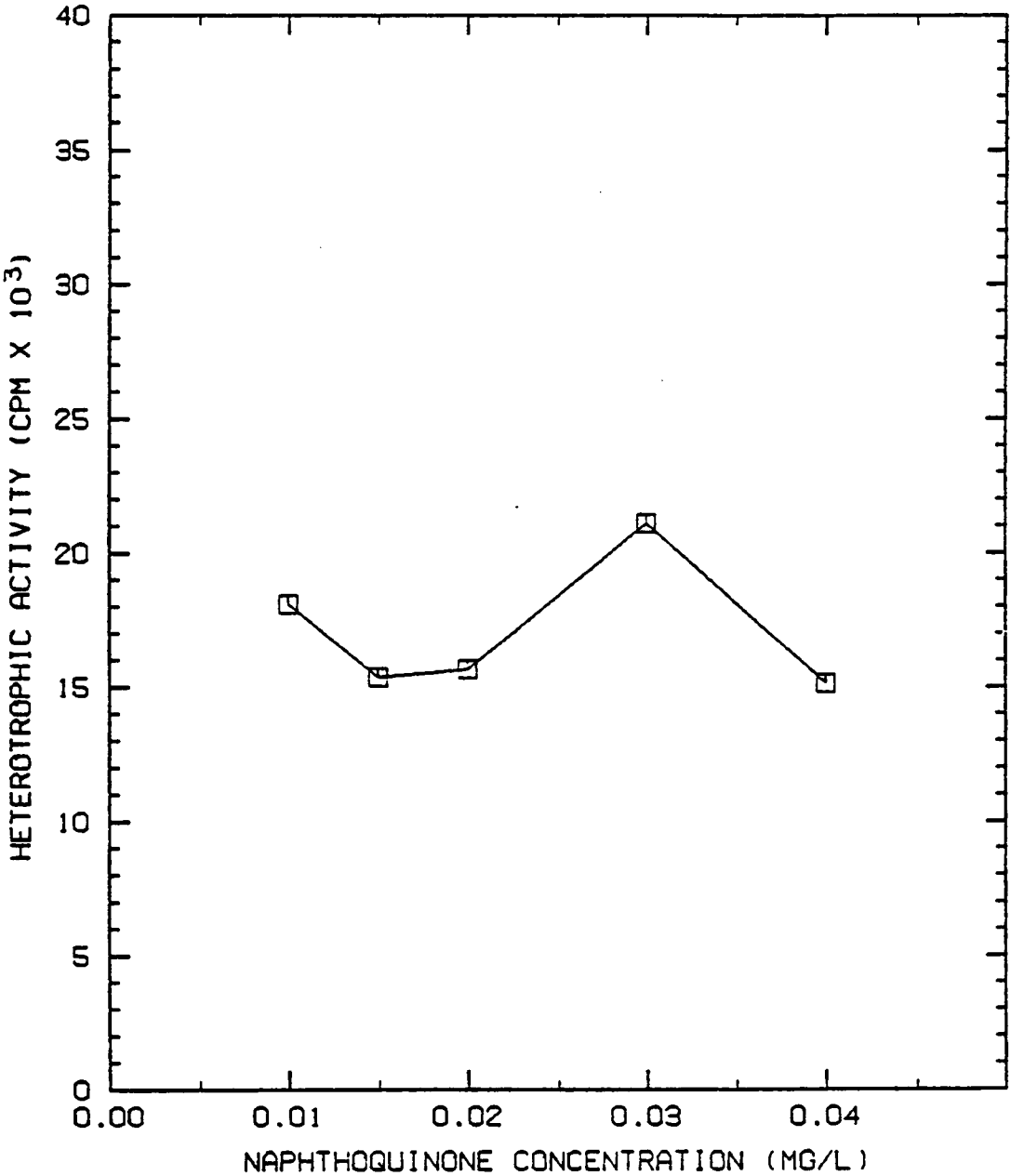

Figure 41. Mean heterotrophic activity in microcosms with naphthoquinone added for each concnetration pooled over all weeks (n = 18-24).

Effects on alkalinity, pH and carbon limitation. Alkalinity values and minimum pH values in the microcosms with naphthoquinone exhibited no significant trend with naphthoquinone concentration. Since these two variables primarily determine carbon dioxide concentration and neither was affected by naphthoquinone concentration, it was assumed that carbon dioxide was not limited due to effects by naphthoquinone addition.

Effects on community composition. Cladocerans were found in all microcosms with naphthoquinone. Ostracods were found in approximately one-half of the microcosms, but their presence did not seem to be related to naphthoquinone dose. Attached algae were found in all microcosms and planktonic algae were found in a few.

Naphthoquinone at these levels does not appear to have affected the community composition.

Summary. The concentrations of naphthoquinone were low because of its low solubility, and these low concentrations had less of an effect on the variables indicative of the aquatic community. The concentration of naphthoquinone had decreased by 60-95% by the third week of its addition. The chemical depressed primary production slightly and depressed heterotrophic activity significantly in the first two weeks after addition of the naphthoquinone. The heterotrophic activity was not decreased enough, however to affect ostracod populations.

The heterotrophic activity and the production recovered to levels equal to or greater than those in the controls by the fourth week.

Statistical Comparisons

Comparisons of nonparametric and parametric analyses.

The statistical comparisons of data were made using net production values and maximum dissolved oxygen values, as data and statistics were calculated using the "Univariate" procedure from the Statistical Analysis System (Ray, 1982).

In a comparison of measures of central tendencies the parametric mean was a higher value than the nonparametric median 82% of the time in analyses of net production values, and was higher 50% of the time in analyses of maximum dissolved oxygen values.

The measures of dispersion, the parametric standard deviation and the nonparametric interquartile range were also different. The interquartile range (a measure of dispersion in nonparametric analyses) was higher than the standard deviation 91% of the time for net production and 55% of the time for maximum dissolved oxygen data.

Statistics representing tests of normality and characteristics of the distribution included the D and W

statistic for the Kolmogorov-Smirnof goodness-of-fit test, skewness, and kurtosis (Sokal and Rohlf,1969).

The probability of these data being normally distributed was less than 0.01% for 100% of the values. The normal probability curves indicated graphically that these data were not normally distributed (Hollander and Wolfe,1973 and Tukey, 1973).

The skewness statistic indicated that there was a significant skew in 100% of the values studied (Sokal and Rohlf,1969). The production values were skewed to the right (greater than the mean) 95% of the time. The maximum dissolved oxygen values were skewed to the right 59% of the time.

The kurtosis statistic indicated a significant kurtosis in 100% of the production values and was positive in 64% of the values (indicating a sharp peaked curve, (Sokal and Rohlf,1969)). This statistic was positive 77% of the time in the maximum dissolved oxygen values.

All of these statistics indicate that those data were not normally distributed making the use of parametric statistics in these analyses questionable.

Two other characteristics of the data that must be considered when one questions whether assumptions regarding statistical procedures have been met are the requirement for homogeneity of variances and similarity

of sample sizes.

A test for homogeneity of variances is the F-Test in which the F statistic is equal to the proportion of the two variances being tested (Downie and Heath, 1974). The greater the F value the less homogeneous the variances. The comparison of the variances of data collected from analysis of the control microcosms (averaged over all sediments and weeks) and each toxicant concentration variance (also averaged over all weeks and sediments) showed that the F-value was significantly greater than 1.Ø (= Ø.Ø1) in 14 out of 16 cases tested, indicating nonhomogeneous variances. The highest F value was 33.Ø4 (quinoline, level 4 replicates) and the lowest F value was 1.6Ø (naphthoquinone, level 1 replicates). Eight out of twelve of the F tests are significant at the = Ø.Ø1 level. In a comparison of variances within a toxicant and between concentrations, the highest F value was 17.9 (quinoline levels 2 and 3).

The sample sizes varied considerably too, depending on the combinations of the data used. For example if the data from the controls are combined by averaging all sediment types and weeks the number of samples is 1,934, and the sample size using the same combination for phenol level one is 44. The sample size could be as small as three when a mean was calculated for a specific toxicant concentration, specific sediment and specific week, or as

large as 1,934 when the mean combines all controls for all sediments and all weeks.

Comparison of confidence intervals. Two different levels of statistical significance were compared: 95 and 80 percent. When the means between treatments are tested using a t-test, more treatments become significantly different from the control when the 80% confidence limit is used.

When the heterotrophic activity in microcosms with phenol were compared to the controls only the heterotrophic activity in microcosms with phenol at levels three and four are significantly different from the controls at the 95% level but the highest three levels were significantly different from the controls at the 80% significance level. If the 95% significance level was used, the EC20 criterion for regulation of phenol concentration in our water supply would be between 18 mg/L and 108 mg/L, however, if the 80% significance level was used the criterion would be between 10 mg/L and 18 mg/L phenol.

A comparison of mean values for heterotrophic activity in microcosms with triethylene glycol (TEG) also indicates different criteria for the two significance levels. The EC20 criterion for regulation of TEG would be between 17 mg/L and 30 mg/L if the 95% level were applied, but between 10 mg/L and 17 mg/L if the 85% level

were used.

A comparison of heterotrophic activities in the quinoline treated microcosms reveals a similar situation. If 95% confidence limits are used, the EC20 would be between 20 mg/L and 60 mg/L. However, for the 80% confidence limits for the EC20 would be between 10 mg/L and 20 mg/L.

There would be no difference in the criterion based on naphthoquinone microcosms' heterotrophic activity at 95% and 80%.

Finally the 95% and 80% confidence levels would not change the EC20 criterion for any other toxicants, if it were to be based on suppression of net production.

Ranking of Chemical Additives According to Toxicity

There are a number of ways these toxicants can be ranked. One is to compare the production and heterotrophic activity values graphically. However, this method does not take into account actual differences in the dissolved concentrations of the toxicants. Naphthoquinone will not be included in the rankings here because the EC20 data were inaccurate due to the low concentrations that were applied. If this method of ranking the relative toxicity to the heterotrophic activity were used, the toxicants would be ranked as follows: phenol > quinoline > TEG. The net production measurements would predict a different ranking: TEG >

quinoline > phenol. One reason for this particular ranking of toxicants based on net production values is because net production in microcosms with level 2 of quinoline and phenol was higher than net production in level 1 (a lower concentration)(see figure 20A and 33) and this ranking is based on reduction of net production or heterotrophic activity.

A better way to rank the toxicants is based on the individual EC20 values predicted from the data for each toxicant. These values are based on the concentration of the toxicant that causes at least a 20% difference (increase or decrease) between the treatment value and the control value. If the acute EC20 values for net production were used the toxicants would be ranked as follows: phenol (EC20 = 18 mg/L) > quinoline (EC20 = 31 mg/L) > triethylene glycol (EC20 > 250 mg/L). The acute EC20 values predicted by using the heterotrophic activity parameters reverses the ranking: triethylene glycol (EC20 = 12.6 mg/L) > quinoline (EC20 = 31 mg/L) > phenol (EC20 = 50 mg/L).

If the chronic EC20 values (based on 6 weeks exposure) are compared for the three toxicants a different ranking for net production would result. The ranking would be: quinoline (EC20 = 4 mg/L) > phenol (28 mg/L) > triethylene glycol (EC20 = 35 mg/L). The chronic EC20 for heterotrophic activity would produce a

ranking of toxicity identical to the acute EC20 values: triethylene glycol (EC20 = 15.5 mg/L) > quinoline (EC20 = 29 mg/L) > phenol (EC20 = 49 mg/L).

Measurement of Ecosystem Properties Comparison

The two ecosystem properties used to study the main components of the microcosms were net oxygen production, which is a measure of autotrophic activity, and carbon dioxide production, which is a measure of heterotrophic activity. The EC20 values predicted by these two ecosystems were different and their statistical characteristics, such as variance, were different.

The production values were more variable than the heterotrophic activity values, with production values exhibiting a median coefficient of variation of 60% (range: 40% to 248%) and heterotrophic activity exhibiting a median coefficient of variation of 32% (range: 12% to 85%).

These two ecosystem properties predicted different EC20 values for the toxicants. The acute and chronic EC20 values were higher for quinoline and phenol for heterotrophic activity than for net production. The reverse was true for microcosms with triethylene glycol. For triethylene glycol microcosms the EC20 values for production were higher than the EC 20 values for heterotrophic activity. These differences yielded different toxicity rankings for the toxicants studied.

Rankings based on suppression of heterotrophic activity placed TEG as the most toxic for both acute and chronic effects. However rankings based on net production placed phenol as the most toxic for acute effects on production, and quinoline the most toxic for chronic effects.

V. DISCUSSION

A number of comparisons were made and hypotheses tested during this study. Each of the comparisons will be addressed separately in the discussion. When reference is made to comparisons with control microcosms, carrier-control microcosms (those to which acetone was added) are not considered unless otherwise stated. Lower net production values were measured in carrier-control microcosms, but did not differ from the controls insofar as heterotrophic activity was concerned.

Sediment Type Effect

Three types of sediment were compared as to their effects on net production and heterotrophic activity and their influence on the toxicity of four organic toxicants. Significantly lower production and slightly lower heterotrophic activity were measured in the microcosms with clay only than was measured in the sand or the sand/clay microcosms. Taub and Crow (1980) found the opposite to be true in their complex sediment microcosms, which contained two kinds of clay. They

found significantly greater net production and chlorophyll concentration in their complex sediment, which included other components (i.e. gravel) in addition to the two kinds of clay. The greater diversity of particle sizes and carbon sources in their complex sediment could account for the greater production observed by Taub and Crow (1980). However the clay sediment as use in the study described here differed from the other two sediments only by the amount of clay in the sediment. The sand sediment had no clay and the clay/sand sediment had 49.5% clay. The clay sediment microcosms with 99.5% kaolinite clay had a much smaller mean particle size (≈0.06 mm) than the other two sediments. A smaller particle size results in a greater surface area for absorption of nutrients and a higher cation exchange capacity. Both of these characteristics of a sediment remove nutrients from the surrounding water column. The suspicion that nutrient levels were lower in the clay sediment microcosms than in the other two types of microcosms is substantiated by the orthophosphate analyses during phase two. Orthophosphate concentration in the clay microcosms were half those in the microcosms with clay and sand. There was no analysis of phosphate made in the sand microcosms phase. Other supporting information for the nutrient depletion is that although the clay and the clay/sand microcosms were initiated with

the same alkalinity levels, lower alkalinity was measured in every microcosm with clay sediment only than the clay/sand sediment microcosms with identical treatment. The potentially lower nutrients may have resulted in a lower net production which also caused a lower heterotrophic activity. Lower production may also have been caused by light attenuation due to suspended clay particles.

The effect of sediment type was evident in the toxicant-treated microcosms also. Triethylene glycol, quinoline, and naphthoquinone caused greater deviations of production and heterotrophic activity from the controls of the same sediment type than the toxicant-treated microcosms containing sand. When an aquatic microcosm community is already stressed by reduced nutrient concentrations, the perturbation induced by a toxicant resulted in a greater deviation from the normal operating range of the controls.

Variability is another important characteristic of experimental studies that must be considered when an experimental test system is designed. The coefficient of variation (Downie and Heath, 1974) in the microcosms containing clay or the clay and sand sediment was significantly higher (CV = 60% and 63% respectively) than those containing only sand microcosms (CV = 45%). Taub and Crow (1980) also found a high variability in their

complex sediment with clay. One of the important characteristics of microcosms in research is their replicability (Giesy, 1980); the lower the variability, the more useful the microcosm system is.

With these characteristics in mind, serious consideration must be given to the selection of an easily standardized sediment type which has little or no effect on the toxicities of the test chemicals. Based on these criteria, the sand sediment as prepared would seem to be the best for use in a standardized microcosm test-system.

Phenol Effects

Phenol was added as a toxicant in Phase one of this experiment at four different levels. Phenol is a single ring compound with a hydroxyl group and is highly soluble in water (1 gram/15 mL of water).

Phenol was measured in the microcosms as the following: 10 mg/L, 18 mg/L, 108 mg/L and 501 mg/L. These values reflect the actual concentrations in the water column determined by gas chromatography. The amount of phenol added was much higher than that indicated by the chromatographic analyses, and this was the case for all four toxicants. The phenol that was added but not measured may have remained undissolved in the bottom of the microcosm or may have not been measured

accurately by the chromatographic analyses (the range of the coefficient of variation for phenol = 26%-74%). The concentration of phenol decreased by 75% after three weeks and became negligible after six weeks except in the microcosm with highest phenol concentration (reduced from 501 mg/L to 160 mg/L)

Net production was significantly affected by the addition of phenol at all four levels. Phenol concentration and net production exhibited a significant inverse relationship, except in microcosms dosed with phenol level two (18 mg/L). At phenol concentrations of 18 mg/L net production values were no different than that in the control microcosms. This apparent stimulation may have been caused by the reduction of the number of algal species due to the toxicity of phenol which would allow an increase in growth and production in tolerant species of algae. Data is not available in this study to document a possible dominant species replacement. Phenol levels three and four (108 mg/L and 501 mg/L) reduced net production to almost zero (0.6 mg/L oxygen and 0.2 mg/L oxygen respectively) throughout the experimental period. A portion of the decrease in net production may have been due to light attenuation occurring in the microcosms with high levels of phenol because of the property of phenol to turn dark brown when exposed to light.

Net production values deviated more than twenty

percent from the control's net production values throughout the experimental period in microcosms with the three highest levels of phenol. The acute EC20 value for phenol's toxicity to net production in the microcosms was 18.4 mg/L and the chronic EC20 value was 28.0 mg/L. The toxicity was greatest immediately after addition of phenol. The decrease in phenol concentration with time and the frequent reinoculation of the biotic community would explain the difference in acute and chronic toxicity, but the chronic toxicity was lower than would be expected if recovery had occurred.

Phenol is used as a bactericide and would be expected to significantly decrease heterotrophic activity. As increased levels of phenol were added, heterotrophic activity decreased, but as phenol concentration in the water column decreased, the heterotrophic activity recovered in microcosms dosed at levels 1 and 2 (10 mg/L and 18 mg/L respectively). Heterotrophic activity in microcosms with the highest levels of phenol addition (108 mg/L and 501 mg/L) decreased to near zero and remained low throughout the experimental time. When the heterotrophic activity in the two lower levels of phenol recovered by week four it recovered to levels significantly higher than the control microcosms. Heterotrophic activity in microcosms with the highest levels of phenol was a hundred times lower

than the controls microcosms heterotrophic activity. The acute EC20 value for phenol's toxicity as predicted by the heterotrophic portion of the community was 48.5 mg/L and the chronic toxicity EC20 was 50 mg/L which were not significantly different. The higher values of the EC20 for heterotrophic activity than the EC20 values for net production may be due to the greater capacity of the heterotrophic community (mainly bacteria) to acclimate and adapt to new carbon sources and new environments.

The algal populations and the grazer populations either decreased significantly or disappeared totally in the microcosms dosed with the three highest levels of phenol. Microcosms dosed at levels three and four (108 mg/L and 501 mg/L) contained no crustaceans and no algae. One-half of the microcosms dosed with 18 mg/L of phenol had no crustaceans and algae, while those dosed with 10 mg/L contained both algae and crustaceans. These changes in the aquatic community were not surprising in view of the observed ecosystem level properties (production and heterotrophy).

Giddings (1982) found similar effects caused by the addition of a crude-oil liquefaction product (98% phenols in the water-soluble fraction) to large aquatic microcosms. The dissolved oxygen measurements changed in such a way as to indicate a negative net primary production. Giddings (1982) stated that respiration

followed a similar pattern, but the decrease in respiration was less severe.

In summary, phenol is a significant toxic substance affecting both the autotrophic and the heterotrophic community. Concentrations greater than 18 mg/L significantly alter the ecosystem health. Effluents with phenol should have permit standards set at the concentration that does not cause observable ecosystem level effects.

Triethylene glycol effects

Triethylene glycol (TEG) was added as a toxicant in phase one and two of this experiment. The actual amount of TEG added was much higher, than those measured by gas chromatographic analysis as was previously described with phenol addition. The measured concentration of TEG had decreased by 30 to 60 percent of the original concentration by the sixth week. The gas chromatograph analyses of triethylene glycol was highly variable due to the chemical's highly polar nature. The potential degradation of triethylene glycol may have contributed to the low concentrations measured. Aliphatic compounds such as triethylene glycol can be used as a carbon source and degradation as mentioned above may occur (Alexander, 1981). If TEG were degraded microbiologically, a

potential breakdown product could be oxalic acid (Windholz, 1983). The presence of oxalic acid would account for the decrease in pH observed by Taub and Read (1982) and Leffler (1981). The pH decrease by as much as 3.2 units, was observed in this study in microcosms containing TEG. The pH decrease was measurable the fourth woek after TEG was added and by the sixth week, the pH was as low as 4.Ø in microcosms dosed with TEG level 3.

The carbon dioxide concentration in microcosms was dependent on alkalinity and diurnal pH fluctuations. The alkalinity was low in microcosms (8 mg/L and 1Ø mg/L in some) with TEG added which caused the pool of inorganic carbon to be low. In the carbonate equilibrium system CO_2 is used by the producers and the other forms of inorganic carbon (HCO_3^-) and $CO_3^=$) are available to form more CO_2). In these microcosms the pH decreased to as low as 4.Ø and would have shifted all the HCO_3^- and $CO_3^=$ to CO_2. The water was then supersaturated with CO_2 and lost CO_2 to the atmosphere. The algae eventually depleted the CO_2 and there was no HCO_3^- or $CO_3^=$ to shift to CO_2. Each time this occurred those organisms such as blue green algae (Moss, 1972 and 1973 refers to them as eutrophic species) which do not require high levels of carbon dioxide, have a competitive advantage. Fraleigh and Dibert (1982) found that low inorganic carbon does not

change the standing crop of algae but does alter the successional pattern in aquatic systems.

The low pH and the low alkalinity found in those microcosms dosed with triethylene glycol could have resulted in a condition in which inorganic carbon was limiting and therefore carbon dioxide concentration would have been limited. The observation of blue green algae in these microcosms support the possibility of carbon dioxide limitation having changed the successional pattern of the microcosms.

Triethylene glycol had a significant effect on net production in the microcosms. Lower mean net production pooled over the experimental period was found in all microcosms dosed with TEG. No linear correlation was found between TEG concentration and decrease in net production. In fact lower net production was measured in microcosms dosed with level 1 and 3 (10 and 30 mg/L) than measured in levels 2 and 4 (17 and 250 mg/L). However, net production values began to decrease in all microcosms with TEG four weeks after the addition of the chemical. This decrease could be attributed to the corresponding decrease in the minimum pH in these microcosms, as discussed earlier. The pH in some microcosms went down to 4.0, which decreased primary production, causing CO_2 depletion and decreasing the population of some algal species.

The acute EC20 value could not be calculated for TEG's effect on production; however, the chronic EC20 was 35 mg/L. Another EC20 value was calculated for the last week of the experimental period only (sixth week) and was 26 mg/L, which is lower than the chronic EC20 value and, is further evidence of the increased toxic effect of TEG after the fourth week following addition.

Heterotrophic activity significantly lower than that in the control microcosms was measured in microcosms containing the three highest levels of TEG (17, 30 and 250 mg/L. Heterotrophic activity in the microcosms with 10 mg/L TEG was not significantly different than the heterotrophic activity in the control microcosms. In week four and six, a greater decrease in heterotrophic activity was measured and the percent deviation from the controls was greater than the measurements that were made during week 1 and 2, which, again, could be explained by the decrease in pH in these microcosms at week four.

The acute EC20 predicted for heterotrophic activity for TEG was 12.6 mg/L, and the chronic EC20 was 15.5 mg/L. These EC20 values were much lower than those for net production which indicates that the heterotrophic community was more sensitive to TEG than the autotrophic community. However, the heterotrophic activity did not decrease significantly as would have been expected if the pH decrease then had caused it.

The composition of the algal populations in the microcosms with TEG were different than that in the control microcosms, as previously mentioned. Elimination of the grazing crustaceans (cladocera, copepods and amphipods) was also observed. Taub and Read (1982) recorded similar effects of TEG as the "elimination of small algal species and Daphnia. "

Triethylene glycol significantly depressed both the autotrophic and the heterotrophic communities in the microcosms. It was more toxic to the heterotrophic community, as was evidenced by the lower chronic EC20 value. An additional effect of TEG was a shift in algal species dominance to blue green algae. Triethylene glycol also eliminated all crustaceans except the detritivore ostracods.

In summary TEG was toxic to the heterotrophic community at concentrations greater than 12 mg/L and threshhold limits should be set to regulate TEG in effluents.

Quinoline Effects

Quinoline concentration in the microcosms containing sand increased with time instead of decreasing as it did in the other microcosms. One explanation for this anomaly may be that the quinoline was added in quantities

greater than was measured in the chromatographic analyses. This additional quantity of quinoline may have resulted in undissolved quinoline to remain in the microcosms with the sand sediment and continue to dissolve into the water column. The microcosms with the other sediments were not dosed with quinoline at the highest dose as the microcosms with sand sediment were and did not exhibit this anomaly.

Net production was significantly lower in all microcosms with quinoline than the control microcosms and this reduced net production was significantly correlated with increased quinoline concentration. However, net production was higher in microcosms containing quinoline level 2 (14-25 mg/L) than level 1 (10 mg/L). The apparent stimulation of production by low levels of a toxicant was observed also in the microcosms containing phenol, as was discussed earlier. An explanation for this occurrence could be the reduction in the number of competing species of primary producers, thus allowing a species tolerant of quinoline or a species that can utilize quinoline as a carbon source to increase its growth potential. Net production values were greater in all microcosms containing quinoline than 20 percent deviant from the control microcosms the first week after the quinoline was added. However, by the third week net production began to increase to levels nearer to the

control microcosms' net production. Microcosms containing quinoline level three began to recover by the fifth week, but net production was near zero in microcosms at level four and deviated more than 90 percent from the controls throughout the study.

The acute EC20 for quinoline, as can be predicted by net production values, was 32 mg/L, and the chronic EC20 was 21 mg/L. These EC20 values appear to contradict the apparent recovery of net production mentioned above. One would expect the chronic EC20 to be higher than the acute EC20. One explanation of this contradiction could be that a lower correlation coefficient ($r = 0.54$) represented the least squares calculation of the chronic EC20 than the correlation coefficient for the acute EC20 ($r = 0.996$), which indicates that the acute EC20 value was likely more valid than the chronic EC20 value.

Leffler (1981) reported acute EC20 values determined for single species bioassays of quinoline to be 25 mg/L when Selenastrum capricornutum was the test organism and 117 mg/L when Microcystis aeruginosa was used. He did not see any significant effect on net production in microcosms when quinoline was added at levels equal to or less than 10 mg/L. His data does not contradict with the results of this research.

Quinoline effects on heterotrophic activity were so dramatic that there was little doubt that it was toxic.

Heterotrophic activity in the microcosms decreased significantly as quinoline concentration increased (r = .83 to .99). Heterotrophic activity in the microcosms dosed with the highest level of quinoline was 70 times less than that in control microcosms. Lower heterotrophic activity was observed in the microcosms dosed with 10 mg/L (level one) than the control microcosms but the reduction was not significant. Heterotrophic activity values deviated more than 20 percent from the control microcosms in all microcosms with quinoline. However in microcosms containing quinoline at levels one, two and three, recovery of heterotrophic activity to levels nearer that in the controls was observed by the fourth week after the toxicant was added. In fact microcosms with quinoline added at level three had heterotrophic activity almost 100% greater than the controls. Microcosms at level four never recovered, and heterotrophic activity remained 98-99% deviant from that in the controls throughout the experimental period.

The acute EC20 values for quinoline, as was predicted by heterotrophic activity, was 36 mg/L and the chronic EC20 was 30 mg/L. Again the acute EC20 value was higher than the chronic EC20 value for quinoline. The chronic EC20 value would have been higher if the deviation from controls caused by the stimulation of

heterotrophic activity had not been included. The term 'toxic' would not be an accurate description when effects due to stimulation are included. These values are not significantly different from each other, because their confidence limits overlap.

Mean alkalinity was significantly correlated with quinoline concentration. As quinoline increased the alkalinity increased to concentrations as high as 6.4 mequiv/L, six times greater than the original alkalinity (1-1.2 mequiv/L). The increased alkalinity in the microcosm may be explained by the possible degradation of quinoline by breaking the ring with nitrogen and/or by the formation of ammonia. Either of these processes could increase the alkalinity by increasing the proton capturing ability of the microcosms with quinoline. Quinoline was measured as 206 mg/L which would if degraded, account for 1.6 mequiv/L addition of ammonia to the alkalinity. However, quinoline was added at a higher level than the chromatographic analyses indicated, and could potentially provide as much as 77 mequiv/L of alkalinity. A corollary chemical indicator would be the mean pH in the quinoline containing microcosms. However, there was no significant difference in the maximum or minimum pH in the microcosm containing quinoline with high alkalinity and the control microcosms with lower alkalinity. The aquatic microcosms with high alkalinity

appear to have been well buffered during the diurnal cycle. Quinoline not only affected the biotic part of the microcosm but it also affected the abiotic part of the microcosm by changing the alkalinity.

Quinoline would be toxic to aquatic ecosystems if ammonia formation is taking place. On the other hand the high alkalinity associated with quinoline addition may buffer the aquatic system and protect it from dramatic pH shifts.

Quinoline appeared to eliminate all crustaceans when dosed at levels 2 (14-20 mg/L), 3 (33-88 mg/L) and 4 (206 mg/L) and to reduce crustaceans numbers in microcosms dosed at level 1 (10 mg/L). Microcosms with quinoline added exhibited a decrease in both the attached and planktonic algal populations. A portion of the toxicity to algae may have been caused by the light attenuation in microcosms containing high levels of quinoline. Quinoline, like phenol, turns dark brown when exposed to light.

Quinoline was toxic to both the autotrophic community, as measured by net production, and the heterotrophic community. The acute and chronic EC20's were not much different from each other, so each community was affected at similar concentrations of quinoline. Quinoline did appear to cause an increase in alkalinity. This increase may be toxic if it is caused

by ammonia formation. Quinoline appeared to alter the components of the aquatic microcosm by eliminating grazers, reducing production and heterotrophic activity. Threshhold limits for effluents should be less than 21 mg/L.

Naphthoquinone Effects

Naphthoquinone was added at much higher levels, but because it is sparingly soluble, the measured concentration was quite low. The experimental design called for naphthoquinone to be added at levels lower than the other toxicants but not as low as the actual concentrations measured.

Significantly lower net production was measured in microcosms with naphthoquinone compared to controls, especially the first week after addition of naphthoquinone. The values for net production in the microcosms with naphthoquinone added were not significantly lower than those in the acetone control microcosms. There was no correlation between net production values and naphthoquinone concentrations.

The percent deviations in production from the control microcosms fluctuated around 20% but net production seldom deviated more than 20% for very long.

Based on these results it seems reasonable to

conclude that naphthoquinone is toxic to the autotrophic community, but at the low actual concentrations used in this research the effects were too variable to be used in making predictions. Therefore, no EC20 values can be calculated from the net production data.

Naphthoquinone effects on the heterotrophic community in the microcosms were mixed. The lower levels of naphthoquinone (0.01 and 0.015-0.20 mg/L) apparently stimulated heterotrophic activity to levels greater than that in the control microcosms. However, heterotrophic activity was less in microcosms with the highest concentrations of naphthoquinone (0.02-0.03 and 0.04 mg/L) than the controls. Heterotrophic activity was significantly correlated with naphthoquinone concentrations and microcosms at all four concentrations when pooled over weeks and sediment type deviated more than 20% from the controls. The deviation from the controls exhibited by the microcosms containing naphthoquinone was greatest the first two weeks after naphthoquinone was added.

Naphthoquinone is toxic to the heterotrophic community, but at these low concentrations the extent of the effect is difficult to predict and the correlation is so slight that is was impossible to calculate EC20 values for heterotrophic activity.

There were no observable effects of naphthoquinone

on the algal or grazer populations in the microcosms.

Although naphthoquinone temporarily depressed net production and heterotrophic activity, the systems quickly recovered. Naphthoquinone was added at levels that resulted in very low concentrations in the water column and the ecosystem measurements made provided inconclusive results regarding naphthoquinone toxicity.

Alkalinity, pH and Carbon Limitation Effects

The potential for carbon dioxide or inorganic carbon limitation in microcosms is dependent on alkalinity and diurnal pH fluctuations. In static microcosms with potentially high net production, carbon dioxide limitation may occur when the pH goes up to 9 or 10 at the end of the day.

The low pH and the low alkalinity found in those microcosms with triethylene glycol added could have resulted in a condition in which inorganic carbon was limiting and therefore carbon dioxide concentration would have been limited. The observation of blue green algae in these microcosms support the possibility of inorganic carbon limitation having changed the successional pattern of the microcosms.

Microcosms with quinoline added had unusually high levels of alkalinity. The significant increase (to

levels around 77 mequire/L) in alkalinity due to quinoline addition may be due to degradation of quinoline or a change in the solubility of inorganic carbon caused by the solution of quinoline into water.

Triethylene glycol and quinoline not only affect the biotic part of an aquatic ecosystem but they also affect the abiotic part of the ecosystem by changing the alkalinity and the pH. These alterations could have far reaching effects on a aquatic ecosystem by influencing successional patterns in aquatic ecosystems.

Statistical Analyses

Two types of statistics that are most commonly used for comparing and describing data are 1) central tendency (e.g. mean and median) and 2) dispersion (e.g. standard deviation and interquartile range). These four statistics mentioned are parametric (mean and standard deviation) and nonparametric (median and interquartile range). These statistics were generated in comparisons of both raw data (maximum dissolved oxygen concentration) and calculated data (net production). The comparison of nonparametric and parametric statistics indicated differences in predicting toxicities of chemicals. The median was always greater than the mean, causing skewed results. When the statistics (i.e. mean and median) are

different, the conclusions based on statistical tests were also different.

The use of parametric statistics such as mean, standard deviation, t tests and F tests requires that certain assumptions regarding the data be met. The assumptions in parametric statistical analyses are: 1) the assumed model is correct, 2) the individual observations are independent of each other, 3) the data values recorded are continuous variables, 4) the variances of two tested populations are homogeneous, and 5) the data are normally distributed (Sokal and Rohlf, 1969)

Data do not have to be normally distributed if one chooses to use nonparametric statistical procedures. Neither do the variances need to be homogeneous for most nonparametric procedures (Hollander and Wolfe, 1973). When homogeneity of variances is not a requirement, uneven samples sizes are not as much of a problem in statistical analyses (Sokal and Rohlf, 1969). When nonparametric and parametric statistical procedures are compared using data that are not normally distributed, the nonparametric procedure has a much higher predictive efficiency and accuracy.

The data derived from this study were not normally distributed according to the Kolmogorov-Smirnof goodness-of-fit test (Sokal and Rohlf, 1969; and Ray,

1982), as was evidenced by the right skew of the data to values greater than the mean and the positive kurtosis, which indicated a sharp, peaked distribution.

The assumption of homogeneity of variances was also not met by the data. In a majority of the statistical comparisons between means of the experimental data, the variances were significantly different. One of the reasons was that the sample sizes were not the same, a fact that was inherent to the experimental design.

These data collected during this study of aquatic microcosms were not normally distributed, which likely is characteristic of most ecological data. Therefore nonparametric statistics should be used more often for analyzing data of this type. In addition, confidence limits other than the 95% confidence interval should be considered. Statisticians traditionally have used the 95% level (Sokal and Rohlf, 1969) almost exclusively in the past but this tradition poses problems in the testing and regulation of toxic chemicals. A more conservative approach should be taken when protection of the aquatic ecosystem is at stake. The term "conservative" in this context is used differently than statisticians use it (Sokal and Rohlf, 1969). A "conservative approach" to toxic-substance screening would mean restricting the use of all chemicals that are toxic to the environment. Invariably the use of some chemicals that may not be

toxic to the environment will be restricted as well.

To the statistician, the use of some confidence limit less than 95% would mean decreasing the Beta (β) error (or power) while increasing the alpha (α) error (Sokal and Rohlf, 1969). In other words, the null hypothesis (Ho) that there is no effect of some chemical on the ecosystem would be rejected more often if the lower confidence bounds were to be used, and effects would be demonstrated when there were no actual effects. If 95% confidence limits were used, in analysis of data collected during the study, the Ho that there were no measureable effects on the ecosystem would have been rejected less often and therefore, toxic effects would not have been indicated when there actually were toxic effects. Thus the use of 80% confidence bounds would result in restriction of more chemicals than use of the 95% confidence bounds. The 80% limits are still within the range that the statisticians call "significant" but barely so (Sokal and Rohlf, 1969). If the 80% level were adopted, more chemicals would be restricted until more detailed tests could be made.

Comparison of Other LC50 and EC20 Data

Phenol. The EC20 concentrations predicted from this study for acute effects (7 days) of phenol on production

was 18 mg/L and for the chronic effects (6 weeks) was 28 mg/L. The EC20 for both the acute and chronic effect on heterotrophic activity was 49-50 mg/L. The acute EC20 for an algal bioassay with Selenastrum capricornutum was between 7.7 mg/L and 31 mg/L in studies by Leffler (1981). Milleman et al. (1984) reported an EC50 for this species as 287 mg/L phenol, and an EC50 for a diatom species (Nitzchia palea) of 185 mg/L. The acute LC50 for Daphnia magna was 19.8 mg/L, and the LC50 for the amphipod Gammarus minus 37.4 mg/L according to Milleman et al. (1984). They found Gambusia sp. and Pimephales promelas (mosquito fish and fathead minnow) to have LC50 values of 11 and 25.6 mg/L, respectively. The acute LC50 values for Daphnia magna as cited in Buikema et al.(1979) were 9.6-31 mg/L phenol. Chronic toxicity tests for phenol were made using Daphnia longispina and effects of fecundity were found at 5 mg/L (as cited in Buikema et al., 1979). The fathead minnow was tested for chronic effects due to phenol and an LC50 value of 1.8-3.6 mg/L was predicted (EPA, 1983)(Table 2).

Triethylene glycol. The predicted acute EC20 for the effects of triethylene glycol on net production was greater than 250 mg/L (the highest level added in this study); however, the chronic EC20 was 35 mg/L. The dramatic change in the EC20 with time may have been due to the significant alkalinity and pH decline apparently

Table 2. Minimum threshold toxicity values (EC20, LC50 and LD50) for the four toxicants tested.

Type of Criterion	Phenol (mg/L)	TEG (mg/L)	Quin (mg/L)	Naphth (mg/L)
EC20-Acute Prod.	18	>250	32	>0.037
EC20-Chronic Prod.	28	35	21	"
EC20-Acute Heter.	52	12.6	35	"
EC20-Chronic Heter.	45	15.5	29	"
EC20-Net Production	--	500(1)	>10(1)	2.8-7.3(1)
EC20-Algae Single(A)	7.7(1)	--	25(1)	0.222(1)
	31.0(1)	--	117(1)	0.025(1)
EC50-Algae Single(A)	287(3)	--	202(3)	--
	185(3)	--	104(3)	--
LC50-Daphnia(A)	19.8(2)	--	34.5(2)	0.037(1)
LC50-Daphnia(A)	9.6-31(4)	--	--	--
LC50-Daphnia(C)	5.0(4)	--	--	--
LC50-Amphipod(A)	37.4(2)	--	40.9(2)	--
LC50-Mosquito Fish(A)	11(1)	--	--	--
LC50-Fathead Minnow(A)	25.6(2)	--	0.44(2)	--
LC50-Fathead Minnow(C)	1.8-3.6(5)	--	--	--

Note: (A) = Acute
(C) = Chronic
TEG = Triethylene glycol
Quin = Quinoline
Naphth = Naphthoquinone

(1) Leffler (1982)
(2) Millemann et al. (1984)
(3) Giddings and Washington (1983)
(4) Buikema et al. (1979)
(5) EPA (1983)

caused by triethylene glycol decomposition. The EC20 calculated for only the sixth week after addition of triethylene glycol was even lower (26 mg/L). The heterotrophic activity of the triethylene glycol microcosms predicted even lower EC20 values with an acute EC20 of 12.6 mg/L and a chronic EC20 of 15.5 mg/L. Triethylene glycol's toxicity has been studied little. However, Leffler (1981) found that 500 mg/L caused a significant decrease in production, respiration and pH in aquatic microcosms, and Taub and Read (1982) found that triethylene glycol induced acidic conditions after four weeks and eliminated some algal species and Daphnia.

Quinoline. Quinoline was found to have an acute EC20 value predicted for the net production of 32 mg/L, but a lower EC20 value (24 mg/L) for chronic exposure. The EC20 values for heterotrophic activity were slightly higher than those for production. The acute EC20 predicted for net production in microcosms with quinoline was 35 mg/L and 29 mg/L for the chronic EC20.

Quinoline was also tested by Leffler (1981) who found the acute EC20 value for production and respiration to be greater than 10 mg/L, which was the highest level he evaluated. Leffler (1981) did predict the acute EC20 value of pH at 5.6 mg/L. Leffler (1981) reported results of bioassays using quinoline as an acute EC20 for Selenastrum capricornutum to be 25 mg/L and 117 mg/L for

another algal species, Microcystis aeruginosa. Milleman et al. (1984) reported on EC50 value for Selenastrum capricornutum as 202 mg/L and for the diatom, Nitzchia palea, an EC50 value of 104 mg/L. They reported the acute LC50 for Daphnia magna was 34.5 mg/L, and for Gammarus minus was 40.9 mg/L.

Naphthoquinone. The analysis of both production and heterotrophic activity data predicted acute and chronic EC20 values greater than the highest concentration actually added to the microcosms during this study (0.04 mg/L). Therefore, to avoid inaccurate extrapolation, the acute and chronic EC20 values for naphthoquinone were not calculated. Leffler (1981) suggested an acute EC20 for production to be 2.8 to 7.3 mg/L and 3.5 to 15.7 mg/L for respiration based on the amount of naphthoquinone he initially added but he did not measure the actual concentration. Leffler (1981) reported the alga Selenastrum capricornutum bioassay indicated an acute EC20 of 0.022 mg/L and the alga Microcystis aeruginosa predicted an EC20 of 0.025 mg/L. The acute LC50 for Daphnia magna was found to be 0.037 mg/L (Leffler, 1981) (Table 2).

Toxicity Rankings

The relative ranking of toxicants based on their

toxicity in relation to some mildly toxic substance (bench mark chemical) is an important first step in a multitiered approach similar to that suggested by Cairns (1981, 1983). The toxicants that are the most toxic should be tested first and in more detail, but the least toxic ones may not need further testing. Another possible approach which is probably favored by most regulatory agencies, is to restrict the release or manufacture of the most toxic chemicals and require further testing of the least toxic ones.

Two important questions for both government regulatory agencies and industries to consider are what test system should be used for the ranking chemicals, and what parameter should the relative rankings be based on. In this research three-relative toxicity rankings were predicted on the basis of the EC20. In each instance the ranking was different, and depended on which parameter was selected and whether acute or chronic effects were considered. If the ranking was based on acute effects on net production phenol was the most toxic. Quinoline and TEG were next, in that order. Toxicity ranking based on the long-term effects on net production (chronic) placed quinoline as the most toxic, phenol as the second most toxic, and TEG as the least toxic. If the parameter for heterotrophic activity was the basis for the ranking, regardless of whether acute or chronic EC20 values are

used, triethylene glycol is the most toxic, quinoline is the second most toxic and phenol is the least toxic. Recall that naphthoquinone was not ranked because there was no correlation between the measured changes in the parameters and the naphthoquinone concentration.

The single species bioassay data and microcosm data of Leffler's (1981) would rank naphthoquinone the most toxic because of its low EC20 and LC50 values. The next most toxic would be phenol and the least toxic of these three chemicals would be quinoline. Although no single species bioassay data for triethylene glycol was available, the assumption could be made that is would be the least toxic because in single species bioassays it is used most commonly as a solvent in these tests of organic chemicals (Taub and Read, 1982).

Rankings of these chemicals toxicity to humans, based on the LD50 test on rats, place quinoline as the most toxic, phenol as the second most toxic and TEG as the least toxic (Windholz, 1983).

The variability of the ranking procedures used in aquatic toxicology indicates that toxicants should be ranked in more than one way and perhaps by more than one test system. The lowest EC20 or LC50 predicted by any of the ecosystem measurements or single species bioassays, should become the criterion for setting the standard for that chemical. Further toxicity testing would follow,

and the criterion and, therefore, the standard, may or may not change.

VI. SUMMARY AND CONCLUSIONS

The purpose of this research was to develop a test system for screening toxic substances by predicting their aquatic ecosystem effects. The system tested was a static, one liter microcosm with a diverse species assemblage. The microcosm was composed of biotic inoculum, chemically defined medium and sediment. The biotic inoculum was from a 38 L stock microcosm. The stock microcosm was originally inoculated from many different aquatic ecosystems and contained primary producers, grazers, carnivores and decomposers. The medium was developed by Taub and Read (1982) to allow growth of algae and crustaceans. Three different types of sediment: sand, clay and clay plus sand were compared according to their effect on predictions of chemical toxicities.

The toxicity of four organic chemicals: phenol, triethylene glycol (TEG), quinoline and naphthoquinone were evaluated with this test system. The toxicities of the TEG, quinoline and naphthoquinone were compared for each sediment type. Phenol was added only to the microcosms containing sand alone. Toxicity was evaluated in terms of the chemicals' effects on primary productivity and heterotrophic activity, though other

observations were made.

The toxicities of the chemicals demonstrated in this study were compared to those from other toxicological evaluations. The toxicity of a chemical was measured as the concentration of the chemical which caused a twenty percent or greater deviation from the control microcosm for an ecosystem level measurement such as net production (EC20). The other toxicological evaluation systems compared, included one-liter microcosms with no sediment (Leffler, 1981) and single-species bioassays using species such as Daphnia magna (Giddings, 1984).

The test systems were compared on the basis of their response to the chemicals. The EC20 predictions provide the basis for ranking three of the chemicals according to their relative toxicities. Naphthoquinone was omitted from the ranking because an EC20 could not be calculated because there was no dose response correlation.

Nonparametric and parametric statistical analyses of a portion of the data were compared. Confidence intervals of 95% and 80% were compared according to their effect on the significant differences between controls and the treatments.

The hypotheses tested in this research were:

(i) The three screening systems compared do not demonstrate significantly different toxicity ranking for the same potentially toxic substances;

therefore, there is no need for site and/or region-specific screening tests.

(ii) The three, screening systems compared do not demonstrate significantly different toxic concentrations (EC20 or LC50) for the same potentially, toxic substances. Therefore, a generalized screening system could be used for determining criteria for standards of organic chemicals.

(iii) The toxicity rankings and threshold toxicity concentrations (EC20 or LC50) for the same potentially toxic chemicals are not significantly different when the sediment type in an aquatic microcosm differs.

The conclusions from testing these hypotheses are:

1. The three screening systems could not be compared based on their rankings of the chemicals. Because naphthoquinone addition did not exhibit a dose response correlation, no EC20 could be predicted. Naphthoquinone had to be omitted from the rankings of chemical toxicity, and, as a result, only two chemicals could be compared among the three systems.

2. The screening systems did not demonstrate significantly different toxic concentrations (EC20, EC50 and LC50) for the same chemicals; therefore, a

generalized screening system would give similar predictions for threshhold concentrations of chemicals. The threshhold values (EC2Ø, EC5Ø and LC5Ø) were not identical, but their 95% confidence intervals overlapped. Therefore they were not significantly different.

3. The toxicity ranking of concentrations (EC2Ø) for the chemicals was the same regardless of sediment type. However, the threshhold toxicity concentrations (EC2Ø) were different when the sediment type differed. The toxicity of the chemicals was greater in the microcosms with the clay sediment than in those containing the other two sediments. The clay sediment may have acted as a nutrient sink or may have reduced light levels because of the suspension of clay particles in the water column, either of which could have caused decreased primary production.

The screening system evaluated during this research is a valuable tool that could be included in a multitiered approach to the testing of toxic substances (Cairns,1982). The information derived from this test system could be used to rank chemicals according to their toxicity and in relation to another chemical of known toxicity. Such information would be useful in decision-making by industry and the government, for example, if the chemical tested is more toxic than the chemical of known toxicity the release of the toxic

chemical would be restricted.

This test system would not be as useful for developing water quality criteria however. In the multitiered approach, a multispecies, microcosm test-system would be used in combination with single-species bioassays of target organisms such as Rainbow Trout. The combination of bioassays and test systems, such as the one tested here, would provide predictions of ecosystem health effects.

VII. LITERATURE CITED

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Appendix 1. Gas chromatographic analyses of the organic chemicals, used these characteristics with a Supelco SE 30 Chromatographic Column.

Organic Chemical	Injection Temperature	Oven Temperature
phenol	100 C	100 C
quinoline	150 C	150 C
naphthoquinone	150 C	150 C
triethylene glycol	150 C	175 C

Appendix 2. Community Composition Observations on microcosms, from the 4th week of Phase I (3-4 replicate microcosms for each treatment).

Phase I

Treatment	Level	algae		Daphnia	Ostracods	Other
		all	att&pl	all	all	amphipods
controls	-	4/4	*	3/4	3/4	
phenol	1	4/4		1/2	2/4	
	2	4/4		none	1/4	
	3	1/4		Ø/4	Ø/4	
	4	Ø/4		Ø/4	Ø/4	
TEG	1	4/4		3/4	3/4	
	2	4/4		1/4	1/4	
	3	4/4	(1/2=BG)	Ø/4	3/4	
	4	4/4	(3/4=BG)	1/4	3/4	
Quinoline	1	4/4		3/4	2/4	
	2	4/4		Ø/4	Ø/4	
	3	Ø/4		Ø/4	Ø/4	
	4	Ø/4		Ø/4	Ø/4	
Naphtho-	1	4/4		4/4	3/4	
quinone	2	4/4		4/4	2/4	
	3	4/4		4/4	3/4	
	4	4/4		3/4	2/4	

note: att. = attached algae
pl. = planktonic algae
BG = blue green algae

* 4/4 indicates presence in four out of four microcosms.

Appendix 3. Community Composition observations on microcosms from the 4th week after toxicant added in Phase II.

Phase II

Treatment	Level	Algae	Daphnia	Ostracods
Controls	-	6/6 pl	4/6	6/6
TEG-clay	1	3/3	3/3	2/3
	2	3/3	3/3	1/3
	3	3/3 BG	Ø/3	1/3
TEG-clay/ sand	1	3/3	3/3	2/3
	2	3/3	3/3	1/3
	3	3/3 BG	Ø/3	1/3
Quinoline- clay	1	3/3	2/3	3/3
	2	2/3	2/3	3/3
	3	Ø/3	Ø/3	Ø/3
Quinoline- clay/sand	1	3/3	2/3	3/3
	2	2/3	2/3	3/3
	3	Ø/3	Ø/3	Ø/3
Naphtho- quinone- clay	1	3/3	3/3	3/3
	2	3/3	3/3	3/3
	3	3/3	3/3	3/3
Naphtho- quinone- clay/sand	1	3/3	3/3	3/3
	2	3/3	3/3	3/3
	3	3/3	3/3	3/3

Note: pl = plankton
BG = bluegreen algae
ratio 2/3 means two out of three microcosms.

Appendix 4. Comparison of Environmental Conditions for the test system studied in this research, Leffler's (1982) and Taub and Read's (1982).

	Thomas	Leffler (1982)	Taub & Read (1982)
light	12h-12h	12h-12h	12h-12h
temperature	20+0.5 C	20 + 3 C	20 + 3 C
size	1 L	1 L	3 L
sediment	yes ?	no	yes
sediment type	sand	-	sand
biotic inoculum	derived	derived	gnotobiotic
chemical medium	Taub #82	Taub #36	Taub #81 or 82
Pretest time	6 weeks	6 weeks	1 week

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