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DETERMINATION OF THE ENVIRONMENTAL IMPACT OF SEVERAL SUBSTITUTE CHEMICALS IN AGRICULTURALLY AFFECTED WETLANDS

by

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Procedures have been developed for processing of anaerobic wetland sediments for pesticide recovery along with formulation of simulation models of anaerobic/aerobic soil and sediment environments to study pesticide degradation. Redox conditions of soils and sediment-water systems have a significant effect on in situ persistence of synthetic organic pesticides. Chemical and microbiological characteristics of wetland sediments have equally important consequences on mobility and degradation of toxic compounds.

The total invertebrate community of selected backswamp regions has been examined as affected by Guthion and other pesticides. Effect of xenobiotics on leaf litter decomposition in pesticide-supplemented field plots has been examined.

A system of continuous-flow and static microcosm systems have been developed for quantitative analyses of the effect of selected toxic substances, including Guthion, methyl parathion, and Kepone. Decomposition of ecologically-significant substrates such as chitin is variously affected by different toxic substances as shown in microcosm investigations. Enzymatic tests, i.e., dehydrogenase and phosphatase, and ATP measurements, are sensitive indicators of biotransformation processes. Significant correlations are seen with microbial diversity indices and specific microbial groups such as filamentous fungi. Factorial analyses of physicochemical and microbial processes and xenobiotic interaction have demonstrated the application of the microcosm as a protocol or "tool" to simulate pristine and impacted in situ ecosystems.

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants required that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze (ERL,GB), contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environment.

Many agricultural chemicals are applied in close proximity to sensitive wetland regions. This report describes field and laboratory studies of the fate and behavior of selected xenobiotics in the coastal wetlands of Louisiana. Data from these investigations are used by the U.S. Environmental Protection Agency to develop strategies that will minimize the harmful impact of toxic substances on the environment.

Henry F. Enos
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ABSTRACT

This research program was developed with the overall objective of examining toxic substances, especially organophosphorus compounds, in wetland regions and ascertaining fate and effect in situ and in controlled laboratory microcosm systems. Major attention has been given to azinphosmethyl (Guthion), including effect of abiotic and biotic factors on compound stability and behavior under diverse pH and oxidation-reduction conditions as affected by the microbial biomass.

Procedures have been developed for processing of anaerobic wetland sediments for pesticide recovery along with formulation of simulation models of anaerobic/aerobic soil and sediment environments to study pesticide degradation. Redox conditions of soils and sediment-water systems have a significant effect on in situ persistence of synthetic organic pesticides. Analyses indicate that chemical and microbiological characteristics of wetland sediments have equally important consequences on mobility and degradation of toxic compounds.

The total invertebrate community and leaf litter decomposition of selected backswamp regions has been examined as affected by Guthion and other pesticides. Ash-tupelo litter decomposition was not adversely affected by Guthion. Dissimilar responses of the complex invertebrate biota and its individual taxa to Guthion and other toxic substances demonstrates the importance of a total community analysis in terms of xenobiotic impact.

Systems of continuous-flow and static microcosm systems have been developed for quantitative analyses of the effect of selected toxic substances, including Guthion, methyl parathion, and Kepone®. Laboratory/field protocol have included formulations of microbial/enzymatic protocols to analyze xenobiotic effect. A data analysis program has been developed to demonstrate significant correlations between in situ observations and microcosm-generated information. Decomposition of ecologically significant substrates, such as chitin, is variously affected by different toxic substances as shown in microcosm investigations. Enzymatic tests, i.e., dehydrogenase and phosphatase, and ATP measurements, are sensitive indicators of biotransformation processes. Significant correlations are seen with microbial diversity indices and specific microbial groups, such as filamentous fungi. Factorial analyses of physicochemical and microbial processes and xenobiotic interaction have demonstrated the application of the microcosm as a protocol or "tool" to simulate pristine and impacted in situ ecosystems.

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SECTION 1

INTRODUCTION

Increasing emphasis on use of a variety of chemicals in agricultural practices presents potential environmental and real problems in terms of land use and danger to adjacent wetland systems. This is further complicated by increasing discharge of an array of toxic substances (xenobiotics) from industrial facilities and chemical disposal areas in close proximity to such sensitive wetland regions. An example of the complexity of the problem is evident in the coastal zone of Louisiana, comprising more than seven million acres of marshes and estuaries and representing approximately 40% of the total coastal wetland area of the contiguous United States. The significance of the region as a fishery resource is well established. This ecosystem is close to major economically significant agricultural and industrial sites in the fertile Mississippi delta. A wide variety of organic materials, including potentially hazardous chemicals, are brought into the region by the gulfward movement of water. In spite of the widespread use of these chemicals, limited data are available on the fate of pesticides and other chemicals in such agriculturally affected wetlands.

Important chemical changes take place during passage of water through the aforementioned wetlands. Suspended sediment settles with decreased water flow, and inorganic nutrients, especially nitrogen and phosphorus, are utilized by plant growth. Major modification of xenobiotics, via abiotic hydrolysis and microbial degradation, in all likelihood is most active in this area.

Degradation of xenobiotics in the environment occurs by abiotic as well as biotic processes. The degree of physical adsorption of compounds to colloidal mineral and humic materials strongly influences the susceptibility of soil- and sediment-bound pesticide residues to chemical or biological attack. Although there is considerable information on persistence and fate of pesticides in soils, limited data are available on the effects of pH, oxidation, and salinity levels and the combined interactive effects of chemical, physical, and biological conditions affecting degradation processes in wetland soil/sediment ecosystems.

Organophosphorus insecticides, the major class of compounds studied, generally are known to be less persistent in soils than many of other chemical classes of pesticides, such as chlorinated hydrocarbons. However, some organophosphorus compounds have been shown to persist in soils for several months; furthermore, chemical and biological properties of soils and sediment-water systems affect such persistence.

Our research is intended to provide information on the fate and behavior of selected xenobiotics in the agriculturally affected coastal wetlands of Louisiana. The major area of study is shown in Figure 1. An understanding of the biological, chemical, and physical conditions conducive to degradation of pesticides may enhance the predictive capability for assessment of potential environmental impact and aid development of management practices that minimize adverse effects.

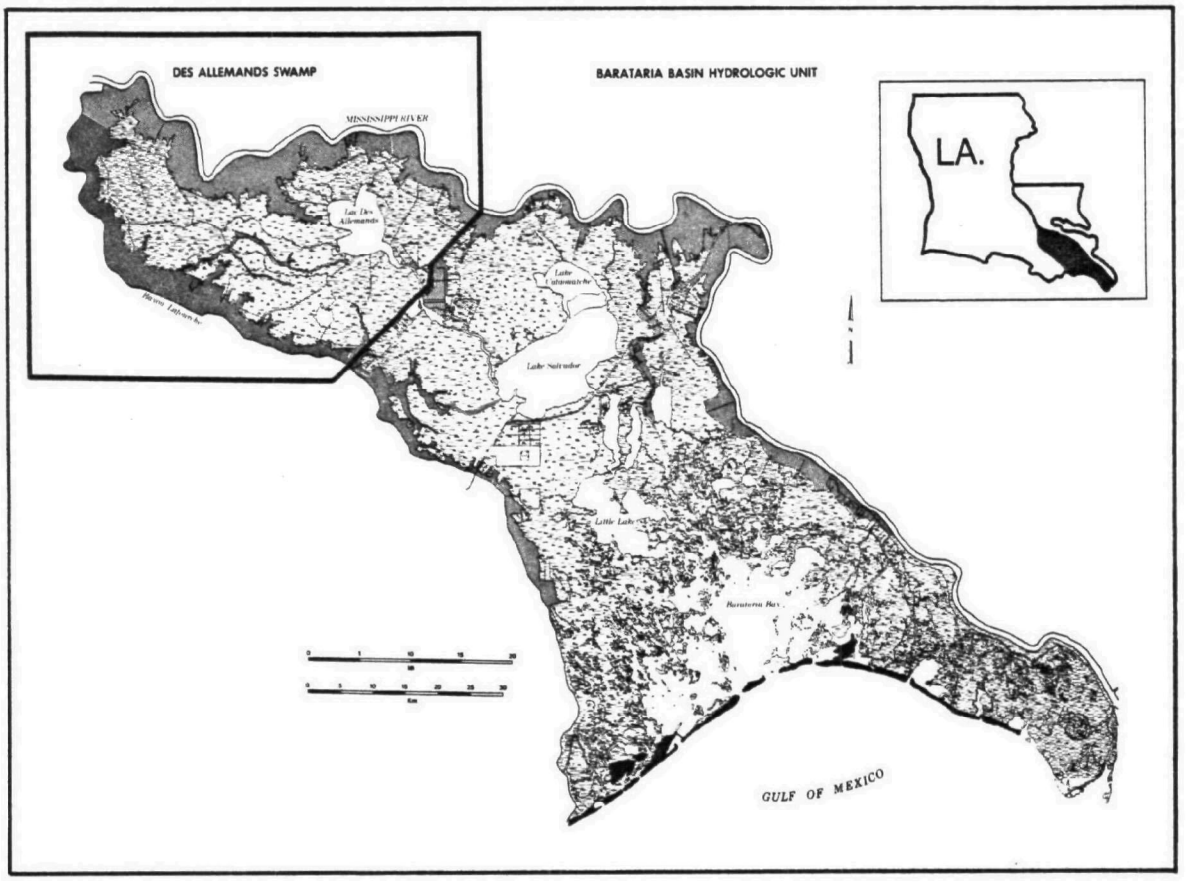

Figure 1. Map of Barataria Bay Hydrologic Unit with Des Allemands Swamp Sampling Site.

The organization of the project involves linkages of research between three major components of this study, namely, microbiology, in situ investigations, and physicochemical studies. The microbiological portion comprises both laboratory and in situ approaches involving correlation analyses and controlled simulation studies. The three field and laboratory approaches developed are designated as: Effect of Oxidation-Reduction Conditions on Pesticide Persistence, In Situ/Microcosm Investigations and Microcosm Evaluation and Validation, and In Situ Effects of Guthion on Backswamp Aquatic Invertebrates and Shallow Water Leaf Litter Decomposition. For clarity, these major subjects are initially treated in separate sections of this report, but as noted subsequently, are closely related topics within the composite coastal zone management area.

Within this interrelated framework of research several important component relationships among the individual projects need to be emphasized, in order to provide a transition between major blocks of research data and to attenuate these major project outputs into a concise package. The contribution of each individual research projects in achieving the research goals originally proposed are addressed in the final project discussion.

Beginning with physical/chemical studies, the primary research objective, as noted, was to ascertain the effect of oxidation reduction conditions on pesticide persistence. An understanding of the underlying physical/chemical characteristics affecting pesticides in aquatic systems provides a framework for further studies within the microbiological/biological component and, in combination, provides an insight into pesticide effects within the general ecology of the target wetland system, an important consideration for coastal zone management. Specific objectives for the physical/chemical studies included:

- Development of efficient and reproducible extraction methodologies which eliminate problems associated with anaerobic sediments.

- Development of procedure for processing of anaerobic sediment samples to recover pesticides without introduction of degradation artifacts.

- Formulation of physical/chemical simulation studies, emphasizing oxidation reduction conditions, of anaerobic/aerobic soil and sediment environments to study pesticide degradation.

Microbiological investigations were two-fold in nature. Our overall objective was to ascertain the effect and fate of a pesticide on the microbial component in an aquatic microenvironment. In doing so, we felt an important linkage between biotic and abiotic factors impinging on pesticide effect and fate could be ascertained, providing further insight into pesticide effects in higher trophic communities. To accomplish this, it was necessary to design and validate a laboratory/field protocol which would provide quantitative analysis of pesticide fate and effect. To do so, the following objectives were proposed:

- Development of laboratory/field protocol for pesticide analyses (including microbial/soil enzymatic activity and response, ^{14}C-respiration and bioaccumulation interaction, and complimentary residue analysis by GC methods established in physical/chemical studies).

- Development of laboratory/field protocol for <u>in situ</u>/laboratory analysis of key organic substrates, i.e., chitin and cellulose, as affected by xenobiotic addition under aerobic and anaerobic conditions.

- Development of selective media for <u>in situ</u>/laboratory sampling.

Our earlier studies have demonstrated the unique microbial composition of the salt marsh ecosystem and its extremely active chitinoclastic (chitin-decomposing) heterotrophic population. Significant rates of detrital turnover into the trophic food web have demonstrated the important role of the extant microbial biomass and the impact of stress factors on microbial ecosystems and specific transformation processes. With the completion of preliminary studies with the organophosphate, Guthion, two additional research objectives were proposed:

- The analysis of heterotrophic activity/enzyme activity profiles in several ecologically significant *in situ* soil microenvironments to correlate these observations with variations in controlled microcosms due to xenobiotic addition.

- Formulation of a statistical and computer-base program for data analysis of microbial and enzymatic activity variations.

Field studies of indigenous invertebrate populations primarily involved the analysis of *in situ* effects of the organophosphate, Guthion, on backswamp aquatic invertebrates, as well as providing indications of the effect of this pesticide on shallow water leaf litter decomposition. Specific objectives of this research group included:

- Development of total invertebrate community analyses of population dynamics with pesticide input.

- Development of laboratory cultures of selected wetland invertebrates and their possible use as indicators of environmental stress.

- Analysis of sampling errors in studies of *in situ* effects of pesticides in wetland communities.

Also included in the above research objectives were the analysis of techniques and problems associated with biochemical stress analysis (energy charge ratio) in pesticide affected food webs in estuarine ecosystems.

In all facets of the study, major attention has been given to the organophosphorus compound Guthion (0,0-dimethyl S-[4-oxo-1,2,3-benzothriazin-3 (4H)-ylmethyl] phosphorothioate) used in control of the cane borer on sugarcane (*Saccharum officinarum* L.) in Louisiana. Guthion (azinphosmethyl) represents 80% of the total volume of insecticides applied to sugarcane, being substituted for Endrin over a decade ago because of problems in fish toxicity and insect resistancy. Other xenobiotics, such as methyl parathion, have been used to develop comparative data in terms of methodology and dose response.

SECTION 2

EFFECT OF OXIDATION-REDUCTION CONDITIONS ON PESTICIDE PERSISTENCE

R. P. Gambrell

INTRODUCTION

The objective of our studies was to determine the effect of oxidation-reduction conditions and variations in the persistence of selected pesticides in soils and sediments typical of Louisiana wetlands. Soil and sediment materials were treated with pesticide compounds and incubated in special laboratory reaction chambers as suspensions under conditions of carefully controlled pH and redox (oxidation-reduction) potential. Sample aliquots were withdrawn periodically over a 2 to 3 week incubation period and extracted to determine how compound concentrations changed with time under the imposed physicochemical conditions.

The compounds studied included Guthion, as well as Trifluralin (δ, δ, δ-trifluoro -2, 6-dinitro-N, N-dipropyl-p-toluidine), methyl parathion (0, 0-dimethyl-0-p-nitrophenyl phosphorothioate), and 2,4-D (2,4-dichloro phenoxy-acetic acid).

Guthion, as noted previously, is used to control the caneborer in sugarcane, an important crop grown adjacent to freshwater wetlands in south Louisiana. Trifluralin is an important herbicide for weed control in soybeans, an increasingly important crop in south Louisiana. Though perhaps not used extensively near wetlands, methyl parathion was selected because it is an effective insecticide which has found increasing demand as some of the more persistent chlorinated hydrocarbon insecticides were phased out because of environmental problems. The herbicide 2,4-D is widely used to control broad-leaf weeds. This and its related compounds are also important in controlling aquatic weeds in many waterways, especially in the southern U.S.A.

It is well known that pH affects the dominant microbial populations and their activities in soils and sediments. In general, a soil pH between 6.5 and 8.0 is considered optimal for microbial activity (Alexander 1961). The oxidation-reduction potential of a soil or sediment-water system also affects the overall kinetics of microbial populations. As oxygen becomes depleted and the soil becomes reduced, aerobic organisms are inactivated, and facultative and obligate anaerobes predominate. As a result, a different array of microbial biochemical processes occur with substitutes for oxygen as the terminal electron acceptor, different metabolic pathways, and end products.

In this way, the microbial biomass of reduced systems are substantially altered from those of aerobic systems (Patrick and Mikkelsen 1971). Since different redox and pH conditions have been shown to strongly affect the breakdown of some pesticides, it is necessary to study the combined effect of these physicochemical factors on decomposition of the "target" pesticides.

Changes in oxidation potential may produce both quantitative and qualitative alterations in humic materials (Reddy and Patrick 1975; Stevenson and Ardakani 1972). Pesticides are reported to be strongly bound with soil and sediment organics; thus, the availability of a given pesticide to microorganisms, or the fauna of wetland ecosystems, may be regulated to a considerable extent by the extant physicochemical environment affecting these adsorption processes (Bailey and White 1970; Pionke and Chesters 1973; White and Mortland 1970).

Pionke and Chesters (1973) in their extensive review, suggested that colloidal hydrous oxides of iron may be effective adsorbents for some pesticides. These oxides are thought to form as reduced ferrous iron is transported into oxidized sediment-water systems. Greater degradation rates for DDT in reduced environments compared with those in oxidized environments has been reported by a number of investigators (Fries 1972). Though less work has been conducted with organophosphorus insecticides, there is some indication that the aforementioned may also apply to this class of compounds. Sethunathan (1972) noted that Diazinon persisted for 180 days in unflooded soils but disappeared in as little as 60 days in flooded soils. Presumably, this organophosphate was reduced. An effect of pH on the persistence of this compound also was noted. Walker (1976) reported on the chemical and microbiological degradation of malathion and parathion in seawater and sediments. Malathion disappearance in the systems described was attributed primarily to chemical rather than to microbiological processes, and its rate of loss was in direct proportion to increasing salinity. Parathion was more persistent, with rate of loss less dependent on salinity levels.

Walker and Stojanovic (1973) studied microbial versus chemical degradation of malathion in three Mississippi soils. Microbial degradation was found to be the chief mechanism of insecticide dissipation in all soils tested. Malathion was quite stable under neutral or acid pH conditions, but was susceptible to hydrolysis under alkaline conditions. Chemical degradation appeared due to both alkaline hydrolysis and adsorption by the soil particles.

Bourquin (1975) examined malathion degradation in a simulated salt-marsh environment and observed rapid *in vitro* breakdown by a variety of salt-marsh bacteria. Bacterial concentrations were correlated with levels and frequency of malathion treatment, although malathion sole-carbon degrading bacteria comprised only about 10% of the bacterial population decomposing the pesticide. It was suggested that increased numbers of malathion-co-metabolizing bacteria catalyzed a more rapid dissipation of the compound. Chemical hydrolysis of malathion was directly correlated with increasing temperature and salinity. However, at temperatures below 26 C and salinities below 20°/$_{oo}$, biological mechanisms appear to be of greater significance.

Walker (1976) isolated microorganisms capable of degrading malathion and parathion from insecticide-enriched sediment and examined in vitro breakdown. Various degrees of pesticide decomposition were observed. Malathion was essentially completely degraded during 18 days incubation in sterile and nonsterile water, while respective parathion losses were from 16 to 23%. Direct correlation between salinity and compound persistence were reported during the field studies. Malathion degraded faster in higher salinity water, while parathion was persistent up to 25°/$_{oo}$.

Sethunathan (1972) reported persistence of Diazinon for different lengths of time in flooded rice soils depending on whether the soils had been treated previously. Diazinon persisted for 15 days in previously treated soils and about 60 da in nontreated soils. A flavobacterium, isolated from water of a treated rice field had a high capability to metabolize diazinon as sole carbon source.

Weber (1972) reviewed the interaction of organophosphorus pesticides in aquatic and soil systems and noted that these compounds do not accumulate in soil fauna, such as earthworms, and concentrate less in birds and fish, than the chlorinated hydrocarbons. Most are readily degraded and do not accumulate for long periods. Organophosphorus compounds readily adsorb to organic and clay components of the soil. The main routes of disappearance of organophosphorus chemicals are via hydrolysis and microbial degradation.

Pionke and Chesters (1973) observed that pesticide persistence in watersheds is dependent largely on the stability of soil-applied pesticides. Many organic phosphorus pesticides can be chemically hydrolyzed by nonmicrobial means, whereas the longer-lived compounds are degraded by both chemical and microbial routes. Soil conditions, such as unusual pH levels and high pesticide residues not conducive to microbial activity, increase the persistence of such compounds in the soil, thereby increasing the potential for aquatic contamination. Generally, the adsorption of most nonionic pesticides is correlated with soil organic levels.

Runoff from agricultural land is one of the main mechanisms for introduction of pesticides into aquatic environments (Pionke and Chesters 1973). Excessive rainfall from storms soon after application can result in losses within 1 or 2 da greater than that which would normally occur in a year. This may be especially true in southern Louisiana where sudden heavy rains are common. Willis et al. (1975), in studies of losses of four herbicides in surface drainage water in Louisiana, found that seasonal losses ranged from 0.05 to 3.0%. Highest concentrations in runoff were usually associated with rainstorms that occurred soon after application. Willis and Hamilton (1973), in investigations of endrin loss in runoff from sugarcane test plots, noted that on an annual basis, about 0.2% of applied endrin was lost. Rainfall soon after application increased loss rates. The low rates of loss may be attributed to substantial losses by volatilization.

Pesticides are readily adsorbed by clay particles and organic matter. The accumulation of organic matter in the anaerobic zone of lakes and wetlands increases the adsorption of pesticides. Some metal complexes of Fe^{2+} and Mn^{2+} formed under these conditions may stabilize pesticides. Adsorption

by algae and aquatic vegetation is rapid, often with concentrations of 100 to 10,000 times that in the aqueous portion of the ecosystem.

Willis et al. (1976) examined pesticide concentrations in runoff from the Mississippi Delta watershed and noted a linear relationship between pesticide yields in runoff and sediment yields. Emphasis was placed on the limited amount of extant information on the role of sediment in pesticide adsorption, transport, desorption, and degradation.

Organophosphorus compounds may be the least hazardous pesticides in aquatic systems because of their rapid hydrolysis, especially favored at alkaline pH.

Guthion

Heuer et al. (1974), in studies of the effect of pH and temperature on half-life of Guthion in aqueous solutions and on glass surfaces, found that both factors have a significant effect on degradation rates. At pH > 9, half-life is about one month at temperatures of 6 C and 25 C. At pH > 9.5, the half-life is about one week, while at 40 C and pH > 9.5, half-life was less than one day.

Meyer (1965) reported that half-life of a Guthion formulation applied to farm ponds to be about 2 da with a 90% reduction after 4 da. In a report by the manufacturer's research staff (Chemagro Division Research Staff 1974) the half-life in a pond water at 30 C was 1.2 da. The Chemagro paper also reported a 50% reduction in Guthion in a Florida "muck-sand" in one month and that a three-month period was required for a 50% Guthion loss from a clay soil in Louisiana.

Yaron et al. (1974) studied the kinetics of Guthion in soil samples in Israel with a pH of 8.4 and an organic matter content of < 1%. Experiments were conducted with wet and dry, and sterile and natural soils at temperatures of 6, 25, and 40 C. They found that natural, wet, and warmer soils gave higher rates of degradation. The half-life in wet, natural soils at 6, 25, and 40 C was 64, 13, and 5 da, respectively. From these data, it can be postulated that the breakdown of Guthion in warm Louisiana wetland soils (with a mean annual temperature of 20 to 21 C) will be quite rapid. However, since the pH of wetland soils is almost always lower than 9 and often acidic, the half-life of the pesticide may be lengthened.

No breakdown metabolites have been identified for Guthion in studies of animal tissues (Murphy and DuBois 1957; Nakatsugawa and Dahm 1962). Elsewhere, Harvey et al. (1969) examined the decomposition of Guthion *in vitro* to elucidate degradation mechanisms involved, and parameters that affect the decomposition.

Ethyl and Methyl Parathion

The pathway of parathion degradation is likely affected by both availability of an energy source and the presence and size of microbial populations with the ability to hydrolyze parathion. Sethunathan (1973) reported

on the effect of rice straw on the degradation of parathion in a flooded soil. Rice straw amendments inhibited parathion hydrolysis to p-nitrophenol and diethyl thiophosphoric acid in soils inoculated with an enrichment culture with a high ability to hydrolyze the compound. However, in uninoculated soils, the addition of rice straw enhanced parathion breakdown by way of nitro group reduction. By one pathway, p-nitrophenol was a degradation product that included the aromatic portion of the molecule, while p-aminophenol was the corresponding degradation product by the other pathway.

Rajaram and Sethunathan (1976) reported that a factor was present in a flooded soil amended with glucose or rice straw that inhibited the microbial hydrolysis of parathion. This factor developed within 36 hr after flooding the rice straw-amended soil.

Predominant degradation products of parathion are reported to be diethyl thiophosphate, p-nitrophenol, aminoparathion, and p-aminophenol (Saltzman et al. 1974; Rajaram and Sethunathan 1976; Rajaram and Sethunathan 1975). Munnecke and Hsieh (1976) reported p-aminophenol and diethyl thiophosphoric acid were degradation products under low oxygen conditions while p-nitrophenol and either diethylphosphoric acid or diethylthiophosphoric acid (depending on the pathway) were formed under oxidized conditions. The aforementioned research is for ethyl parathion, commonly called parathion. Considerably less work is published on the degradation of methyl parathion, a compound included in our study, though the similarity of the two compounds would suggest a similar environmental chemistry.

Garnas et al. (1977) reported on the fate of methyl parathion in a marine benthic microcosm. Ring-labelled methyl parathion disappeared from the water column after 14 da, mostly as a result of transport and binding of the compound and/or unextractable residues in the sediment. It was shown that methyl parathion degraded rapidly, primarily by microbial processes, to a number of more polar products including aminomethyl parathion and p-nitrophenol.

Sudhakar-Barik et al. (1976) reported on the metabolism of nitrophenols by two bacterial isolates from parathion-amended flooded soil. Of the radioactivity applied as p-nitrophenol, 23% and 80% (for Pseudomonas sp. and Bacillus sp., respectively) was recovered as $^{14}CO_2$ after 72 hr.

Trifluralin

Trifluralin, a selective pre-emergence soil incorporated herbicide, is a prominent compound in the dinitroaniline series of herbicides used as a selective pre-emergence herbicide for the control of broad-leaved weeds and grasses (Cripps and Roberts 1978). The compound is very sparingly soluble in water and thus not subject to leaching in most soils. Trifluralin has a relatively high vapor pressure and some volatilization losses may occur (Weber 1970). Trifluralin is strongly adsorbed by organic soil colloids as demonstrated by a greatly reduced effectiveness with increase in the soil organic matter content (Lambert 1967; Probst et al. 1967).

Parr and Smith (1973) studied trifluralin under various oxidation conditions and reported that aerobic degradation was initially characterized by the sequential dealkylation of propyl groups while anaerobic degradation began with reduction of the nitro groups. In a substrate (energy)-amended soil, degradation was more rapid under anaerobic versus aerobic conditions (99% and 15%, respectively, after 20 da). Experimental evidence indicated microbial involvement in initial degradation.

Probst et al. (1967) reported on the effects of soil aeration on trifluralin. Under oxidized conditions, several metabolites were formed as a result of dealkylation and reduction reactions, but these degradation products were short-lived and were soon converted to polar products. Under anaerobic conditions, the rate of degradation was greater. Reduction reactions predominated and were followed by dealkylation.

Willis et al. (1974) reported on trifluralin degradation under controlled redox potential conditions using a Sharkey clay soil (pH 5.7, 3.4% organic matter). Flooding the soil to exclude oxygen initiated rapid degradation only when the redox potential decreased below a potential between +150 and +50 mv. At +50 mv, the trifluralin recovered decreased to zero in 8 da while approximately 60% of the initial 1 ppm applied was present by 20 da. Examining the degradation pathways under aerobic and anaerobic conditions proposed by Probst et al. (1967), Compound E (one nitro group reduced and one propyl group dealkylated) was found in trace amounts at +250, +150, and +50 mv, and no other degradation products were found in treatments better oxidized than +50 mv. This compound is thought to be an intermediate in both aerobic and anaerobic degradation. Another compound (one nitro group reduced) was found at +50 mv, a compound postulated to be part of the anaerobic pathway.

LaFleur et al. (1978) reported a two-stage process for the loss of trifluralin from a Congaree soil. The initial disappearance process had a half-life of 19 da while the slow process had a half-life of 450 da. This multistage trifluralin disappearance was believed associated with various adsorption-desorption phenomena.

2,4-Dichlorophenoxyacetic Acid

The herbicide 2,4-D is known to degrade rapidly in soils so that its persistence is usually reported to be on the order of a few weeks (Brown 1978).

In a review of phenoxyalkanoate pesticides, Kaufman (1974) reported the major metabolic reactions associated with phenoxyalkanoic acids include ring hydroxylation, B-oxidation of the long chain aliphatic acid moiety, cleavage of the ether linkage, dehalogenation, and ring cleavage. From published information, Kaufman (1974) presented a microbial metabolism chart for 2,4-D that indicates ring hydroxylation is a common and predominant process in initial degradation prior to further degradation to "ecologically acceptable metabolites." Succinic acid is believed to be the final product in 2,4-D metabolism.

Though 2,4-D and related compounds have been extensively studied, we have been unable to find any information on oxidation-reduction effects on its degradation.

Gambrell and Patrick (1978), in their review of the literature, concluded that pH and especially oxidation-reduction conditions of soils and sediment water systems affects the degradation rate of a number of chlorinated hydrocarbon pesticides. From the limited information available on oxidation reduction effects, it may be concluded that if redox potential conditions influence the degradation rate of pesticide compounds, they degrade more quickly under reduced conditions. Recently, Gambrell et al. (1981) have reported that Permethrin, a synthetic pyrethroid, is degraded more rapidly under oxidizing rather than reducing conditions.

From this short literature review, it is apparent that the physicochemical conditions of soils and sediment-water systems affect the fate of many pesticide compounds. Because of the wide range of physicochemical conditions to which residues may be subjected, especially if transported from the site of application, it is important to understand the effects of parameters such as pH and redox potential conditions on the environmental chemistry of pesticides.

MATERIALS AND METHODS

Experimental Reaction Chambers

The effect of pH and redox potential (oxidation-reduction) conditions on the degradation of four pesticide compounds was studied in sediment-water suspensions. The reaction chamber used is illustrated in Fig. 2 and has been described previously (Patrick et al. 1973). This apparatus has been used successfully in our laboratory to study effects of these physicochemical parameters on nutrient transformations in soils and the geochemical distribution of trace and toxic metals in sediments as well as their availability to marsh plants. The sediment-water mixtures were incubated in 2-l, 3-necked flasks and continuously stirred using a magnetic stirrer. To prevent wear to the 5.1 cm-long teflon-coated magnetic stirring bar due to abrasion with sand and silt particles, the stir bar was slipped inside an equal length of flexible polyvinyl chloride tubing. A combination pH electrode was securely mounted in each flask and the pH was checked twice daily and adjusted as necessary using a syringe to add 1N HCl or 1N NaOH through the serum cap located in the center rubber stopper.

A thermometer was mounted in one rubber stopper and the temperature maintained at 28 ± 1°C by inserting thin asbestos sheets as required between the stirring motor and the flask to regulate heat transfer. Each flask was fitted with separately valved inlet tubes for air and nitrogen and an outlet tube which ended in a water trap to reduce oxygen diffusion into the flask.

Redox potential in the suspensions was measured continuously with two bright platinum electrodes (and a calomel reference electrode connected to the suspension with a KCl-saturated agar salt bridge) which were connected to

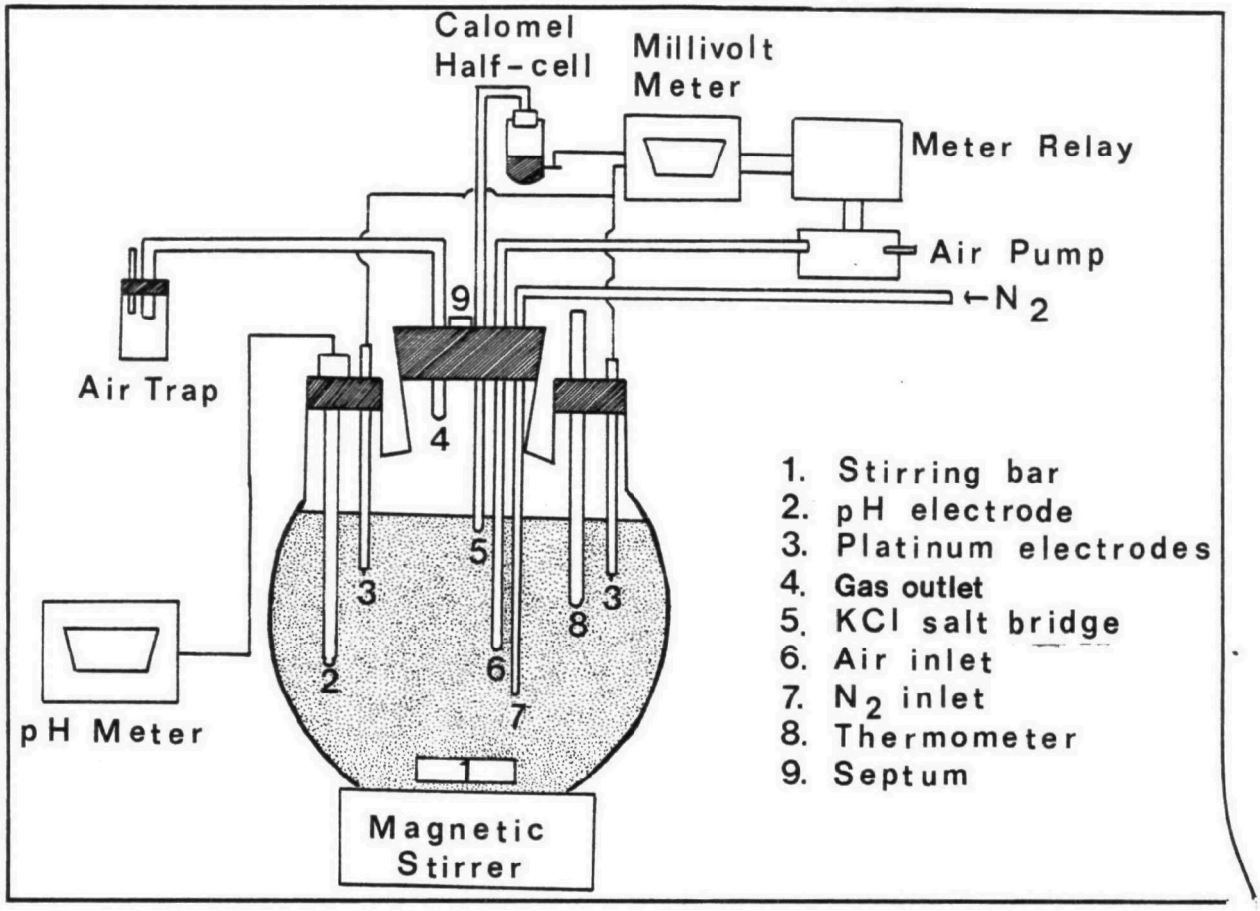

Figure 2. Reaction Chamber Apparatus for Controlling Redox Potential and pH.

a millivolt meter. Automatic redox potential control was achieved by connecting the recorder output of the millivolt meter to a meter relay that switched on a small air pump whenever the redox potential decreased below the set level for each incubation flask. By metering the air flow to about 2 to 4 ml/min, the suspension would slowly oxidize to the set potential during the air pumping cycle until the meter relay switched off the pump.

Soil- and sediment-water systems supporting considerable microbial activity become more reducing in the absence of oxygen. Thus, by regulating the addition of oxygen to soil and sediment-water systems, redox potential can be controlled over a wide range. Redox potential was controlled to within 10 millivolts of the selected potential in this study. Nitrogen gas was continuously supplied to the suspension at a flow rate of about 5 ml/min. This addition was useful in preventing an accumulation of gaseous decomposition products which might adversely affect microbial activity and in purging residual oxygen from the suspension at the end of each air pumping cycle.

Prior to removal of sediment material for loading into reaction chambers, each small storage container was thoroughly mixed on a roller-mixer to insure

sample homegeneity. Based on moisture content, sufficient wet sediment was weighed into the incubation flasks to give 300 g of solids (oven dry basis), following which water was added to give the desired solids to solution ratio (1:6). Stirring was begun for the preliminary incubation which consisted of gradually adjusting both pH and redox potential to preselected levels. After the suspensions were maintained at those levels for one week for the suspensions to equilibrate at the controlled physicochemical conditions before pesticide addition.

Samples were obtained from the flasks by withdrawing suspension aliquots through the serum cap using a 50-ml glass syringe fitted with a 12-gauge stainless steel needle. The samples were stored in a freezer in 112 ml glass bottles sealed with aluminum foil-lined caps.

Soil and Sediment Material

The soil material for the Guthion degradation studies was from the surface, approximately 5 cm depth (after the forest litter layer had been scraped away), of the crawfish farm site described elsewhere in this report. The material had a medium texture and a dark color indicative of appreciable organic matter content. The area is subject to periodic flooding. Large pieces of twigs, roots, and decaying leaves were removed by hand. Sediment material for use with methyl parathion, trifluralin, and 2-4,D was taken from Bayou Chevreuil (near the bridge on Louisiana State Road #22), a site located approximately 3 km from the crawfish farm. The sediment material was fine textured (predominately clay) and contained an appreciable (~6%) organic matter content.

Both soil/content materials were batched when returned to the laboratory, mixed well, and stored in small containers in a refrigerated room at their natural moisture condition.

Pesticide Extraction Procedures

Guthion (soil)--

1. Thaw sample in lukewarm water.

2. Centrifuge in original glass storage bottles at 1650 rpm (IEC #266 rotor) for 25 min.

3. Decant water (into separatory funnels if water is to be analyzed).

4. Mix 2 ml of toluene with soil to retard microbial activity during sample drying; place samples in a forced draft oven at 40 C.

5. Finely grind the dried samples with a mortor and pestle and weigh 2 to 4 g into a tared extraction thimble.

6. Extract for 4 hr with 130 ml of acetone using the Soxhlet extraction apparatus.

7. Rinse the glass Soxhlet extraction tube and thimble with acetone into the Soxhlet boiling flask.

8. Pour the acetone extraction solvent through sodium sulfate into 250 ml flasks to dry the sample.

9. Concentrate as necessary in a water bath heated to 40 C, then quantitatively transfer into graduated test tubes with screw caps.

10. Bring to volume and seal sample with aluminum foil-lined caps.

Guthion (water)--

1. To a 50 ml sample in a 250 ml separatory funnel, add 50 ml of a hexane-methylene chloride (85:15 ratio) and 1 g of sodium chloride and shake for 2 min venting frequently.

2. After phase separation, drain the aqueous layer (lower) into a second separatory funnel.

3. Add 50 ml of methylene chloride to aqueous sample in second funnel and shake for two min.

4. Transfer the hexane-methylene chloride extract of the first separatory funnel to the second and transfer the aqueous layer from the second funnel to the first.

5. Add 50 ml of methylene chloride to the aqueous sample now in the first funnel and shake for 2 min venting often.

6. Drain the aqueous phase into tared containers and determine weight of the water.

7. Combine methylene chloride extracts, rinsing separatory funnels with hexane; filter extracts through hexane-washed sodium sulfate into 250 ml flasks.

8. Add several glass beads and 1 ml of a 10% Nujol (in hexane) solution to the flasks, rinse Snyder column with 5 ml of hexane, and connect columns to flask.

9. Under a fume hood in a boiling water bath, concentrate the methylene chloride extract to 5 ml.

10. Add 100 ml of a 1:1 hexane-acetone mixture and concentrate again to 5 ml. (Note: solvents are changed to protect N.P. detector on GC.)

11. Pour extract from flasks into graduated test tubes and make volume to 5 to 10 ml with acetone.

12. If further concentration is necessary, evaporate some solvent with a stream of dry nitrogen gas.

Methyl parathion--

1. Thaw sample in lukewarm water.

2. Mix 0.5 g NaCl with the thawed sediment-water mixture and centrifuge at about 1500 rpm (IEC #266 rotor) for 15 min.

3. Discard the supernatant solution or pour into separatory funnels if solution phase is to be analyzed.

4. Under a hood, mix 1 to 2 ml of toluene with the wet sediment (to retard microbial activity) and dry in a forced draft oven at a maximum of 40 C.

5. Grind the sediment samples to a fine powder and weight into tared extraction thimbles.

6. Extract for 4 hr on a Soxhlet extraction apparatus with 130 ml of acetone.

7. Rinse extraction glassware and thimble with acetone into a beaker.

8. Dry the acetone extract by filtering the sample through acetone-rinsed Na_2SO_4.

9. Collect in 200 or 250 ml volumetrics and bring to volume with acetone.

10. Concentrate if necessary.

Trifluralin--

1. Add an aliquot of the well mixed sediment-water suspension sample (containing 2.5 to 5.0 g solids on a dry weight basis) to preweighed glass centrifuge tubes.

2. Centrifuge and discard the water (centrifugation speed and time depends on the type of sediment; 6,000 rpm in a Sorval GS-3 rotor for 25 min was necessary to remove fine particles from Bayou Chevreuil sediment suspension. A slower speed probably would have been adequate if NaCl had been added to enhance flocculation.)

3. Add 75 ml of a hexane:acetone mixture (41:59 vol:vol) to each sample, mix well with a Teflon® spatula, and shake for 30 min.

4. Centrifuge at 3500 rpm (Sorval GS-3 rotor) for 20 min. Collect the solvent extract.

5. Repeat steps 3 and 4 and store extract in a dark place or amber bottle; refrigerate if sample is to be stored overnight.

6. Oven dry centrifuge tubes and obtain dry weight of sediment extracted (about 24 hr at 50 C or until a constant weight is obtained).

7. Wash the hexane:acetone extract three times with distilled water (volumes of 100, 30, and 30 ml) in 250 ml separatory funnels, discarding the water:acetone layer (bottom) after each wash.

8. Dry the hexane extract by filtering through hexane saturated anhydrous sodium sulfate into a volumetric flask.

9. Bring to volume and dilute as necessary with nanograde hexane.

2,4-D--

1. Thaw sample in lukewarm water (1-2 g oven dry solids basis).

2. Add 15 ml of 1% KOH, check pH to insure it is near 11.

3. Shake for 15 min.

4. Let samples stand in a water bath at 45 C for 15 min; transfer to glass centrifuge tubes.

5. Centrifuge at 3500 rpm (GS-3 rotor) for 30 min.

6. Decant through glass wool into a separatory funnel.

7. Repeat steps 2-6 and combine extracts.

8. Oven dry residual soil or sediment solids and determine dry weight.

9. Extract KOH with 20 ml of diethyl ether in a separatory funnel by shaking for 1 min and permitting phase separation. Transfer the KOH (bottom) layer to a second separatory funnel and discard ether.

10. Wash the KOH layer twice more with diethyl ether and discard the ether.

11. Transfer the KOH extract into a glass centrifuge bottle or a separatory funnel, add 1 ml of an H_2SO_4 (9N) solution, and check to see that the pH is 3.0 or lower.

12. Add 20 ml of diethyl ether, shake, and allow the solution to clear or centrifuge to separate layers.

13. Remove ether layer with a pipet and save. Repeat step 12 and combine ether extracts.

14. Add a small amount of acidified Na_2SO_4 to ether extract and evaporate to 5-10 ml under N_2.

15. Transfer ether to a 15 ml centrifuge tube and evaporate to dryness.

16. Add 1 ml of diazomethane to each tube, cap tightly, and place in a 45 C water bath for 15 min.

17. Add 1 ml of hexane and evaporate to 1.0 ml.

18. Dilute as necessary with hexane filtered through anhydrous Na_2SO_4.

Gas Chromatograph Analysis

Operating conditions for GC determination of the breakdown products of the four pesticides evaluated are given in Table 1.

RESULTS AND DISCUSSION

Guthion

Guthion was incubated with a swamp forest soil material in reaction chambers under controlled pH (6.0, 7.0, and 8.0) and redox potential (-150, +50, +250, and +450) conditions. Sample aliquots were taken from the microcosms periodically, extracted, and analyzed for Guthion. A decrease in recovery with time was assumed to be a measure of degradation. Data are given in Figs. 3, 4, and 5 for pH 6.0, 7.0, and 8.0, respectively. It was found that with Guthion, a 3-wk incubation was sufficient to determine treatment effects on degradation with the soil material used. The data confirm what is commonly known about the organophosphate insecticides, namely, they are not persistent in the environment. Recovery was less than one-third of initial spiking levels within 3 weeks under physicochemical conditions resulting in slowest degradation.

Redox potential levels had a marked effect on the rate of degradation. Guthion levels decreased most rapidly at +450 mv (well-oxidized conditions) at all pH levels. The degradation rate decreased with redox potential through +250 and +50 mv to -150 mv (strongly reducing conditions), which resulted in the slowest degradation rate. Over the range studied, there did not appear to be a critical potential below which Guthion was stable since the degradation rate increased sequentially with increasing redox potential. Soil pH affected degradation less than redox potential, though it appeared, at redox potential levels of 250 and 450 mv, degradation was slower at pH 6 than at either of the higher pH levels. This effect, if real, is likely attributed to pH levels of 7.0 and 8.0 being more favorable for microbial activity than pH 6.0. Under well-oxidized conditions, Guthion had a half life of about 2 da at each of the pH levels. This increased to about 5 or 6 da under strongly reducing conditions.

Degradation rates observed in this study under oxidized conditions were comparable to those reported for Guthion in soil (Yaron et al. 1974) and pond water (Meyer 1965; Chemagro Division Research Staff 1974), but considerably faster than the 3-month half life reported for a Louisiana clay soil. Redox potential conditions and other experimental parameters for these literature reports are not available, thus, it is not possible to explain the reasons for differences and similarities in degradation rates between this study and earlier reports. However, the temperature, moisture conditions, and organic matter content of the swamp soil were quite favorable for microbial activity, which would enhance biological breakdown. The small increase in stability of

Table 1. Operating Conditions for Gas Chromatograph.

Parameter	Compound			
	Guthion	Methyl Parathion	Trifluralin	2,4-D
carrier gas	zero grade N_2	zero grade N_2	zero grade N_2	zero grade N_2
carrier gas flow rate, ml/min	70 6'	70 6'	70 6'	70 6'
column	1.5% OV-17, 1.95% QF-1	1.5% OV-17, 1.95% QF-1	3% OV-1	3% OV-210
injector temperature, C	230	230	230	230
column temperature, C	220	200	165	170
interface temperature, C	250	250	250	250
detector	Nitrogen/Phosphorus	Nitrogen/Phosphorus	ECD at 275 C	ECD at 275 C

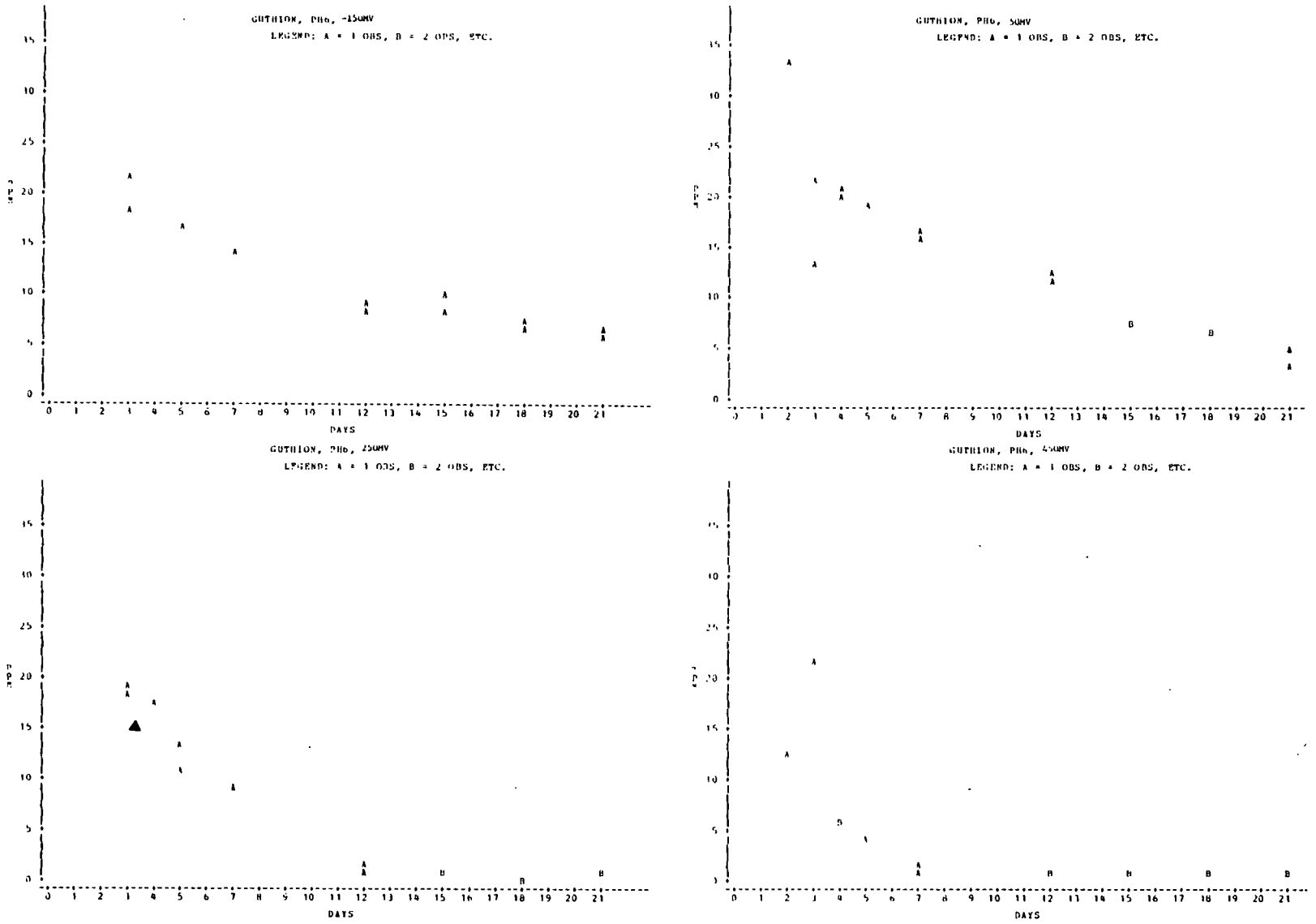

Figure 3. Effects of Controlled Redox Potential on Recovery of Guthion From a Swamp Forest Soil (Incubated at pH 6.0).

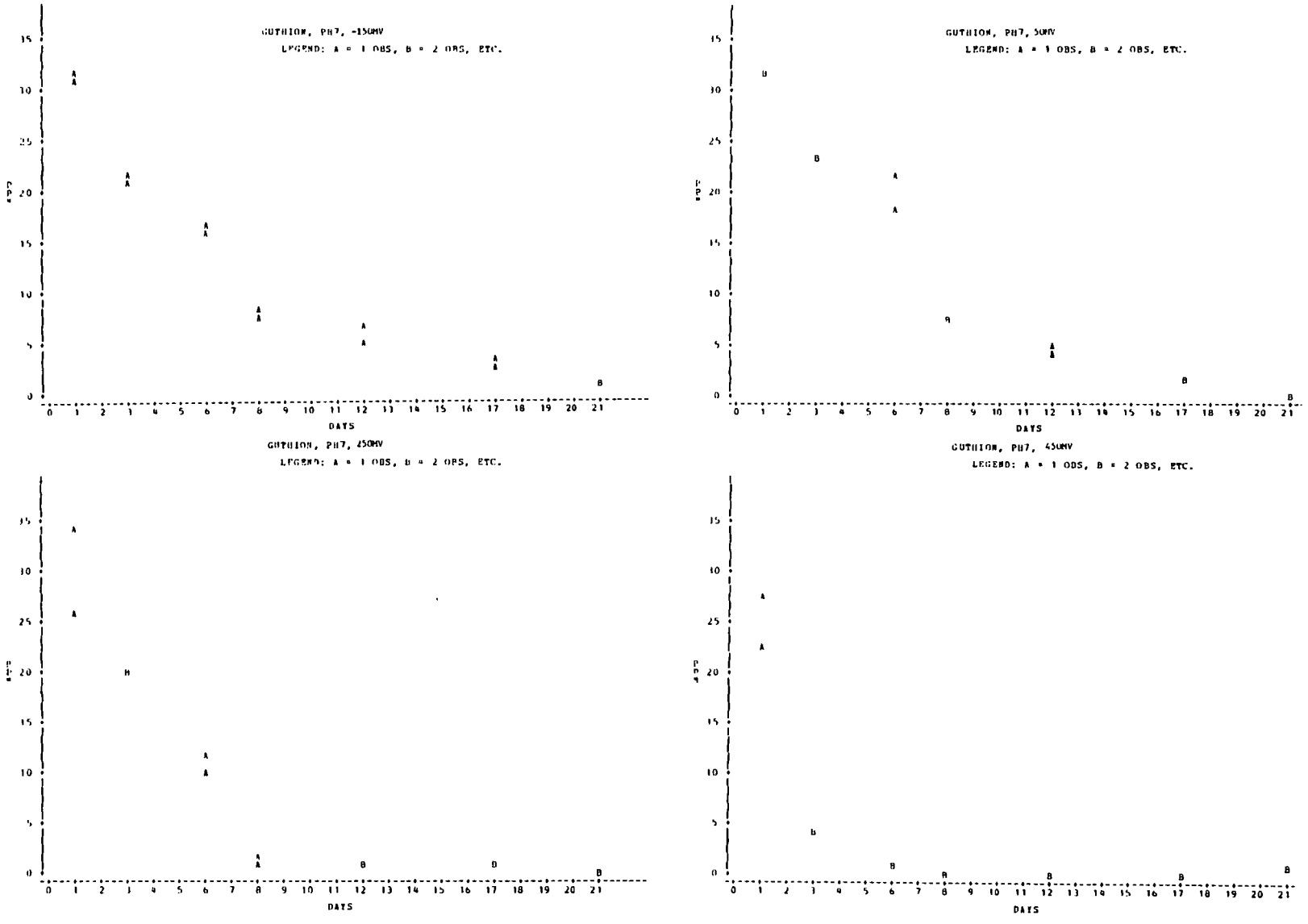

Figure 4. Effects of Controlled Redox Potential on Recovery of Guthion From a Swamp Forest Soil (Incubated at pH 7.0).

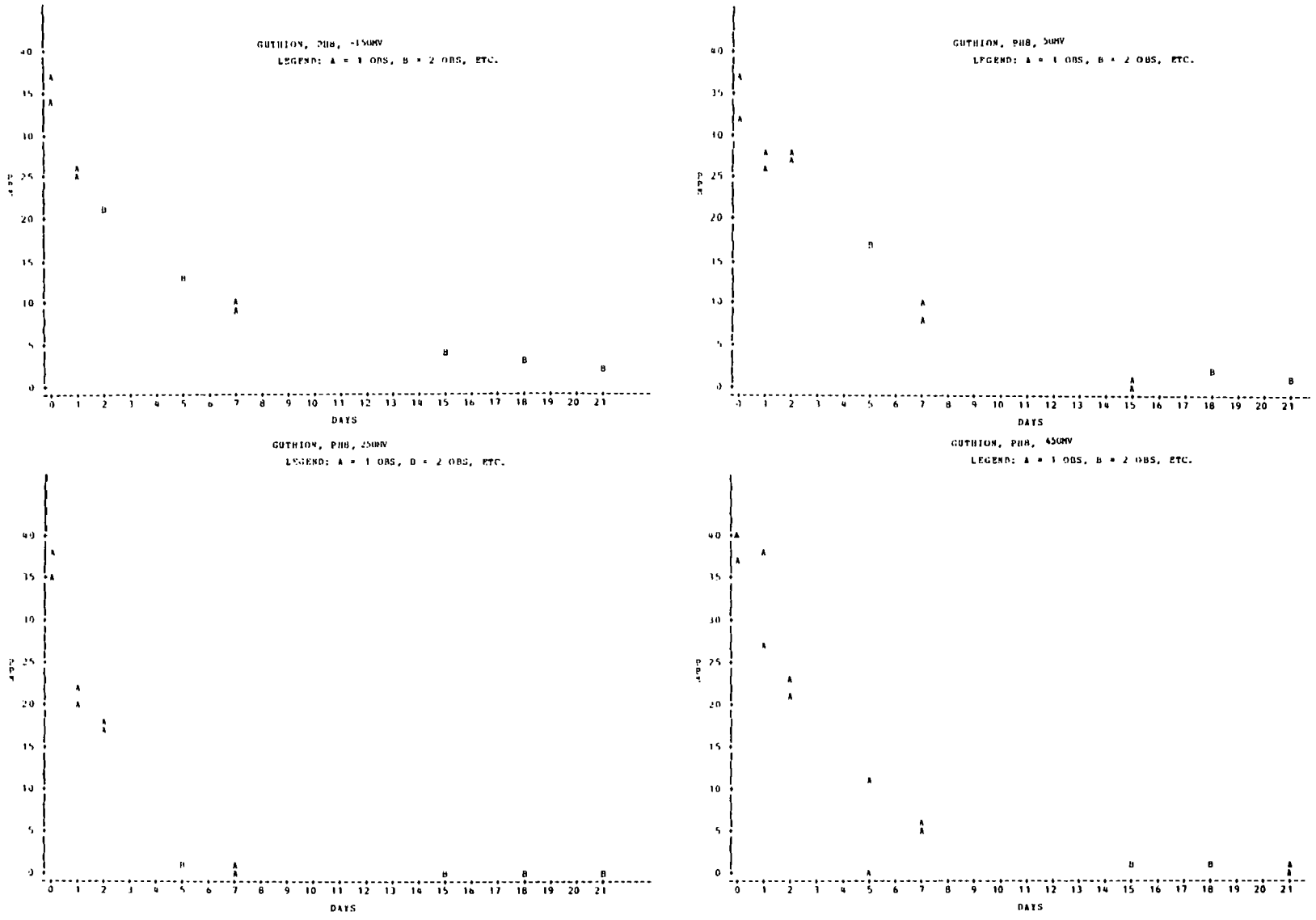

Figure 5. Effects of Controlled Redox Potential on Recovery of Guthion From a Swamp Forest Soil (Incubated at pH 8.0).

Guthion as pH decreased from 8 to 6 corresponds with the limited pH information available for Guthion degradation and reports of pH effects for several other organophosphate insecticides.

Table 2 indicates concentration ratios of Guthion bound to the soil phase vs. Guthion extracted from the aqueous phase of the samples on a few selected sampling dates (μg Guthion/ml in solution phase ÷ by μg Guthion/g solids). In these samples, both bound and dissolved Guthion were measured to

Table 2. Ratio of Soluble to Adsorbed Guthion at pH 6.0 and 7.0 in a Swamp Soil-Water Suspension.*

Incubation Period (in days)	pH	Parameter	Redox Potential, mv			
			-150	+50	+250	+450
0	6	water	0.158	0.098	0.103	0.087
		soil	14.1	11.6	18.8	18.3
		water/soil	0.011	0.008	0.005	0.005
1	6	water	0.025	0.024	0	0
		soil	9.0	9.4	21.0	22.4
		water/soil	0.003	0.003	0	0
3	8	water	1.290	0.920	0.695	0.123
		soil	21.5	23.1	20.0	4.0
		water/soil	0.060	0.040	0.035	0.031
6	8	water	0.813	0.270	0.872	0
		soil	16.1	20.2	10.9	0.9
		water/soil	0.050	0.013	0.080	0
8	8	water	0.259	0.110	0	0.079
		woil	7.9	7.4	1.3	0.1
		water/soil	0.033	0.015	0	0.790

*Soluble (μg/ml solution); Adsorbed (μg/g oven dry solids).

get preliminary information on the possibility that oxidation-reduction conditions affect partitioning between the aqueous and solid phases of sediment-water systems.

Where sufficient Guthion was present in both phases for good measurements, there was an increase in the ratio of solution phase Guthion to solid as the sediment-water mixture became more oxidizing. This suggests that reducing conditions may enhance soluble levels of Guthion in interstitial waters of sediments compared to oxidizing conditions. While the mechanism(s) accounting for this observation were not investigated, two general possibilities may be considered: 1) properties of the anaerobic solid phase, making it slightly less effective in binding Guthion than under oxidized conditions; and 2) some characteristic of the solution phase enhancing soluble levels. For example, it has been reported that soluble humic materials, presumably fulvic acids, are present in higher concentration in anaerobic sediment-water systems than in more oxidized materials (DeLaune et al. 1981). The increased soluble Guthion may be associated with dissolved fulvic acids.

Trifluralin

Trifluralin was incubated at -150, +50, +250, and +500 mv at pH 7.0 in sediment material from Bayou Chevreuil.

Fig. 6 indicates the effect of redox potential on recovery of trifluralin during a 24-da incubation. Under strongly reducing conditions, recovery dropped from initial spiking levels of 8 ppm to less than 0.5 ppm within 1 da. The rate of loss for the three higher redox potential treatments were similar in the Bayou Chevreuil such that approximately one-half could not be recovered after 4 to 5 da. The +50 mv levels were slightly lower with time (about 1 μg/g for most of the incubation) than the two more oxidized treatments which were very nearly identical until the end of the experiment. By 24 da, the +50 mv level was less than 0.1 μg/g while the levels in the +250 and +500 mv treatments were around 0.75 to 1.5 μg/g. Though while it is not a persistent herbicide under any oxidation-reduction condition studied, it is apparent that strongly reducing conditions considerably increase the rate of trifluralin loss.

Willis et al. (1974) identified δ, δ, δ-trifluoro-5-nitro-N^4-propyltoluene-3, 4-diamine as a degradation product of Trifluralin in a Sharkey clay soil incubated under controlled redox potential conditions. This was referred to as "Compound E," a designation used in the present report. The concentration of Compound E increased rapidly to around 2.5 μg/g under strongly reducing conditions by 1 da and then decreased to less than 0.5 μg/g by 6 da (Fig. 7). Comparisons of Figs. 6 and 7 indicate that: Compound E is indeed a degradation product of the parent molecule; its production and possibly degradation rate is dependent on redox potential conditions, (none was measured under well-oxidized conditions); and like Trifluralin, Compound E could not be considered a persistent molecule under the experimental conditions studied.

Fig. 8 shows the relative levels of another Compound (Y) associated with the degradation of Trifluralin. Though present in somewhat lower levels than

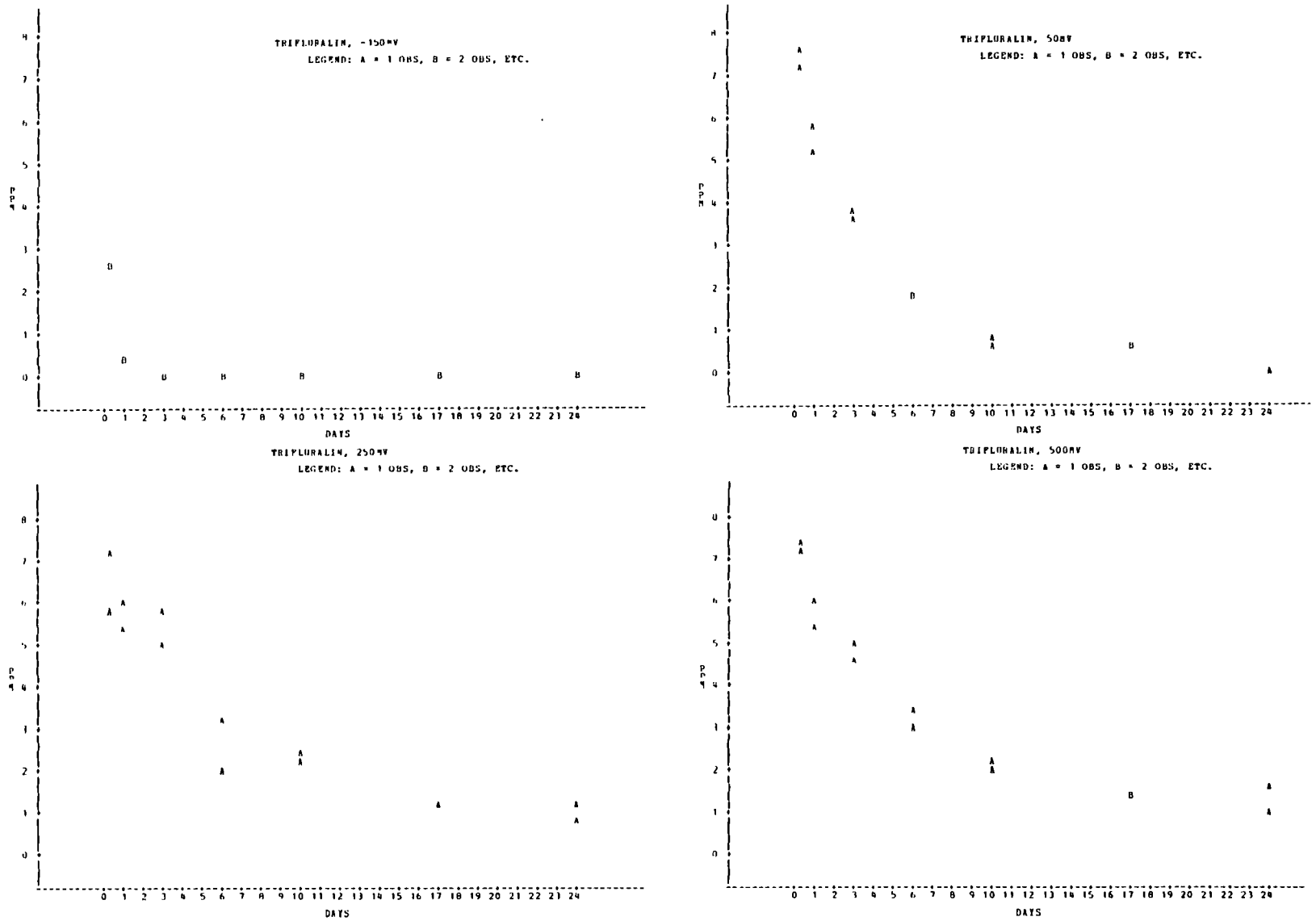

Figure 6. Effects of Controlled Redox Potential on Recovery of Trifluralin From a Bayou Chevreuil Sediment (Incubated at pH 7.0).

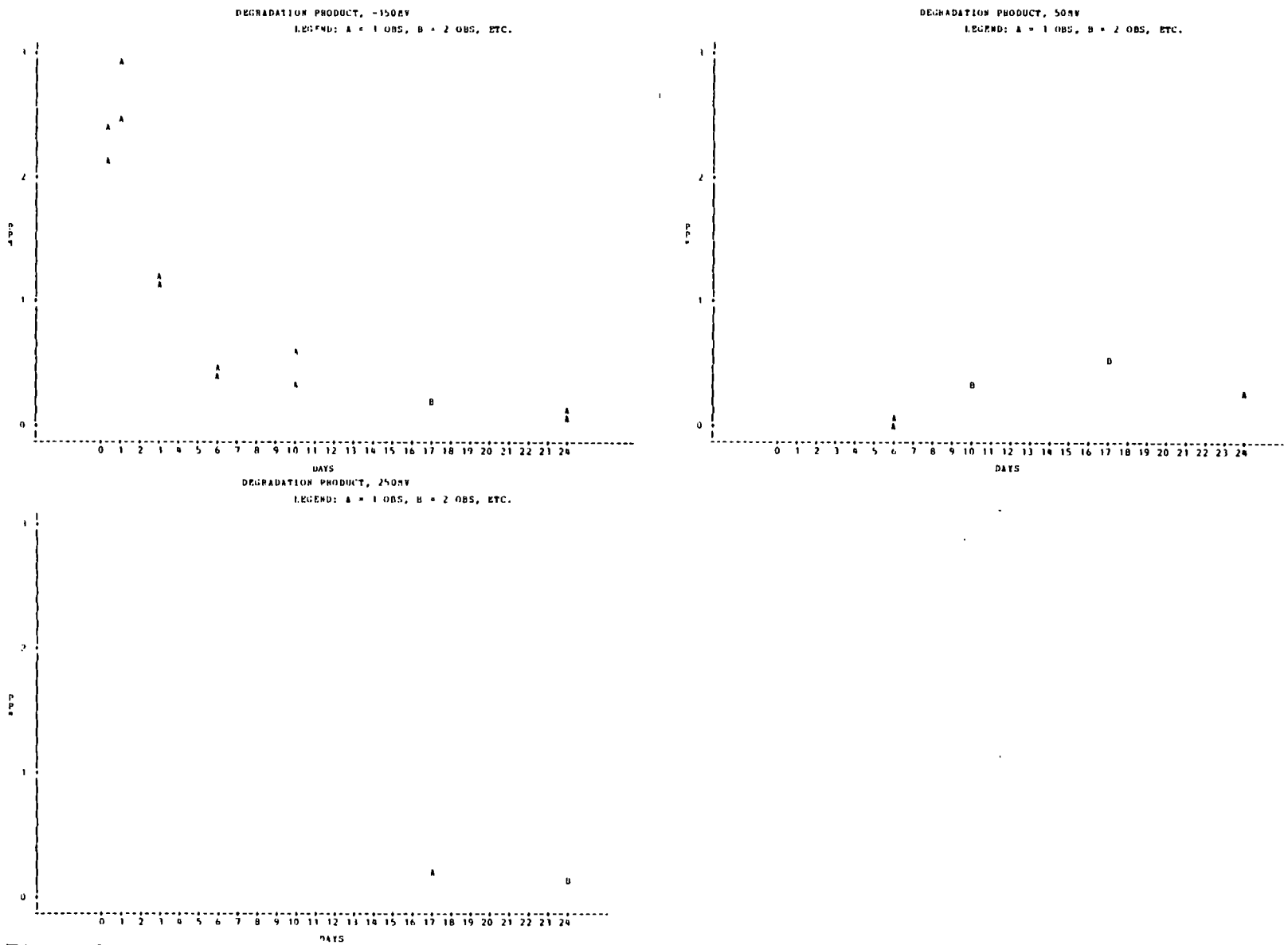

Figure 7. Effects of Controlled Redox Potential on Recovery of Compound E From a Bayou Chevreuil Sediment (Incubated at pH 7.0).

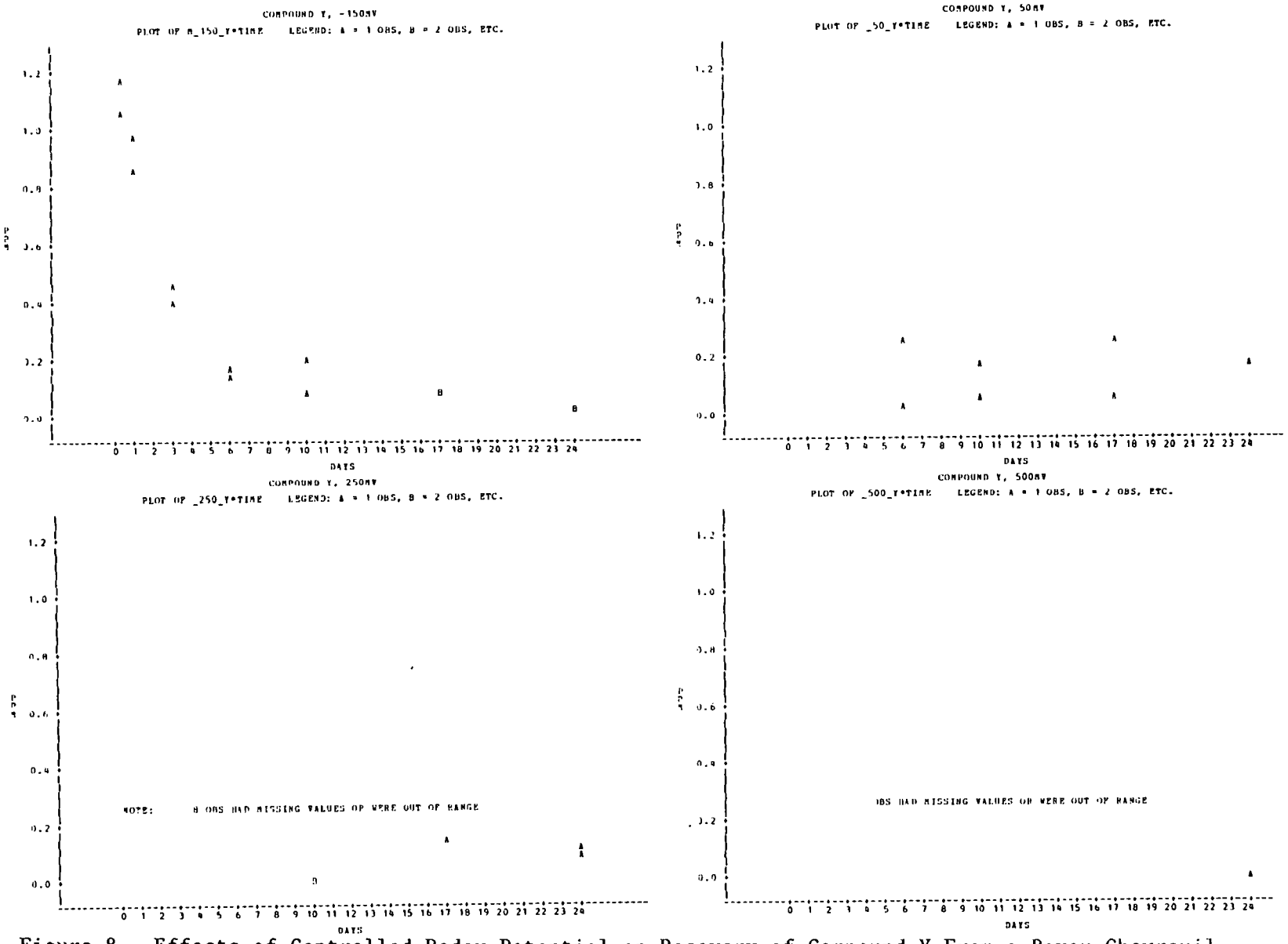

Figure 8. Effects of Controlled Redox Potential on Recovery of Compound Y From a Bayou Chevreuil Sediment (Incubated at pH 7.0).

Compound E, comparison of Fig. 8 with Figs. 6 and 7 indicate the conclusions given about Compound E in the previous paragraph are applicable to Compound Y.

Fig. 9 shows the relative recovery of another compound in the sediment suspensions, shown to be a contaminant or impurity associated with Trifluralin. The original dosing solution was checked for this impurity. This compound, N-nitrosodipropylamine, initially was present at about 2.5 to 3 μg/g for the three highest oxidation treatments. Its low levels or absence at -150 mv may be due to chemical instability under strongly reduced conditions. Because of the health hazard from nitroso compounds, the environmental chemistry of this impurity may be of equal or greater importance than that of the primary compound. If this is an impurity, it is apparent from Fig. 9 that its potential for an adverse environmental impact is negligible in the strongly reduced sediment material and that under better oxidized conditions, its loss from the system occurs at approximately the same rate as Trifluralin.

Methyl Parathion

Methyl parathion was incubated also in the Bayou Chevreuil sediment material at -150, +50, +250, and +450 mv, at pH 6.0 and 8.0 (Figs. 10 and 11). Recovery of this compound decreased very rapidly in the laboratory chambers under all pH and redox potential conditions. Methyl parathion was most persistent under well oxidized conditions, but had decreased to less than one tenth original levels in only 4 da in the most oxidized sediment. Under strongly reducing conditions, recovery decreased to undetectable levels (less than 0.25 ppm as this spiked study was done) at 1 da into the incubation (Fig. 10). At pH 8.0 where spiking levels were around 15 μg/g, recovery had decreased to around 2.5 μg/g within 4 hr. This very rapid loss may be due to factors other than those attributable to biological degradation. It is possible that under the strongly reducing environment, conditions are favorable for rapid chemical or enzymatic decomposition. Based on the similarity of this compound and information available for parathion (ethyl parathion), nitro group reduction may be involved in the rapid alteration of the methyl parathion molecule.

Wahid et al. (1980) reported the almost instantaneous degradation of parathion (ethyl parathion) when the compound was shaken with a pre-reduced soil. Aminoparathion was the major degradation product. The parathion levels decreased to about 44% and 12% of original spiking levels after 5 sec and 30 min of equilibration, respectively.

It is interesting to note that a change in oxidation-reduction conditions affects the apparent degradation rate of Guthion and methyl parathion differently. For example, transport of methyl parathion into anaerobic sediments will enhance the loss of methyl parathion and inhibit the removal of Guthion.

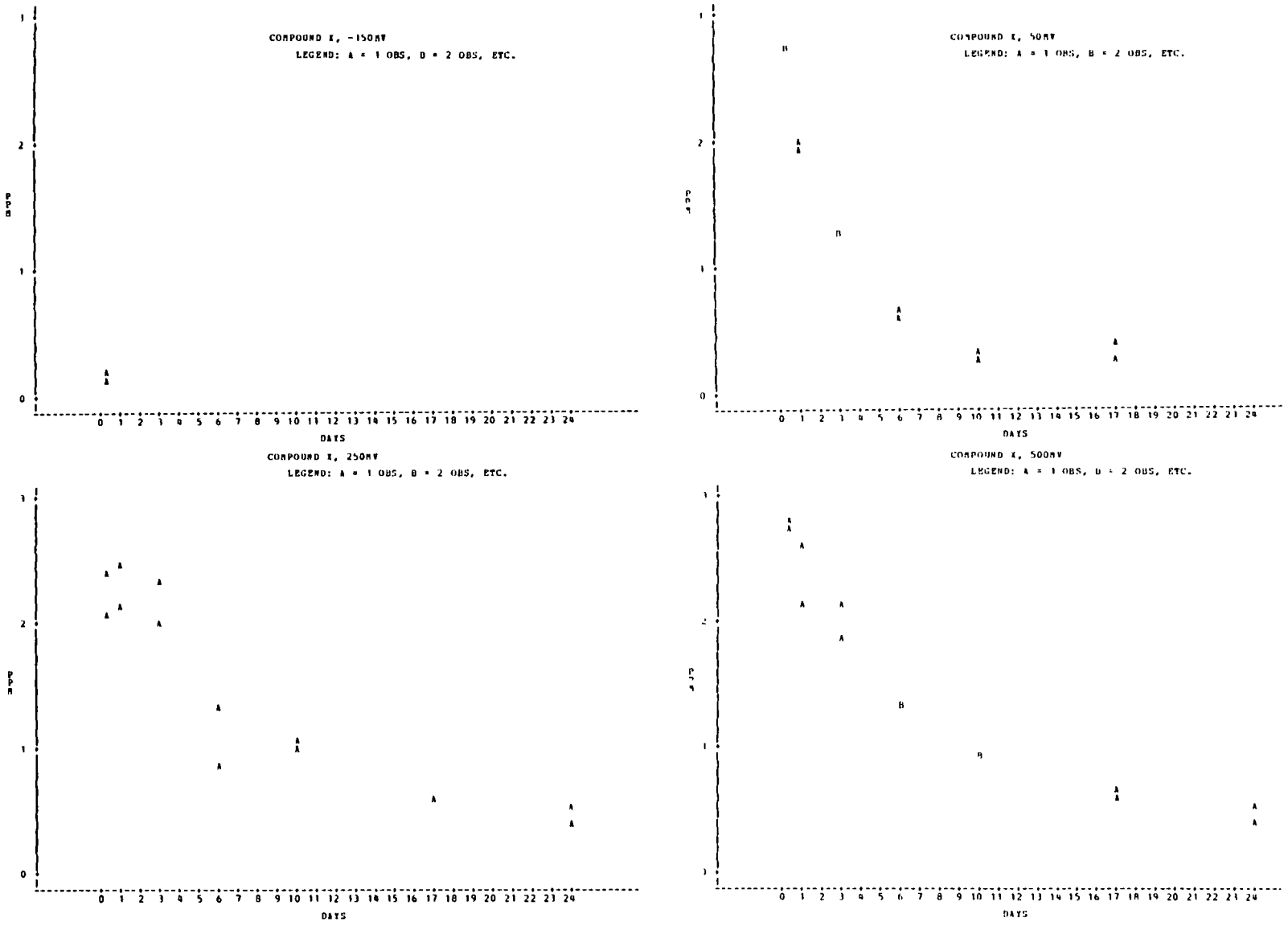

Figure 9. Effects of Controlled Redox Potential on Recovery of Compound X From a Bayou Chevreuil Sediment (Incubated at pH 7.0).

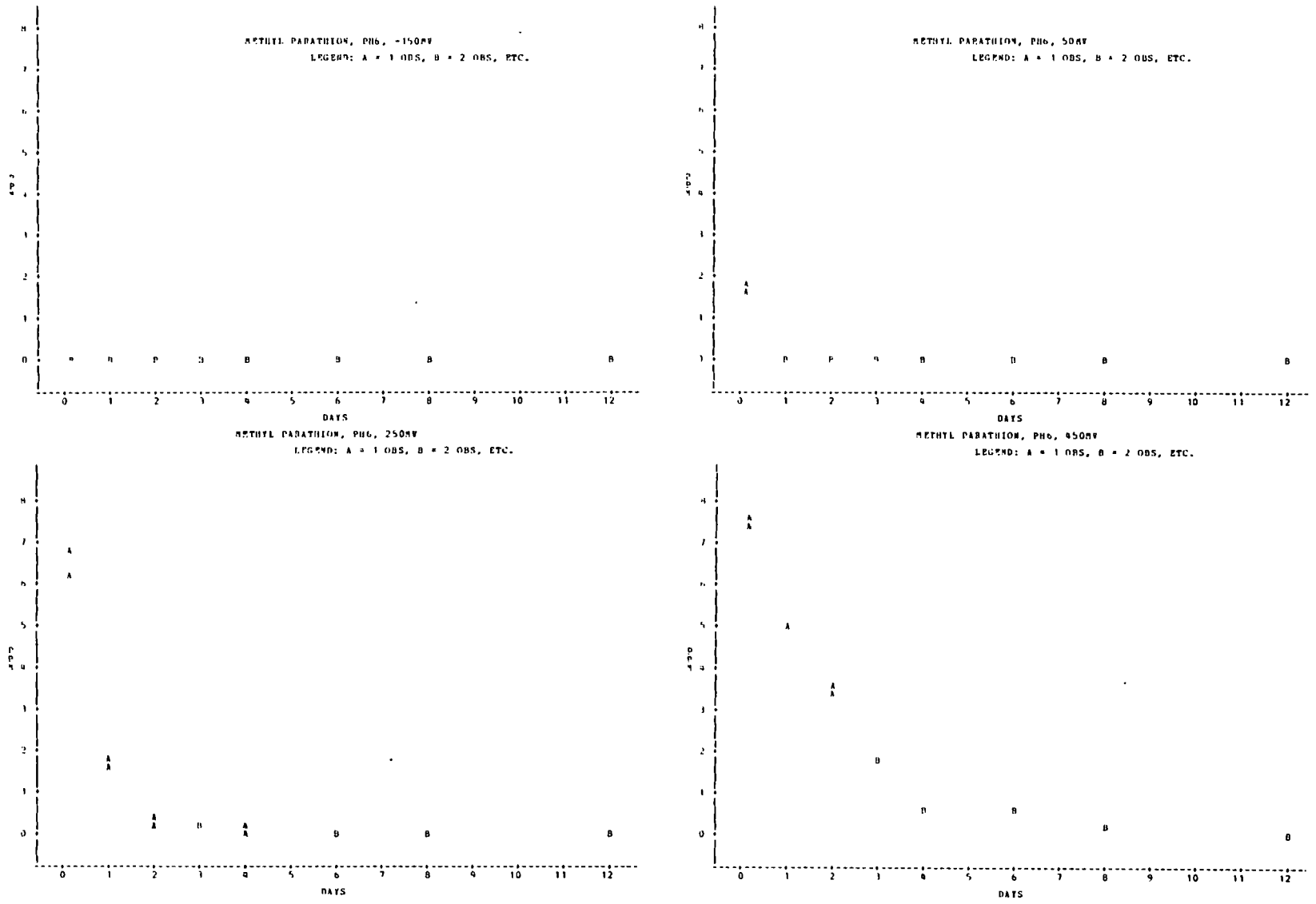

Figure 10. Effects of Controlled Redox Potential on Recovery of Methyl Parathion From a Bayou Chevreuil Sediment (Incubated at pH 6.0).

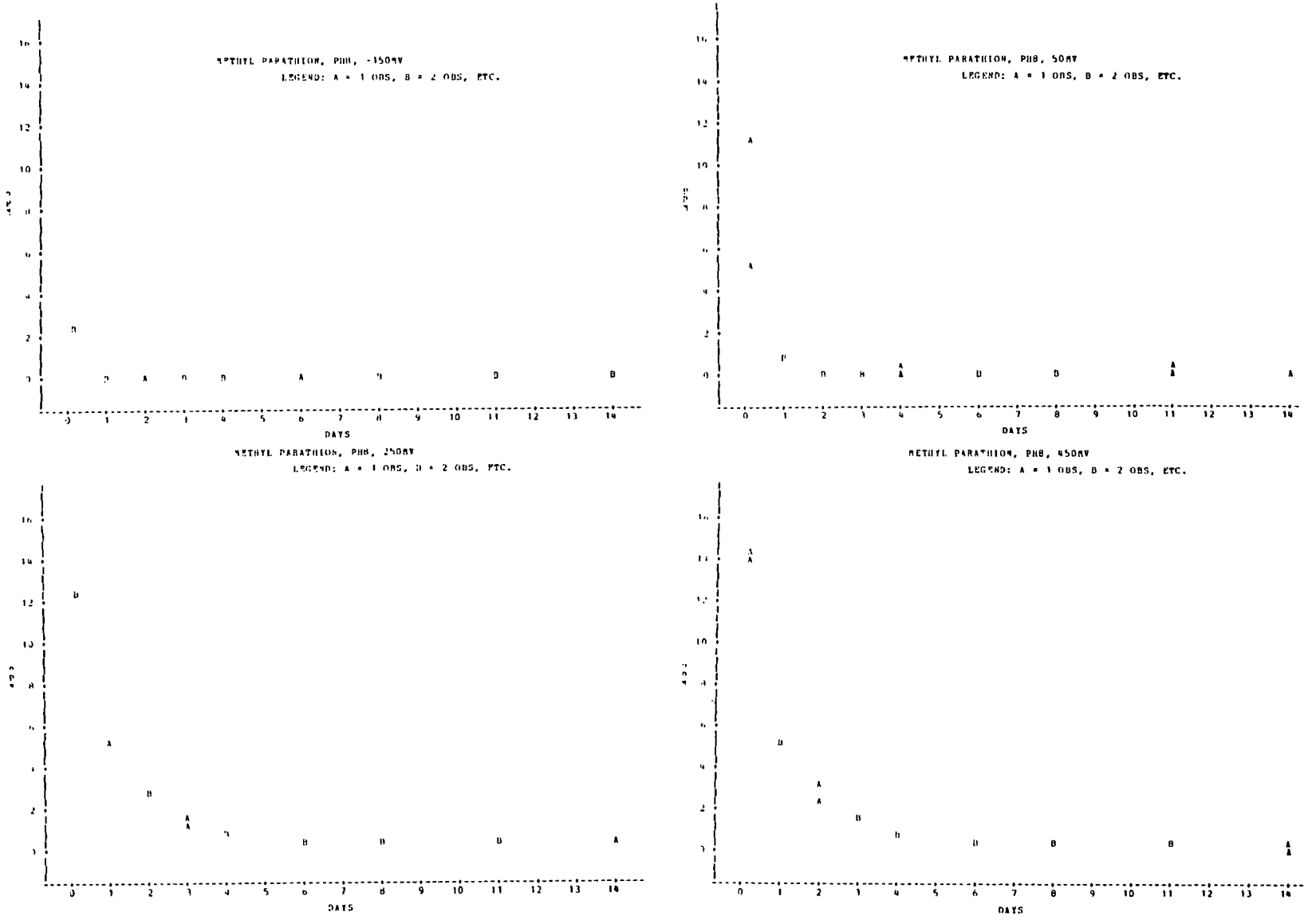

Figure 11. Effects of Controlled Redox Potential on Recovery of Methyl Parathion From a Bayou Chevreuil Sediment (Incubated at pH 8.0).

2,4-D

The herbicide 2,4-D was incubated in a Bayou Chevreuil sediment suspension under controlled pH and redox potential conditions as described for Guthion, Trifluralin, and methyl parathion.

Sample aliquots were stored in a freezer until extraction and methylation procedures were tested to confirm proper reproducible recoveries. Unfortunately, a power failure occurred during this period for an undetermined amount of time and thus it must be assumed that the sample integrity was not maintained during the thaw. A very few samples were extracted to determine if oxidation-reduction effects on degradation were indicated, though any trends must be interpreted carefully. The data indicate that 2,4-D may be more persistent under strongly reduced conditions (Table 3), but further studies of the effect of redox potential on the environmental fate of 2,4-D are needed.

Table 3. 2,4-D Recovery from Bayou Chevreuil Sediment Material Incubated under Controlled pH and Redox Potential Conditions†.

Incubation Time, Days	Redox Potential, mv	
	-150	+500
	2,4-D, μg/g	
2	11.1‡	10.3* ± 0.05
17	4.9 ± 1.6	0.04 ± 0.00
26	3.7 ± 1.1	.08‡

†These samples were subject to thawing for an undetermined period during storage. The data should not be considered reliable except possibly as an indication of relative redox effects.

‡Duplicate sample unavailable.

*Mean and standard deviation.

CONCLUSIONS

This study has demonstrated that oxidation-reduction conditions of soils and sediment-water systems have an important effect on the persistence of synthetic organic pesticides in the environment. Of the insecticides studied, Guthion degradation was enhanced under oxidizing conditions while methyl parathion, another organophosphate insecticide, degraded more rapidly under anaerobic conditions.

The herbicide Trifluralin degraded more quickly under reducing conditions. Preliminary evidence indicates that oxidation-reduction conditions affect the persistence of the herbicide 2,4-D, but this cannot be quantified from the microcosm studies conducted because of problems in storing the samples before analysis. Most studies on the environmental persistence of pesticide residues have been with aerobic, agricultural soils. Compared to typical agricultural soils, sediments, especially those of wetlands, tend to be weakly to strongly reducing, near neutral in pH, and characterized by a somewhat higher clay and organic matter content than agricultural soils of the same area.

The different chemical, and especially microbiological characteristics, of wetland sediments compared with soils can have important consequences on mobility and degradation of toxic compounds. For example, Permethrin, a synthetic pyrethroid, is very toxic to certain aquatic animals and the compound is considerably more stable in anaerobic sediments than in the agricultural soils from which the residue may be transported (Jolley et al. 1978; Gambrell et al. 1981). Persistence studies based on degradation rates in aerobic soils may not accurately indicate the residence time and potential impact of the residues which move into wetlands during periods of runoff.

This was an intensive study of oxidation-reduction effects on a few selected compounds used adjacent to Louisiana wetlands in a very limited number of soil and sediment materials. The importance of oxidation-reduction conditions on the environmental chemistry of pesticide compounds is indicated. However, the different soils and sediments with which residues become associated differ greatly in their physical and chemical properties, thus affecting the fate of pesticides. Moisture content, temperature, and continuous stirring of research materials in laboratory reaction chambers affect chemical and biological processes in that while the data indicate the relative effects of oxidation-reduction conditions on degradation, such information probably do not reflect the actual rates at which the compounds would degrade under in situ conditions.

Because noteworthy oxidation-reduction effects have been demonstrated, additional research is needed to provide information on physicochemical effects on the degradation rates of pesticides under a variety of natural field conditions.

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SECTION 3

IN SITU/MICROCOSM INVESTIGATIONS AND MICROCOSM EVALUATION AND VALIDATION

S. P. Meyers and R. J. Portier

INTRODUCTION

In conjunction with studies on the impact of selected xenobiotics on microbial ecology and substrate turnover, efforts have been directed toward development of analytical approaches to simulate in situ activities in controlled laboratory systems that are both accurate and reproducible and can be subjected to critical program evaluation.

The mechanism used and reported here comprised a microcosm, or microenvironmental system, to simulate the target field environment under closely monitored laboratory conditions. The application of the microcosm approach in assessing effect of toxic chemicals is documented by Hague (1980) and has been discussed by other investigators (Pritchard et al. 1979; Portier 1979). In general, use of microcosm technology in analyses of terrestrial ecosystems and in assessment of fate, transport, and effects of xenobiotics is well established (Giesy 1980), although there is minimal documentation in wetland ecosystems.

A variety of model ecosystems or microcosms have been developed in approaching environmental problems (Gillett and Witt 1977; Bourquin and Pritchard 1979). Close coupling of field and laboratory (microcosm) information, as proposed by Gillett (1980), facilitates development of valid predictive models and provides useful quantitative data on in situ interactions between xenobiotic(s) and the ecosystem. Furthermore, analyses of microcosm data allow a better understanding of abiotic and biotic factors that affect toxic chemical breakdown over a range of salinity conditions. However, methodologies developed for terrestrial studies may not be applicable to investigations in aquatic regimes. Primary differences involve the long-term effects of water itself. Thus, microcosm work in wetland or flooded ecosystems, especially those with diverse salinity gradients, is generally lacking. These and other factors affecting fate of chemicals have been described recently (Anon. 1981).

Our studies involved three correlated areas of investigation: the design and evaluation of continuous flow and static microcosms, presentation of microcosm-generated information on chitin substrate turnover and pesticide hazard, and study of data factors and analytical approaches needed to formulate predictive models for use in hazard assessment. The microbiological

phase has included monitoring of alterations in total heterotrophs and specific physiological groups in both in situ and microcosms, to establish utilization of substrates, such as chitin and enzyme activity related to pesticide levels. Field studies have been designed to detect in situ changes in total microbial biomass and its composition (diversity index) and a preliminary indication of compound half-life.

Major attention has been given to Guthion with comparisons made with another organophosphorus compound, methyl parathion. Additional comparative studies have been made with the organochlorine compound, Kepone®. Use of chitin in our investigations is based on its abundance in aquatic ecosystems and its key role in recycling of carbon and nitrogen in aquatic environments (Seki 1965; Hood 1973; Hood and Meyers 1977, 1977a).

Microcosms for microbiological studies and physicochemical analysis have been used in the work reported here to determine the behavior of the different pesticides under diverse pH, redox, and chemical conditions. The experimental approach combines field and microcosm methods, with the latter employing a variety of determinants, including microbial indexes, enzymatic activities, respiratory response, and transformation of key substances such as chitin and cellulose. Ultimate application of this composite information will be valuable in development of protocols for analyses of xenobiotics, and evaluation of their impact on critical environmental processes.

EXPERIMENTAL PROCEDURES

Study Site and Sample Collection

Soil and sediment collections were made in the Lac des Allemands environment of the Barataria Bay drainage basin, Louisiana (see Map, Fig. 1). This is an interdistributary region bordered by natural levees of the Mississippi and Bayou Lafourche, the latter an abandoned distributary of the Mississippi River. Several distinct vegetation zones are evident. A band of saline marshes borders the Gulf of Mexico; inland, there are successive zones of brackish and freshwater marshes and swamp forest. The regions of the basin most directly affected by runoff are the brackish and fresh areas, located adjacent to intensively cultivated agricultural fields.

Cores consisting of aluminum cylinders, 60 cm tall x 7.6 cm diameter, were inserted into the sediment to the water level, capped and sealed, and stored on ice for transportation to the laboratory for microbial analysis. In drier sites, an inclined trench to a depth of 60 cm was dug with an undisturbed wall at one end for aseptic sampling. All samples were processed within a 12 hr period. Sediment from the particular sites was used to inoculate individual microcosms and to categorize the major microbial groups present.

Enumeration of Microorganisms

For enumeration of total heterotrophic aerobes and anaerobes, sediment samples were appropriately diluted with phosphate buffer, pH 7.2. Replicate 0.1 ml samples were added by the pour plate method to standard plate count

agar (Difco) incubated for three days at 26 C, followed by enumeration of colony forming units (CFU). For anaerobic studies, proper atmospheric conditions were maintained by using a disposable hydrogen/carbon dioxide envelope (Gaspak) in the presence of a cold catalyst in an anaerobic jar. A methylene blue indicator strip was used to insure anaerobiosis. Plates were incubated at 26 C and examined every 24 hr.

Four groups of microorganisms, i.e., bacteria, actinomycetes, filamentous fungi, and yeasts, were enumerated in terms of biomass present based on colony forming units (CFU). Appropriate replicate dilutions were made of all sediment samples using the pour plate method. For enumeration of bacteria and actinomycetes, replicate 1.0 ml aliquots were added to Jensen's agar medium. Cycloheximide (Sigma), an antifungal agent, was added at 40 μg/ml to inhibit overgrowth by filamentous fungi. For enumeration of filamentous fungi and yeasts, Martin's agar medium was used together with streptomycin (Sigma) and chlortetracycline (Sigma) at 30 μg/ml to retard bacterial growth. All plates were incubated at 30 C and examined after three days. Colonies were enumerated using a scan dissection microscope to distinguish the four major morphological groups.

Chitinoclasts were enumerated using a chitin medium containing 2.5% precipitated chitin (Sigma) plus mineral salts (Hsu and Lockwood 1975). Preparation of the precipitated chitin followed the methods of Okutani (1966) and Hood (1973). Pure chitin, ball-milled for 72 hr at 4 C, was dissolved in concentrated HCl in quantities of 10 g/150 ml. The dissolved chitin was added to a large volume of distilled water. The milky white precipitate was washed repeatedly to remove acid, and the solution adjusted to give a value of 10 mg/ml at pH 7.2. The plates incubated at 30 C were inspected after three days. Colonies exhibiting clearing zones were diagnostic of chitin utilization.

Enzyme Assay

Chitinase--

The assay for chitinase activity followed the method described by Okutani (1966) using the N-acetyl-D-glucosamine assay (Reissig et al. 1955). A crude enzyme extract was obtained by filtering 10 ml of culture broth aseptically collected through a sterile 0.45 μ cellulose acetate filter. One milliliter of cell-free liquid was added to an enzyme assay solution (Hood 1973), incubated for seven days at 30 C, and N-acetyl-D-glucosamine determined spectrophotometrically at 585 nm.

Phosphatase--

Phosphatase activity was determined using the procedure outlined by Tabatabai and Bremner (1969), and modified by Atlas et al. (1977). Replicate 2 g aliquots of control and pesticide-treated sediment were placed in 150 ml Erlenmeyer flasks with 0.1 ml toluene, 4 ml sterile distilled deionized water and 0.5 mg/ml p-nitrophenol phosphate (Sigma). Flasks were incubated at 30 C for 100 min. With the addition of 1 ml of 0.05 M calcium chloride, 4 ml of 0.5 M sodium hydroxide and 20 ml of distilled deionized water, each sample

was centrifuged at 10,000 x g for 10 min and the resulting yellow solution assayed at 410 nm for p-nitrophenol production. In subsequent microcosm studies, replicate 1 ml aliquots were assayed for phosphatase activity using the procedure outlined above.

Dehydrogenase--

Dehydrogenase determination was based on formation of 2,3,5 triphenyltetrazolium formazan (TPF) from a standard of 2,3,5 triphenyltetrazolium chloride (TTC). Replicate 10 gm aliquots of control and pesticide-treated soils were placed in 250 ml Erlenmeyer flasks and brought to 100% water-holding capacity with addition of a 0.5% solution of 2,3,5-triphenyltetrazolium chloride (Sigma), as described by Bartha et al. (1967). Each flask was flushed with O_2-free nitrogen, tightly stoppered, and incubated for six hrs (Casida 1977). TTC is converted by dehydrogenase enzymes to 2,3,5 triphenyltetrazolium formazan forming a characteristic red color. The latter was extracted with 50 ml of spectroscopic grade methanol (Baker Chemical), centrifuged and filtered through No. 5 filter paper (Whatman). Absorption was measured at 485 nm and dehydrogenase activity units expressed in mg/ml TPF. In subsequent microcosm studies, replicate 2 ml aliquots were assayed for dehydrogenase activity using the procedure outlined above.

Adenosine Triphosphate--

An adenosine triphosphate (ATP) assay, as advanced by Holm-Hansen (1966) and further presented by Karl (1980) and Stevenson et al. (1979), was used for determination of microbial biomass. In our earlier investigations, releasing reagents from a variety of commercial manufacturers, designed for specific release and quantification of microbial and somatic ATP, were used. These reagents allow selective permeable release of nucleotides without cell lysis. Thus, nucleotides are released without being broken or changed by the enzymes of the cells, and ATP measurement can be achieved with minimal interfering effects as noted by Stevenson et al. (1979). Excellent reproducible assays were achieved with a short preparation time. Use of selective applications of these reagents (somatic and microbial) provides good differential quantifications of ATP. Furthermore, only 200 µl of sample is needed for easy assay.

Gas Chromatographic Techniques

Gas chromatography methods for determining Guthion concentrations in soil and water samples are those outlined in the previous section, along with an outline of these procedures described in detail. The recovery efficiency for azinphosmethyl extraction is 98 to 102% for spiked swamp surface waters and 65 to 70% for both oxidized and reduced sediment materials. Identifications were based on comparisons with known standards of each xenobiotic.

Data Analyses

Statistical methods used have included multivariant analyses to test the validity and reproducibility of the design features of the microcosm, together with SAS and Fortran programming approaches. Evaluations and tests of

the variables considered, including microbial population composition, enzyme response, and tagged compound turnover, have all been based on repeated runs of replicate sets.

Description of the Microcosms

Continuous-Flow Units--

To maintain a more precise controlled environment to study microbial/substrate changes induced by pesticide addition, a continuous flow-through microcosm (Portier 1979) has been developed and evaluated (Fig. 12A). The initial unit designed consisted of a 1000 ml tall form beaker (Pyrex brand, Corning #1060) to which was attached an overflow spout to prevent possible buildup of inoculum due to blockage in the system. Pesticide was pumped into the beaker by means of a peristaltic pump (Harvard Aparatus Model #1203) through a sterile medical grade tubing (Silastic brand, 1/8 ID X 1/4 OD) having negligible pesticide sorption characteristics. Accurate flow rates as low as 0.0042 ml/min were obtained.

Upon entering the reaction vessel, the pesticide is uniformly distributed into the sediment sample as a result of air (or nitrogen) sparging through a Buchner-type funnel (Corning #6060) attached and replacing the bottom of the beaker to promote an aerobic (or anaerobic) environment. Original designs consisted of air sparging of the sample as it was stirred intermittently by means of a magnetic stirrer.

The peristaltic pump maintained the desired level of water and sediment by removing excess water from the system at the same rate as pesticide addition. A sediment trap at the top of the column prevents sediment loss to the system and allows for the re-introduction of possible sediment accumulation to the reaction vessel. Excess water was collected in 4000 ml aspirator bottles (Corning #1220) to monitor pesticide loss to the system and maintain aseptic conditions. A 60 ml separatory funnel (Corning #6420) placed at the top of the sedimentation column effectively recovered lost sediment.

A temperature of 28 C was maintained by means of a heat lamp regulated by a temperature controller (Versa Therm Model No. 2158) with a sensitivity of ±.06 C. In earlier designs, a water bath heated with standard aquarium heaters was used, however, a uniform temperature could not be maintained. The pH of the reaction vessel is maintained at the desired level by a pH meter/controller (Horizon Model No. 5997) connected to another peristaltic pump (Masterflex Model No. 7545). Accuracy is within a 0.05 pH unit.

Samples can be aseptically withdrawn from the reaction vessel by means of a micropipette or syringe. Due to the uniqueness of the surgical grade tubing used, the entire assembly can be autoclaved and sterile conditions can be maintained for prolonged periods of time.

The design of this bench top microcosm allows introduction of a variety of analytical features including: variation in flow rate and sequential

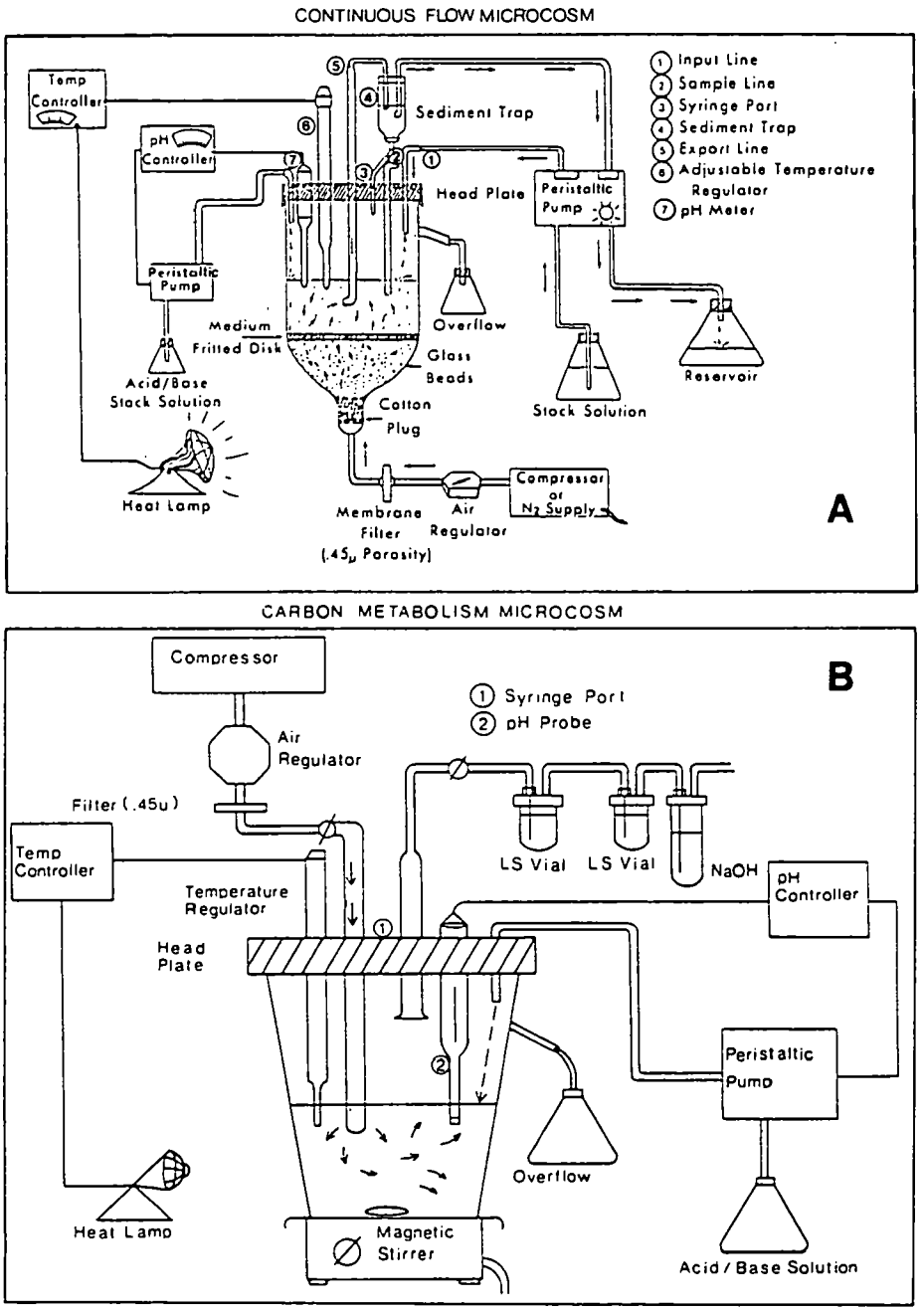


Figure 12. Diagram of Microcosm Units: (A) Continuous Flow; (B) Carbon Metabolism Unit.

compound addition; control of pH/Eh, temperature, O_2, and salinity; maintenance of various soil/water conditions; generation of controlled quantitative data. The entire apparatus is capable of maintaining the desired parameters of temperature, flow rate, and pH for a period of five weeks or longer.

For the controlled microcosm studies, sediment from the Lac des Allemands site was placed into four continuous flow microcosms. Approximately 50 g (wet weight) of sediment was introduced with appropriate amounts of filter-sterilized (0.45 μ porosity cellulose acetate filter) site water to give a final volume of 500 ml. Two microcosms served as controls while test solution of the xenobiotic was introduced into the two test microcosms following equilibration. The test solution, added at varying flow rates consisted of filter-sterilized site water, followed subsequently by site water amended with the xenobiotic for test microcosms. A 10 ppm solution of the pesticide azinphosmethyl (Guthion) was used in preliminary studies. In tests with methyl parathion (Chem Service) or the organochlorine Kepone® (Applied Science Labs., Inc.), solutions were added from a stock solution to 5 ppm and 0.5 ppm, respectively in a final microcosm volume of 1 L. Temperature was maintained at 29 C with the pH at 7.2.

Carbon Metabolism Studies--

A static microcosm (Fig. 12B) was originally designed for studies involving analyses of chitin breakdown rates by production of N-acetylamino sugars in chitin-supplemented reaction chambers. The substrate used was ^{14}C-labeled N-acetyl-D-glucosamine, with subsequent analyses of $^{14}CO_2$ production via liquid scintillation techniques.

Carbon Metabolism Units--

To study effects of pesticide addition on respiration rates and biomass accumulation of labeled ^{14}C-compounds, a static microcosm (Portier 1979) equipped to trap $^{14}CO_2$ by liquid scintillation vials was designed (Fig. 12B). The reaction vessel consisted of a 1000 ml tall form beaker (Corning #1060) firmly supported and immobile to prevent spillage of radioactive material. In the initial experiments N-acetyl-D-glucosamine (glucosamine 1-^{14}C) [New England Nuclear #723], added to non-labeled compound, was introduced into the sealed reaction vessels by means of syringe. Guthion (non-labeled) and other test compounds were also added by this method.

Air sparged into each reaction vessel facilitated entrapment of $^{14}CO_2$ by liquid scintillation vials containing a complete oxidizer cocktail (Oxi-Fluor - CO_2; New England Nuclear). Samples were aseptically removed by means of micropipette or syringe. Temperature and pH were controlled by equipment and methods previously described for continuous flow microcosms.

Sediment from the field site was dispensed in 25 g aliquots into four static microcosms with 400 ml of sterile distilled deionized water. A labeled solution and a non-labeled aliquot of N-acetyl-D-glucosamine (NAG) was added to each microcosm to give a final concentration of 0.1071 μ Ci/500 ml. The specific activity of labeled N-acetyl-D-glucosamine was 58.18 mCi/mmol. The

final concentration of both labeled and non-labeled NAG was 40 ppm. Temperature of the vessels was maintained at 28 C with a pH range of 6.8-7.2. Two microcosms served as controls while the test chambers included a final pesticide concentration of 10 ppm Guthion. Radiolabeled Guthion was not available for these initial studies.

In subsequent carbon metabolism studies with methyl parathion and Kepone, sediment from the field site was dispensed into replicate series of four static microcosms with 500 ml of filter-sterilized site water for each compound. In studies with methyl parathion, a uniformly labeled ^{14}C-methyl parathion solution (Pathfinder Laboratories) was used with a non-labeled solution (Chem Service) to give a final solution of 5 ppm (1.0 μ Ci/500 ml). An air-dried ball-milled chitin material obtained from the freshwater red swamp crawfish (Procambarus clarkii) was added to each microcosm at 0.25 mg/g sediment wet weight to monitor substrate effects. For similar studies with Kepone®, a ^{14}C-Kepone® solution (Pathfinder Laboratories) was used with a non-labeled solution (Applied Science Labs, Inc.) to give a final solution of 0.5 ppm (1.0 μ Ci/500 ml).

A moderate airflow into each microcosm allowed for entrapment of $^{14}CO_2$ by liquid scintillation vials containing 15 ml of complete oxidizer cocktail (New England Nuclear). To determine uptake rates of labeled compound, replicate one milliliter aliquots from 10^{-5} serial dilutions were filtered through 0.45 μ membrane filters. Each filter was then rinsed with sterile distilled deionized water (buffered, pH 7.2) to remove excess compound not utilized by the microbial biomass. Filters were dried and placed in scintillation vials containing 15 ml of phase combining scintillant (Amersham). Membrane filters were previously checked for sorption characteristics. Sorption from standards accounted for less than 0.2% loss.

All scintillation vials were counted for 10 min on a Beckman 250 liquid scintillator. A present error of 0.2% was used in conjunction with the external standard ratio mode for quench correction.

Axenic Flask Study--

Representative members of the four morphological groups, from azinphosmethyl, methyl parathion and kepone microcosms, were isolated using axenic culture techniques to screen for possible compound sensitivity and/or utilization. Sensitivity to the xenobiotics methyl parathion and Kepone® was determined by relative changes in ATP levels compared with controls. Assimilation rates of each radiolabeled compound was determined by methods outlined in the previous microcosm section. Total biotransformation of each compound was based on ^{14}C levels following incubation, and was adjusted for possible abiotic effects using sterile control flasks. Total biotransformation was considered to be the difference between total ^{14}C added minus ^{14}C residual levels and ^{14}C assimilation levels.

RESULTS AND DISCUSSIONS

Initial In Situ, Static, Continuous Flow
And Carbon Metabolism Microcosm Studies

In Situ Enumeration of Total Heterotrophs and Chitinoclasts--

The effect of Guthion on the total heterotrophic and chitinoclastic population in swamp sediments for Field Study No. 1 is shown in Fig. 13.

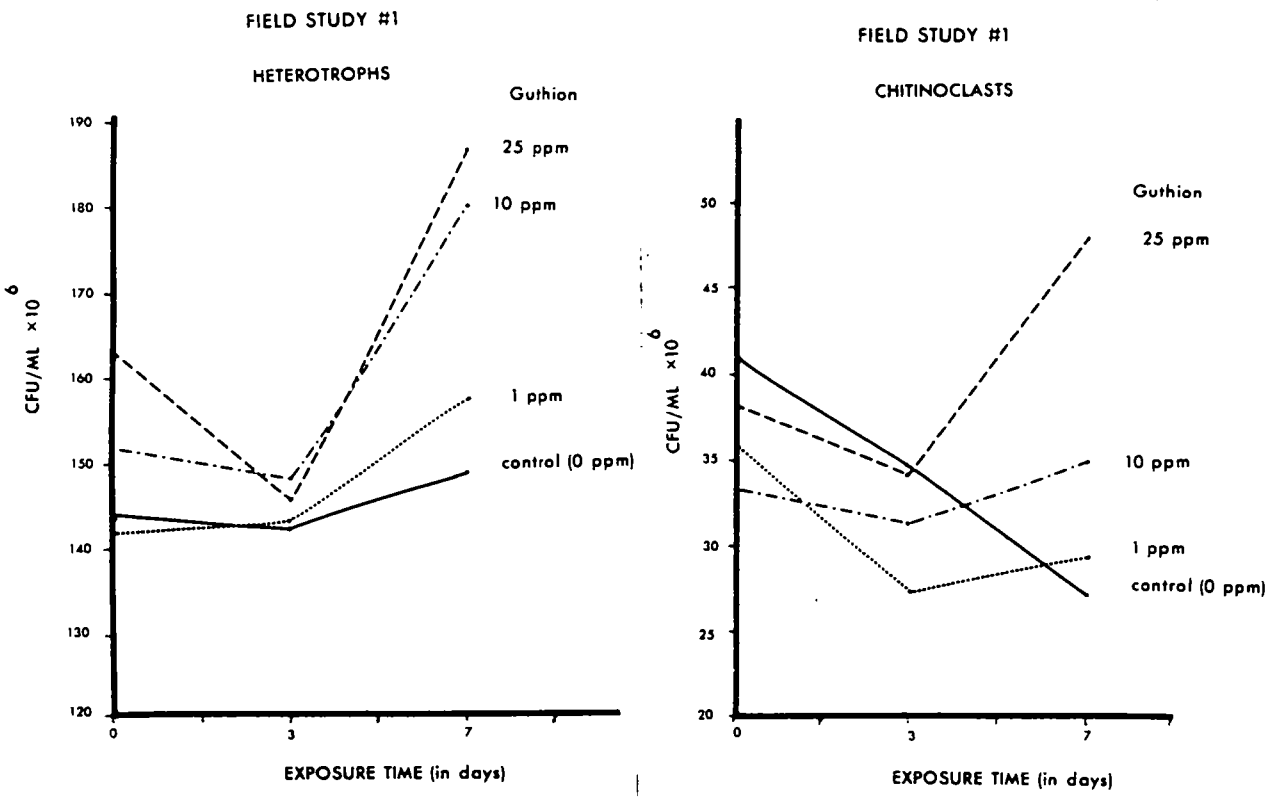


Figure 13. Effect of Guthion on Heterotrophic and Chitinoclastic Population in Swamp Sediments (Field Study No. 1).

Values shown are least square mean values for replicate plots. Statistical analysis revealed a significant difference ($P \leqq .01$) among the treated plots for total heterotrophs. An analysis for chitinoclasts, although not statistically significant ($P \leqq .06$), was strongly indicative of variation within the population due to both presence and level of pesticide. The data suggested that amendment of swamp sediment with Guthion, at concentrations of 1, 10, and 25 ppm, had a pronounced effect on concentrations of both total and chitinoclastic bacteria populations. These rather high concentrations were used based on observations in an earlier study using Guthion, at concentrations of 0.01, 0.1 and 1.0 ppm in which no variations of total heterotrophs or chitinoclasts between control and pesticide amended sites were noted. GC

residue analysis failed to detect Guthion in these sediments. It was uncertain as to whether Guthion indeed had no inhibiting effect at these concentrations or that the concentrations used were too low for our field methodology to detect any variability.

Since the highest variation in bacteria concentrations for Field Study No. 1 occurred at levels of 10 and 25 ppm Guthion, the second field application consisted of additional replicate plots of 0 ppm (control) and 25 ppm, respectively. Fig. 14 shows the effect of Guthion on the total heterotrophic and chitinoclastic populations. Values shown are least square mean values for replicate plots. Statistical analysis revealed no significant differences among the treated plots for heterotrophs and chitinoclasts. The data suggested that pesticide applications at levels of 25 ppm did not contribute to an increase in the bacteria concentration, in contrast with data observed in Study No. 1. There appeared to be a lack of statistically significant increase in bacteria concentrations due to the considerable variation of counts for total heterotrophic biomass within the control plots (0 ppm).

Fig. 15 shows the effect of Guthion in Field Study No. 3 on total bacteria and the chitinoclastic population in swamp sediments amended at levels of 0, 10, 25, and 100 ppm. Values shown are least square mean values

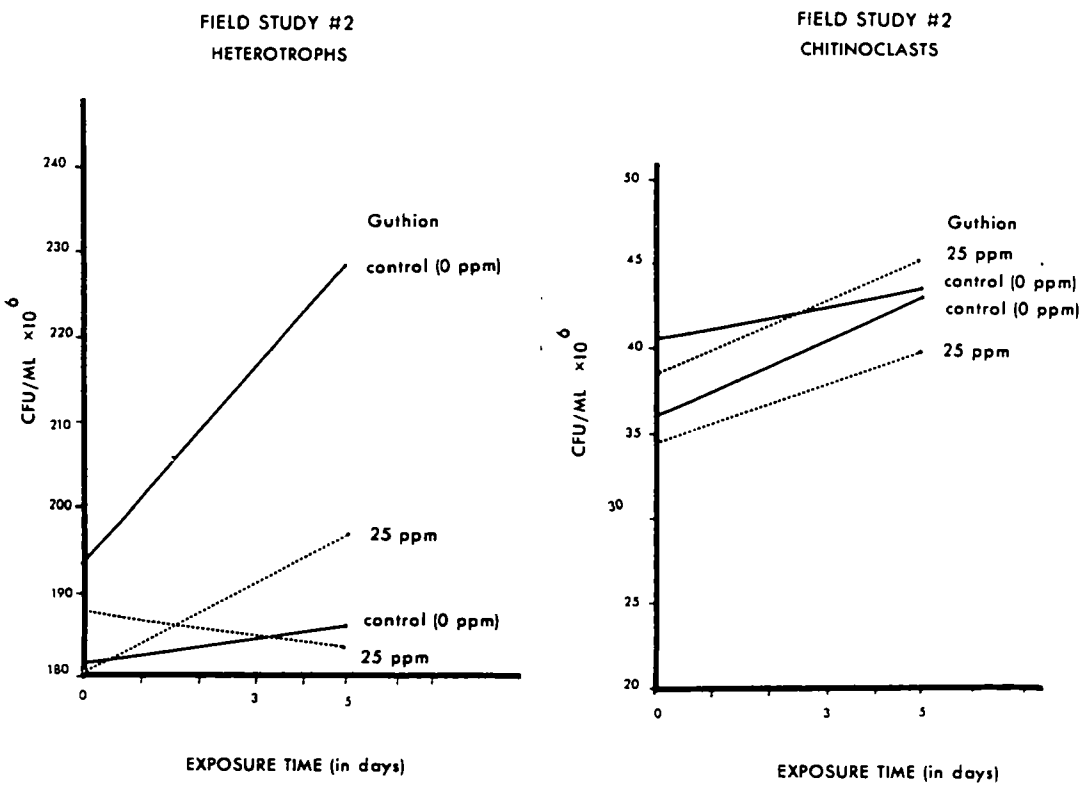


Figure 14. Effect of Guthion on Heterotrophic and Chitinoclastic Population in Swamp Sediments (Field Study No. 2).

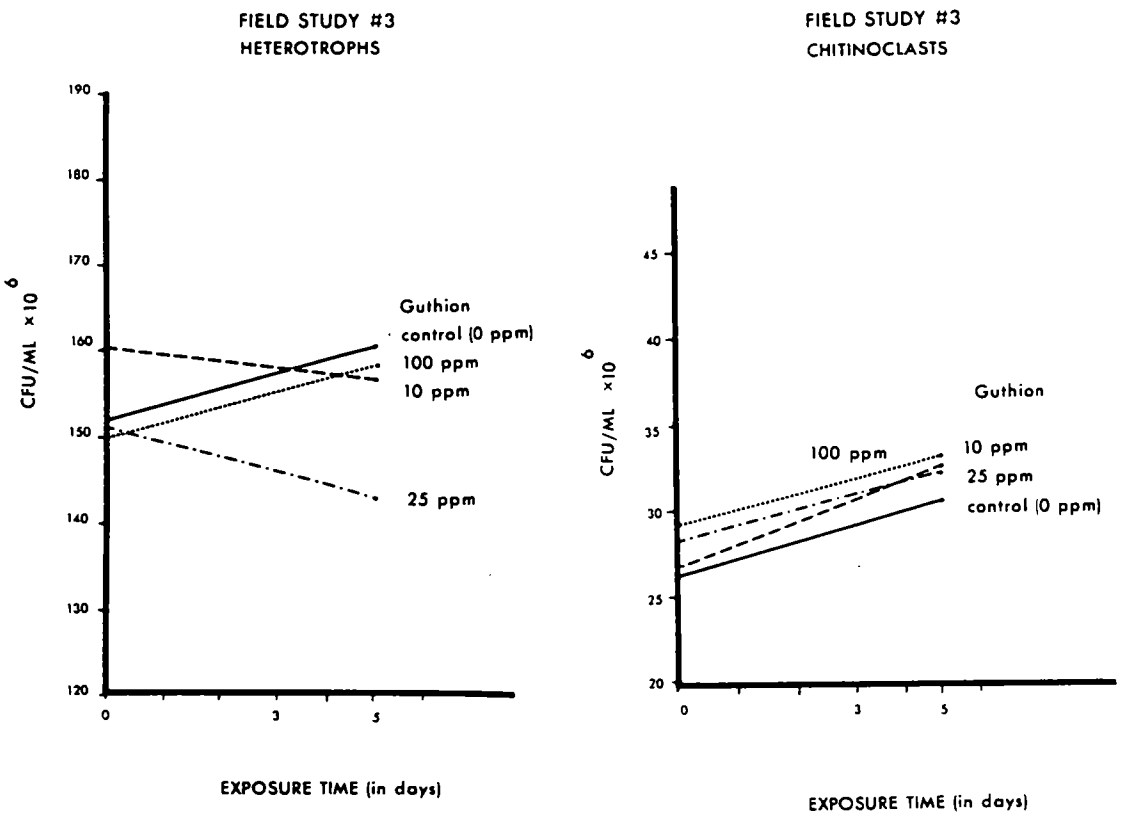


Figure 15. Effect of Guthion on Heterotrophic and Chitinoclastic Population in Swamp Sediments (Field Study No. 3).

for replicate plots. Statistical analysis revealed a significant difference among the treated plots for total heterotrophs ($P \leqq 0.05$), but not for chitinoclasts. The data suggest an inhibitory effect by Guthion in swamp sediments amended at levels of 10 and 25 ppm but a pronounced stimulation of microbial growth in sediments amended at levels of 100 ppm.

As noted, the three field application studies present an inconsistent picture on the effects of Guthion in amended swamp sediments. Field Study No. 1 indicates significant increases in the microbial population due to pesticide levels, in contrast with data from Field Studies No. 2 and 3. Overall, these results are somewhat questionable due to the unknown effects of ground water seepage and intermittent flooding in the impounded swamp. Subsequent microcosm studies have indicated that these sediments enhance breakdown of Guthion, resulting in significant increases in the microbial population. It is entirely possible that such increases in microbial concentration occurred at a very rapid rate prior to sampling for Field Study No. 2 and 3. Sampling may represent a period of population decline following an initial peak.

Changes in microbial biomass as a result of transfer and storage of samples may have contributed to the variability observed. Kaper et al. (1978) noted rapid changes in number of organisms on stored water samples that were not reproducible and did not follow detectable trends. Increases and decreases in counts occurred in samples regardless of whether they were maintained at room temperature, refrigerated, or stored in plastic or glass containers. Immediate sample processing is strongly recommended to minimize variations in the microbial populations of samples from aquatic environments. Numerical data from stored samples are, more or less, crude estimates, although, as noted, field samples were placed in ice immediately and transported to the laboratory for microbial enumeration within 6 hr. for these studies.

Determination of Pesticide Half-Life--

Table 4 shows the residual pesticide concentrations in amended swamp sediments for each field application. Linear regression techniques were used in calculating $T_{\frac{1}{2}}$. Although the small number of samples from each field application provided a limited estimator of $T_{\frac{1}{2}}$ and, hence, reduces the accuracy of half-life prediction, when one looks at all samples, a very short half-life, i.e., from 1 to 2 da, can be seen for all treatments. This is considerably less than the one month half-life for Guthion in water reported by Huer (1974) and Yaron (1974a). Such a short half-life may be attributable to the high microbial activity in the swamp sediment or, as mentioned earlier, to the unknown effects of ground water seepage and intermittent flooding in the swamp forest. Subsequent microcosm studies have demonstrated the high microbial activity prevalent in swamp sediments.

Additional <u>In Situ</u> Studies--

In follow-up <u>in situ</u> studies, estimates of microbial activity, as expressed by numerical estimates of the four major groups of microorganisms, i.e., actinomycetes, bacteria, filamentous fungi, and yeasts, were made at various depths for five distinctive soil environments reflecting variations in land use and management. A brief description of these soil profiles with depth for each sample site are presented in Table 5. It is important to consider the differentiation of these soils which are historically similar by agricultural practices and flooding, both natural and controlled. Most of these soils have considerable concentrations of organic material with varying levels of clay content as a function of depth.

Table 6 shows variations reflected by dissimilarities in total microbiological levels and differences between physiological groups as a function of depth. Table 7 presents analogous site enzyme activity with depth on a dry weight basis. Variations in microbial population correlated with enzyme activity for each soil environment and depth are given in Fig. 16. Soil phosphatase and dehydrogenase activity was examined in terms of bacterial and fungal populations with depth and the particular soil environment. Both of these enzyme levels closely parallel changes in total microbial activity. Bacterial and fungal populations, reflecting microbial population activity, are presented for comparison purposes since they represent distinct microbial groups. However, subsequent analysis of enzyme activity for yeasts and

Table 4. Mean Residual Pesticide Concentrations from Field Application Studies.

Field Application Study No. 1

Enclosure	Initial Conc.	Final Conc.	Percent Recovery	Half-Life ($T_{1/2}$)
A	0	0	0	
B	1 ppm	0	0	
C	10 ppm	0	0	
D	25 ppm	2.48	9.92	2.099 days
			Exposure Time:	7 Days

Field Application Study No. 2

Enclosure	Initial Conc.	Final Conc.	Percent Recovery	Half-Life ($T_{1/2}$)
A	0	0		
B	0	0		
C	25	4.44	17.76	2.005
D	25	3.58	14.32	1.783
			Exposure Time:	5 Days

Field Application Study No. 3

Enclosure	Initial Conc.	Final Conc.	Percent Recovery	Half-Life ($T_{1/2}$)
A	0			
B	10	0	0	
C	25	1.39	5.56	1.199
D	100	4.16	4.16	1.089
			Exposure Time:	5 Days

actinomycetes show a similar relationship. Analyses showed a high correlation ($r = 0.925$) between phosphatase and microbial population activity and between dehydrogenase and microbial population activity ($r = 0.934$).

It is important to note how variations in population/enzyme profiles provide some indication of the impact of land management practices. For example, high levels of activity were noted in the 0 to 5 cm depth for pasture compared with adjacent sugarcane fields. Sugarcane fields were sampled before and after harvesting and burning of the leaf and stem bulk. A 50% drop in microbial activity was noted as a result of this "slash burn" technique. Below the burn zone, microbial and enzyme activity was much higher in

Table 5. Field Sites: Soil Profiles with Depth.

Site Number	Location	Depth	Profile
1	Sugarcane	0-5 cm	fine textured soil, granular, very dry, fire scarred; rapid water runoff
2	Sugarcane	10-20 cm	fine textured soil, granular, moist, considerable root rhizosphere debris
3	Sugarcane	30-40 cm	more pronounced clay content, marbled, iron oxides evident in rusty soil color, moist
4	Pasture	0-5 cm	fine textured soil, highly organic, moist, rapid water runoff
5	Pasture	10-20 cm	fine textured soil, more obvious clay content, moderate root rhizosphere debris, moist
6	Pasture	30-40 cm	high clay content, water table at 50 cm mark, high iron oxides, very marbled
7	Swamp forest	0-5 cm	high litter content, moist but not water-logged; dark, rich soil having a very pronounced musty odor; intermittent flooding
8	Swamp forest	10-20 cm	high clay content, moderate root rhizosphere debris, marbled
9	Swamp forest	30-40 cm	heavy clay below water table
10	Natural swamp	0-5 cm	high litter, overlaying water level 5 cm, fine suspended sediment; perennial flooding
11	Natural swamp	10-20 cm	high clay content, moderate root rhizosphere debris, accumulated litter moderate, moderate clay content
12	Natural swamp	30-40 cm	heavy clay below water table and organic level; very anaerobic
13	Impounded swamp	0-5 cm	extensive litter deposits; finely separated sediment material, overlaying water level, 9 cm-microcosm sediment source; perennial flooding; static water flow
14	Impounded swamp	10-20 cm	moderate litter deposits and root fragments, moderate clay material; microcosm sediment
15	Impounded swamp	30-40 cm	heavy, compact clay below water table and organics level; very anaerobic

Table 6. Fluctuation of Microbial Population with Depth.

Field sites[a]	Filamentous Fungi (10^3)*	Yeasts (10^3)*	Bacteria (10^5)*	Actinomycetes (10^5)*	F,Y:B,A 10^3 : 10^5
1	35.0	2.0	25.0	10.0	1.05:1.0
2	72.0	4.0	335.0	20.0	.21:1.0
3	21.0	<1.0	35.0	14.0	.43:1.0
4	140.0	3.0	226.0	42.0	.53:1.0
5	22.0	<1.0	74.0	17.0	.24:1.0
6	1.0	<1.0	12.0	2.0	.07:1.0
7	88.0	11.0	74.0	18.0	1.07:1.0
8	5.0	1.0	34.0	18.0	.12:1.0
9	1.0	<1.0	10.0	2.0	.07:1.0
10	47.0	12.0	91.0	17.0	.55:1.0
11	3.0	1.0	37.0	14.0	.08:1.0
12	<1.0	<1.0	7.0	6.0	.08:1.0
13	95.0	13.0	145.0	20.0	.65:1.0
14	10.0	1.0	55.0	13.0	.16:1.0
15	<1.0	<1.0	15.0	5.0	.05:1.0

*Colony Forming Units (CFU)/dry weight in grams.

[a]See Table 1: Sites 1-3 Sugarcane; 4-6 Pasture; 7-9 Swamp Forest; 10-12 Natural Swamp; 13-15 Impounded Swamp.

Table 7. Enzyme Activity at Various Sites with Soil Depth.

Site Number	Dry Weight (in grams)	Phosphatase Activity Absorbance	mg/ml*	mg/g	Dehydrogenase Activity Absorbance	mg/ml**	mg/g
1	9.38 g	.170	.030	.032	.198	.06	.064
2	8.89	.465	.075	.084	.674	.17	.191
3	8.80	.263	.047	.053	.324	.09	.102
4	6.30	.367	.073	.116	.584	.125	.198
5	7.12	.457	.044	.062	.291	.165	.232
6	7.68	.257	.013	.017	.104	.085	.111
7	3.25	.287	.067	.206	.489	.100	.308
8	5.59	.345	.037	.066	.244	.121	.216
9	5.99	.203	.014	.023	.116	.075	.125
10	2.40	.209	.048	.200	.329	.076	.317
11	5.40	.231	.044	.081	.289	.080	.148
12	5.49	.087	.010	.018	.094	.030	.055
13	2.22	.254	.057	.257	.391	.084	.378
14	5.26	.407	.022	.042	.159	.140	.266
15	5.34	.130	.007	.013	.065	.045	.084

*p-nitrophenol.

**TTF.

the root rhizosphere area (10 to 20 cm). Differences in moisture content may have played an important role in these variations.

Swamp forest profiles appeared to be comparable to those obtained in pasture except that bacterial and fungal populations are more stratified with depth. This is indicative of the high organic material accumulating on the forest floor as a result of litter fall. Below the 20 cm depth, a significant decrease in microbial and enzyme activity indicates changes in soil organic content and increasing anaerobiosis.

Magnitude of microbial populations between the swamp forest and the impounded swamp were comparable. Higher fungal concentrations were noted for both compared with that present in the natural swamp. Bacterial populations were more comparable in profile, with both the swamp forest and impounded swamp having a higher bacterial biomass than the natural swamp. It is noteworthy that phosphatase and dehydrogenase levels were in close agreement with

microbial population levels, giving indications of the degree of activity of bacteria and fungi present. Dehydrogenase levels were higher for the swamp forest and impounded swamp, probably correlated with the proponderance of high litter fall.

Initial Static Microcosm Studies for Total Heterotroph/Chitinoclast Enumeration--

Fig. 17 shows variations in total heterotrophs in natural and chitin-amended soil to Guthion concentrations of 0, 10, 25, and 100 ppm. Statistical analysis indicated significant variations in total bacterial concentrations ($P < .01$) for both control and chitin-amended soils. Fig. 17 indicates high bacteria concentrations after only two days of exposure to Guthion, especially for soil amended with 100 ppm of the pesticide. A high bacteria concentration after only two days of exposure to the pesticide is seen.

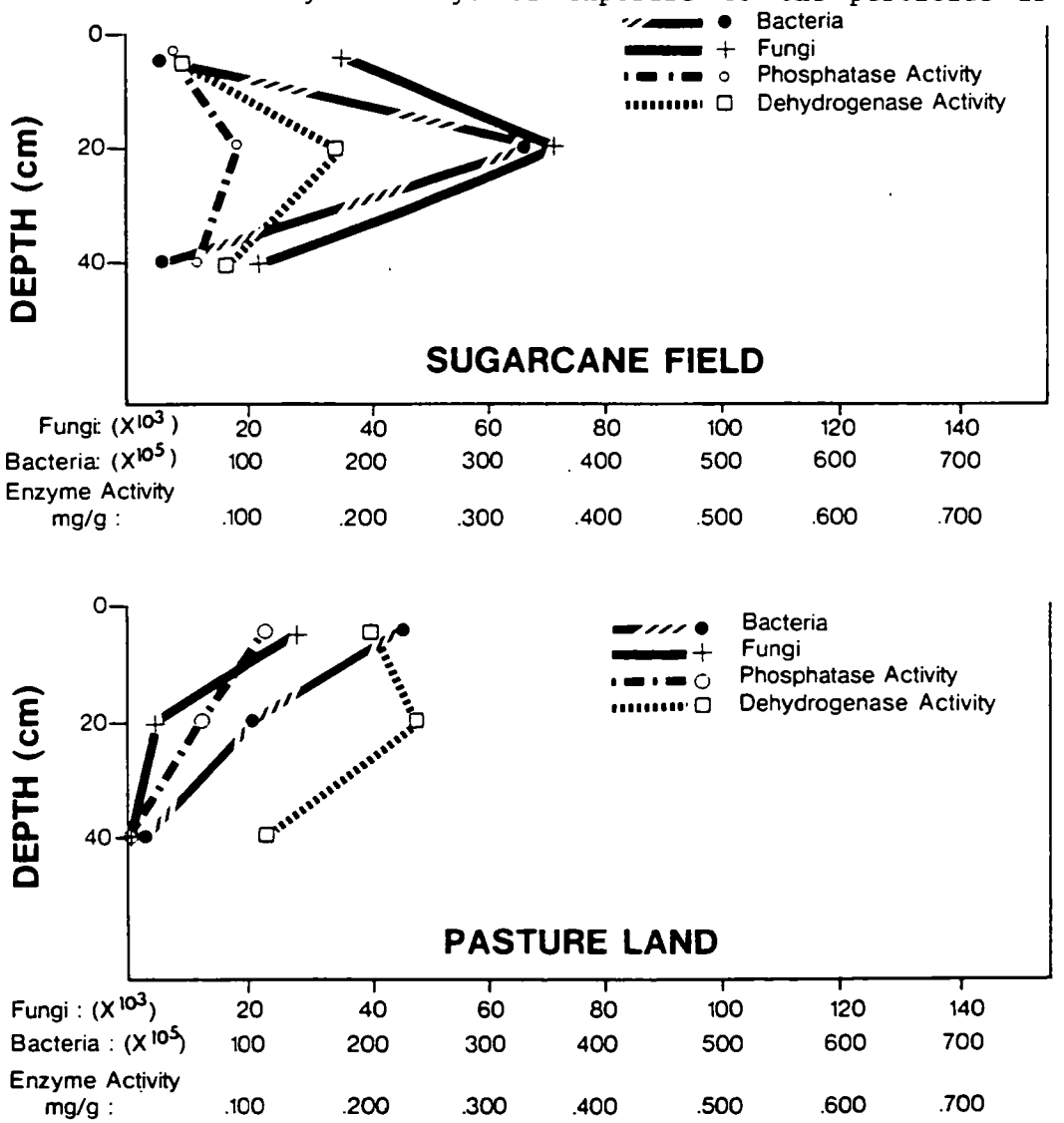


Figure 16. Variations in Microbial Populations and Enzyme Activities.

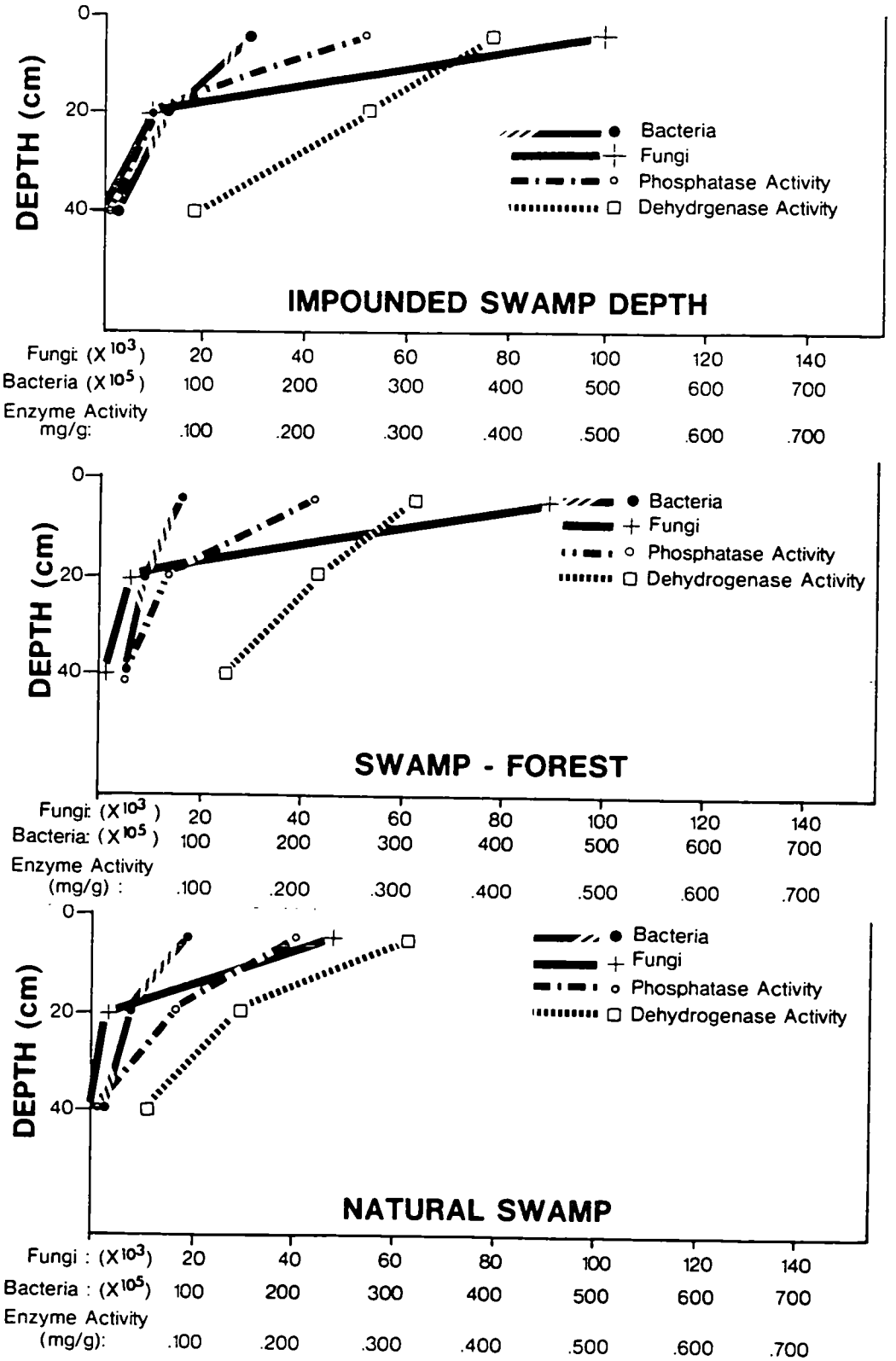


Figure 16 (cont.) Variations in Microbial Populations and Enzyme Activities.

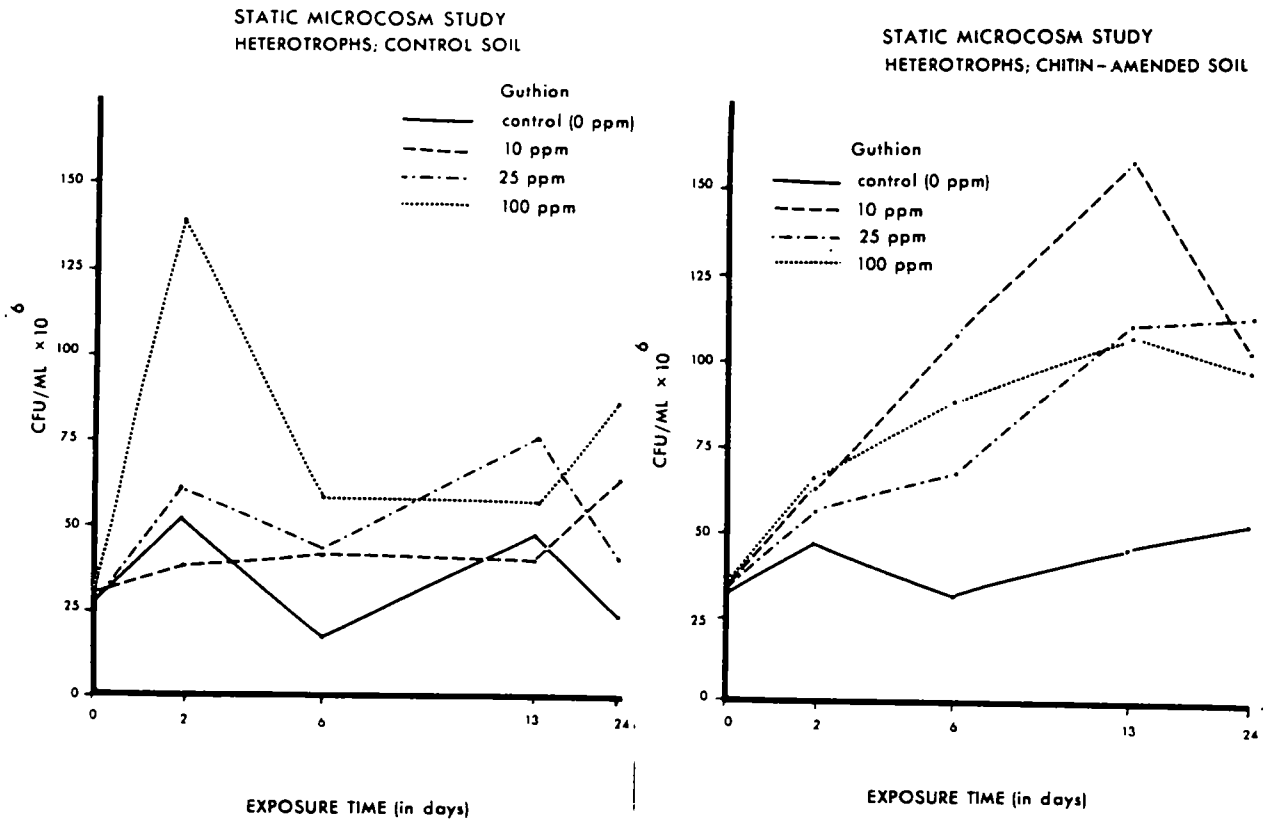


Figure 17. Effect of Guthion on Heterotrophs and Chitinoclasts in Chitin-Amended Soils.

The data indicates that Guthion has a pronounced positive effect on microbial growth. Concentrations of the pesticide above 10 ppm tend to increase microbial biomass. Treatment variations of total heterotrophs in control and chitin-amended soils is shown in Fig. 18. Chitin-amended soil had a higher least square means for each treatment than did control soil.

The static microcosm study indicated that pesticide levels significantly affected microbial concentrations. Since chitin was the only definable variable between soils, Guthion seemed to have a positive effect on chitin turnover and, in all likelihood, on levels of chitinoclasts. However, due to inherent limitations in the static microcosm design, a more definitive conclusion could not be reached. Mortality of invertebrates (nematodes and larger organisms), as noted in data from field community tests, certainly may have contributed to the number of degraders. However, this contribution was not as highly significant in Guthion-amended controls as for chitin-amended soils. Confirmation of this positive effect on chitin turnover was made in subsequent continuous flow microcosm studies.

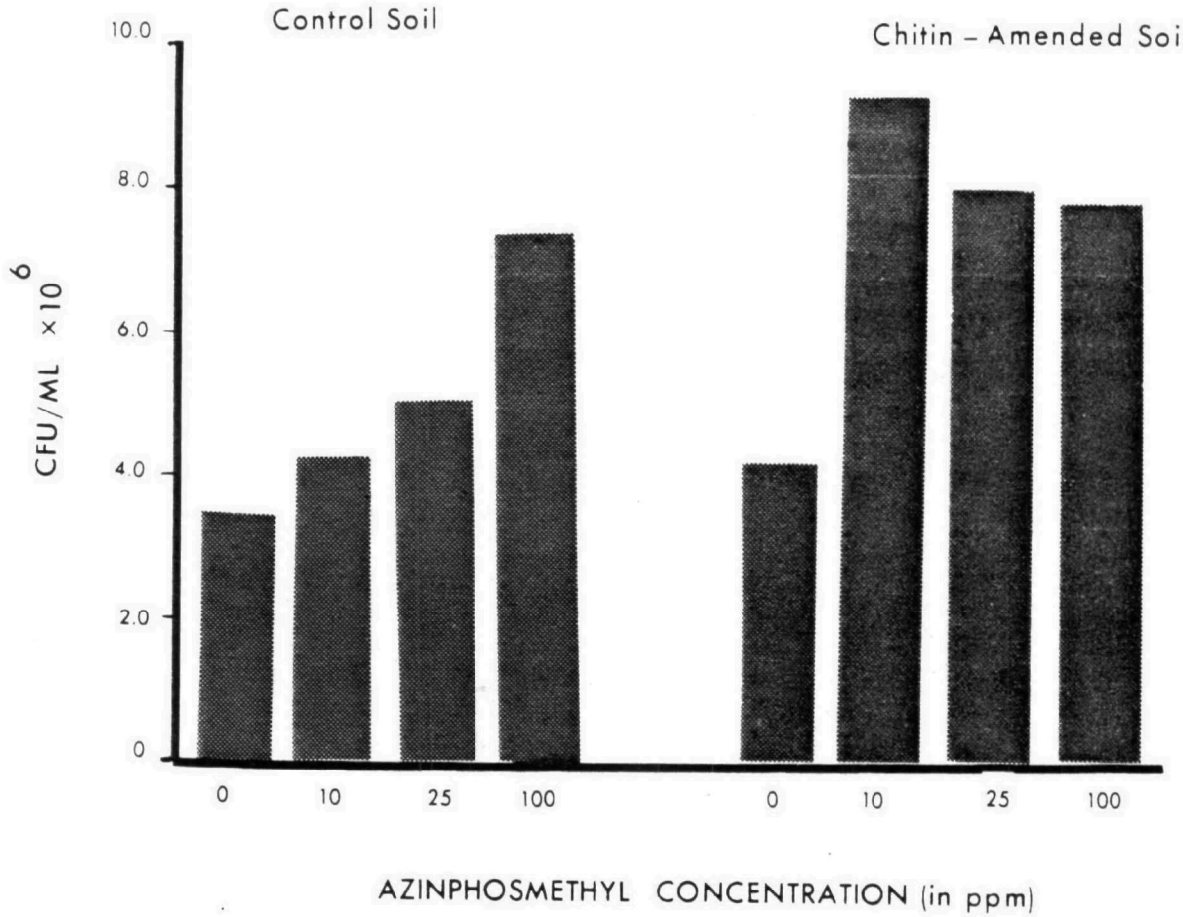


Figure 18. Static Microcosm Study. Treatment Variation of Total Heterotrophs.

Continuous Flow Microcosm Studies for Total Heterotroph/Chitinoclast Enumeration--

Fluctuations in microbial biomass, expressed as colony forming units (CFU), in control and Guthion-fortified (100 ppm) microcosms are shown in Figs. 19 and 20. Data are given for chitinoclasts and heterotrophs under aerobic and anaerobic conditions. Statistical analysis showed significant variations in total heterotrophs and chitinoclasts ($P < 0.01$). A rapid increase in microbial biomass occurred within the first three days of continuous application of the pesticide. After a 10-day application period, microbial concentrations returned to initial levels, with no significant change noted after the ten day period.

Under anaerobic conditions, differences in the heterotrophic and chitinoclastic populations in the control and Guthion-fortified microcosm were evident. Significant increases, attributable to Guthion application, were noted under both aerobic and anaerobic conditions. Statistical analysis

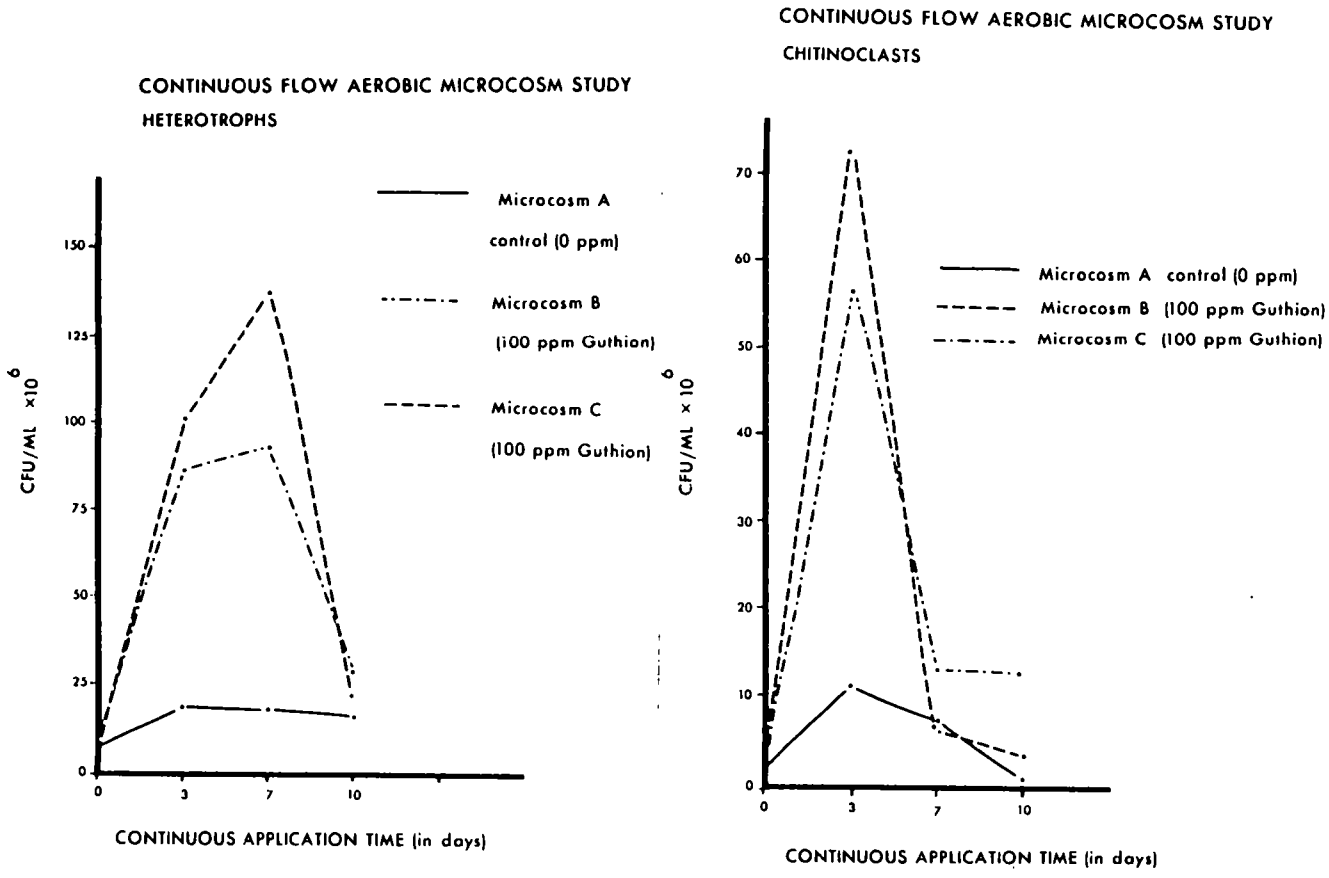


Figure 19. Effect of Guthion on Heterotrophs and Chitinoclasts in Continuous Flow Microcosms (Aerobic).

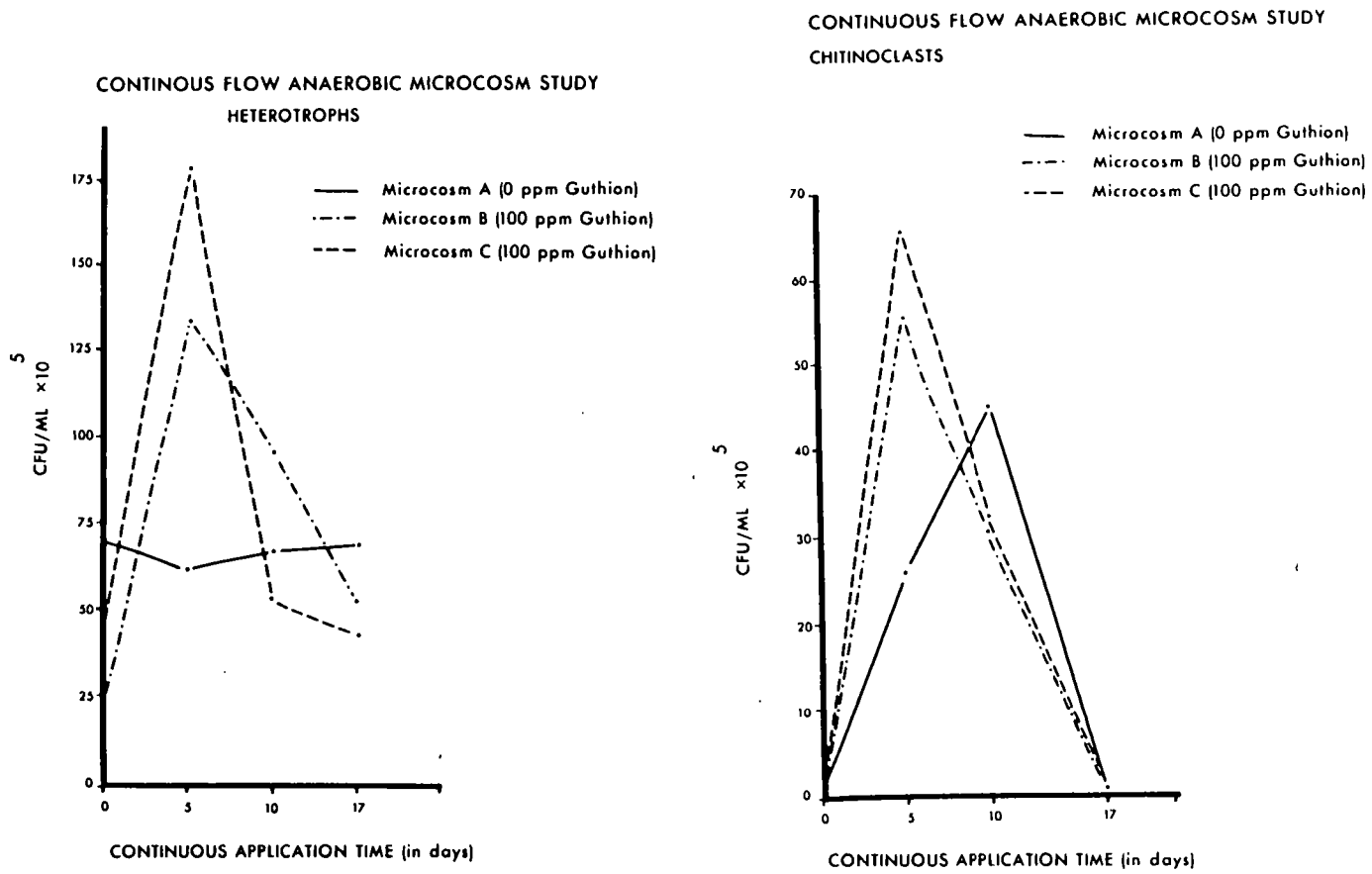


Figure 20. Effect of Guthion on Heterotrophs and Chitinoclasts in Continuous Flow Microcosms (Anaerobic).

shows significant variations in total heterotrophs ($P < 0.01$), however, no significant variation in chitinoclasts occurred after five days of continuous application of Guthion. Populations returned to initial levels, probably due to containerization effect of the microcosm system, and remained invariant after 17 da.

Chitinoclasts comprised a significant portion of the aerobic microbial biomass prior to pesticide application and increased with continuous Guthion application. A correlation between substrate turnover and enzyme activity with increased pesticide application can also be projected. Fig. 21 shows variations by treatment of heterotrophs under aerobic and anaerobic conditions and represents a comparison of relative population concentrations between aerobic ($x10^6$) and anaerobic ($x10^5$) conditions. Equipment limitations prevented tests under aerobic and anaerobic conditions to be conducted simultaneously. Significant increases attributable to Guthion application were noted under both aerobic and anaerobic conditions. Numerical values of total heterotrophs were, as expected, higher for aerobic than for anaerobic

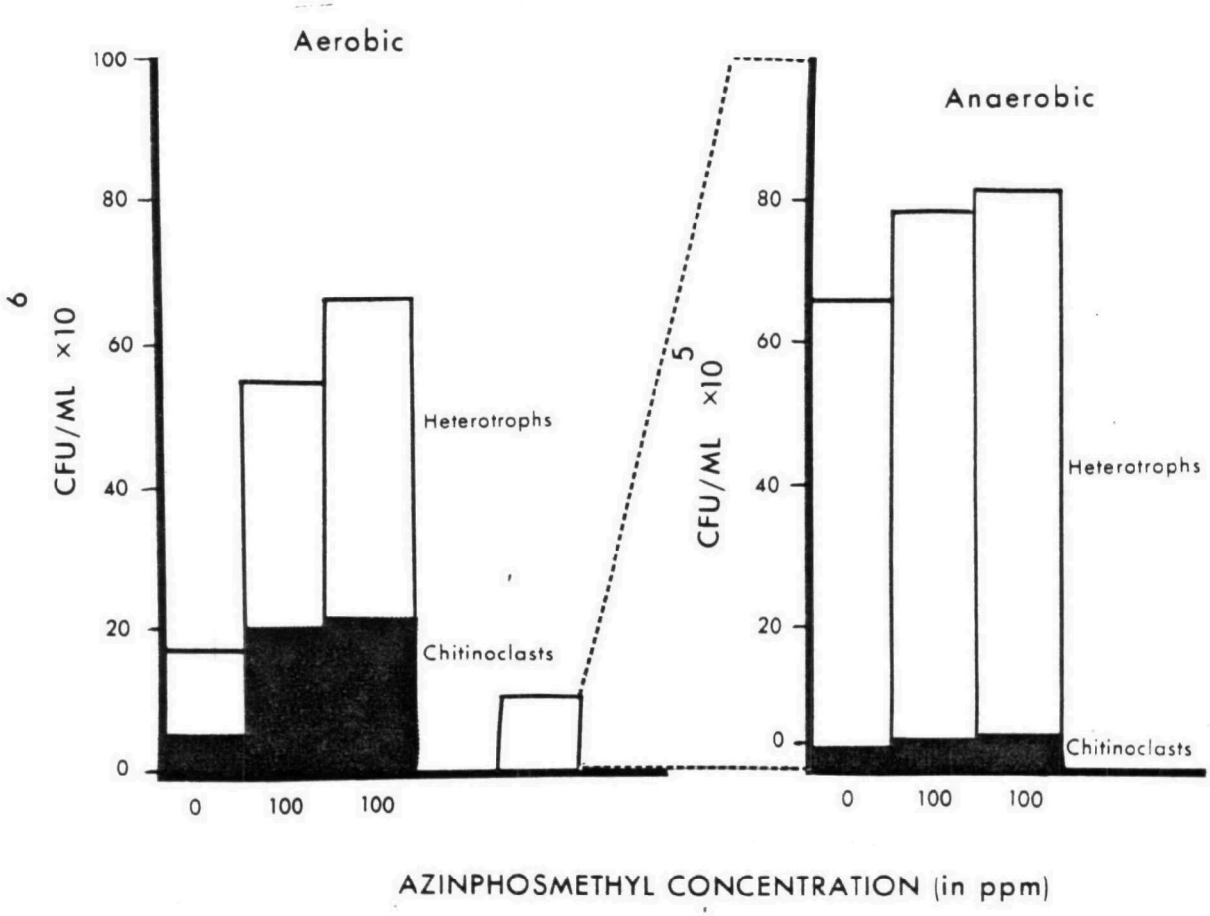


Figure 21. Treatment Mean Variations for Heterotrophs and Chitinoclasts in Guthion Supplemented Continuous Flow Microcosms.

conditions. Variations in chitinoclasts by treatment with the total population were apparent. Declining CFU reflects, of course, the utilization of the available food source, chitin and additional dead invertebrates, in Guthion-amended microcosm units. These concentrations are important for two reasons. In all likelihood, variations by a major physiological group within the total population indicates selectivity of pesticide interaction within the total population. A correlation between substrate turnover and enzyme activity with increased pesticide application can also be projected. Chitinoclasts constituted a significant percentage of the aerobic population prior to pesticide application. This percentage of the total population increased after continuous application of Guthion. Chitinoclasts are not a significant physiological group within the anaerobic portion of the biomass and remained invariant.

It can be postulated that variations in the chitinoclastic group, as affected by Guthion, is indicative of the selectivity of pesticide interaction within the total microbial population. These and other data support the hypothesis that substrate turnover, whether from chitin itself or from invertebrate mortality and specific enzyme activity, are directly affected by Guthion application.

Changes in Sensitivity and
Utilization of Azinphosmethyl--

A test for changes in bacterial sensitivity to, and utilization of, azinphosmethyl was conducted in conjunction with enumeration of total heterotrophs for the aerobic continuous flow microcosm study. Table 8 depicts these changes by the microbial community at different pesticide levels and type of growth media. Colonies were transferred from sample plates to pesticide-amended plates using the Laderberg replica plating technique. The values expressed are the percentage of transferable colonies enumerated after incubation. Media amended with acetone served as a control to avoid possible inhibitory effects attributable to acetone alone.

Slight variations in sensitivity to Guthion at concentrations of 10, 25, and 100 ppm noted were not statistically significant. Minor variations in growth of transferable colonies (Table 8) indicate a relatively low microbial sensitivity to Guthion by sample treatment populations.

Slight variations in utilization of Guthion at levels of 10, 25, and 100 ppm were noted but were not statistically significant. The growth of transferable colonies indicate a relatively small percentage of the total population can utilize Guthion as a sole carbon source. These numbers increased slightly for pesticide-treated microcosms, giving some indication of adaptation by the microbial community to Guthion utilization.

Selected cultures obtained from the above tests for Guthion utilization were grown in three types of media amended with the pesticide to a level of 225 ppm. Table 9 presents the relative growth of these selected axenic cultures. A few isolates exhibited good growth rates using the pesticide as a sole carbon source. Two actinomycete cultures exhibited good growth for

Table 8. Changes in Sensitivity to and Utilization of Azinphosmethyl During Continuous Flow Aerobic Microcosms.*

Application Time (Days)		MEDIA							
		N.A** Acetone	N.A 10 ppm	N.A 25 ppm	N.A 100 ppm	M.S.*** Acetone	M.S 10 ppm	M.S 25 ppm	M.S 100 ppm
Microcosm A (100 ppm Guthion)	0	90.1	90.1	90.1	90.1	24.3	29.7	29.7	27.0
	3	99.0	96.0	96.0	91.0	22.2	19.8	22.6	18.8
	10	98.3	95.1	95.1	88.5	30.3	30.3	18.1	18.1
Microcosm B (100 ppm Guthion)	0	89.2	89.2	79.6	86.0	45.1	40.3	41.3	36.5
	3	99.0	98.7	98.2	97.4	42.2	50.5	49.5	49.5
	10	99.0	98.3	96.7	95.9	48.6	51.8	55.6	54.1
Microcosm C (100 ppm Guthion)	0	98.3	92.5	87.5	87.5	23.4	28.1	26.6	15.6
	3	99.0	98.2	98.2	97.3	42.3	52.3	46.6	45.5
	10	99.2	99.2	99.2	99.2	48.0	50.3	52.6	52.6

*Expressed in Terms of % Transferable Colonies Enumerated After Incubation.

**Nutrient Agar (Difco, Inc.).

***Minimum Salts & Basal Agar (Difco, Inc.).

Table 9. Axenic Culture Study. Growth of Selected Axenic Cultures Isolated from Continuous Flow Aerobic Microcosms.

Isolates	MEDIA: Nutrient Broth and 225 ppm Guthion	MEDIA: Minimum Salts and 225 ppm Guthion	MEDIA: 0.1% Chitin Minimum Salts and 225 ppm Guthion
H_1 P_2	XXX	XX	0
H_2 P_2	XXX	XX	0
H_3 P_2	XXX	0	0
H_4 P_4	XXX	XX	0
HG	XXX	XXX	XXX
A_{11} P_4	XXX	XX	XXX
A_{20} P_4	XXX	XXX	XXX
C/16	XXX	XX	XXX
C/19	XXX	0	XXX
C/9	XX	X	XXX
C_{11} P_2	XX	0	XX
C_{14} P_2	XX	X	XXX

Time: 8 Days

0 = No Growth
X = Adequate
XX = Moderate
XXX = Good Growth
H = Heterotroph
A = Actinomycete
C = Chitinoclast

all three types of media. This is noteworthy in view of the relative abundance of chitinoclastic actinomycetes in both upland and flooded soil microbial populations. Chitinoclasts and heterotrophs grew well in the presence of the pesticide. No estimation of actual degradation rates was made of those cultures growing solely on Guthion.

Pesticide Accumulation--

The flow pattern of the continuous flow microcosm involved removal of an amount of fluid from the reaction vessel equivalent to amounts introduced. Preliminary tests in pesticide loss from the system due to flushing showed a depletion rate of 10%. At the prescribed rate of pesticide addition (0.105 ml/min) for 500 ml, complete replacement of water within the microcosm with 100 ppm pesticide amended water would occur in 3.31 da. The detectable amount of pesticide after three days was considerably less. A significant accumulation of pesticide by the flooded soil occurred as a result of the continuous application, with high (1000 ppm) pesticide levels noted in sediment in both aerobic and anaerobic microcosms. Similar accumulation phenomena was noted in physical/chemical studies. This indicates that the sediment was an effective reservoir (sink) for the pesticide. It is noteworthy that the total microbial population under both aerobic and anaerobic conditions was not adversely affected by the extremely high concentrations of Guthion.

Initial Enzymatic Studies--

Dehydrogenase Activity. Measurement of dehydrogenase activity provides an insight into the metabolic activity of microorganisms in soil. Fig. 22 shows the mean treatment effect on dehydrogenase activity of two amended and control soils, indicating no significant changes in dehydrogenase activity.

Starch was used as a second nutritive source to chitin to make orthogonal comparisons on dehydrogenase activity between amended and control flooded soils. Although not statistically significant, inferences can be made on dehydrogenase activity being affected by amending with chitin and starch. Dehydrogenase levels for chitin-amended soils were perceptibly higher than those for control and starch-amended soils. Dehydrogenase levels in chitin-amended soils increased with pesticide level, but not to significant levels.

Phosphatase Activity. Fig. 23 shows the mean treatment effect on phosphatase activity of three soil types with addition of Guthion at levels of 0, 10, 25, and 100 ppm. Statistical analysis of treatments for each soil type indicated no significant changes in phosphatase activity. Similar to dehydrogenase activity, inferences can be made on phosphatase activity being affected by amending the natural soil with chitin and starch. Slight decreases in phosphatase activity are noted for pesticide-treated soils due to the increased availability of phosphorus provided by the pesticide.

Determination of Pesticide Half-Life. Residual pesticide concentrations in soil from dehydrogenase and phosphatase activity studies are shown in Table 10. Half-life for the pesticide for each soil type at different levels

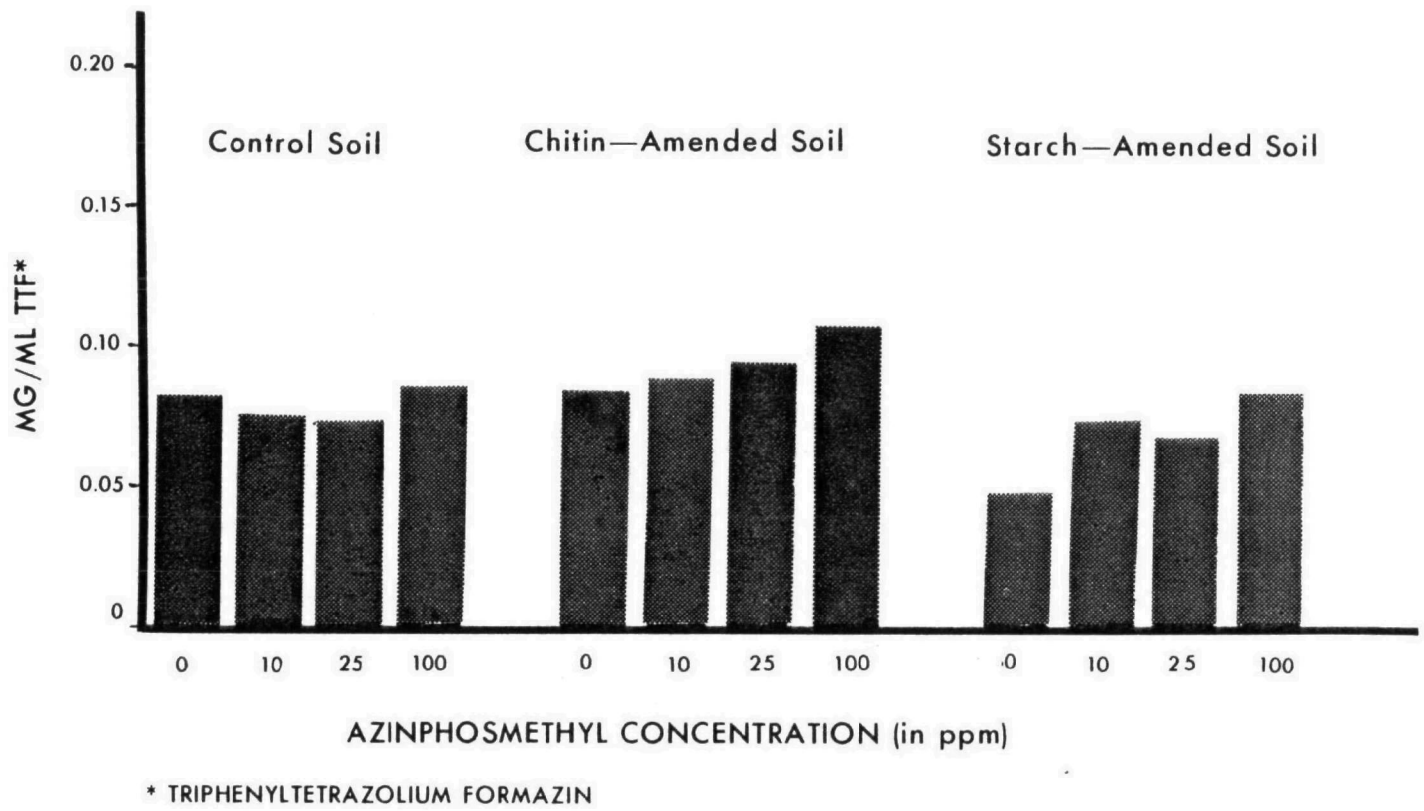


Figure 22. Effect of Chitin-Addition on Dehydrogenase Activity In Situ.

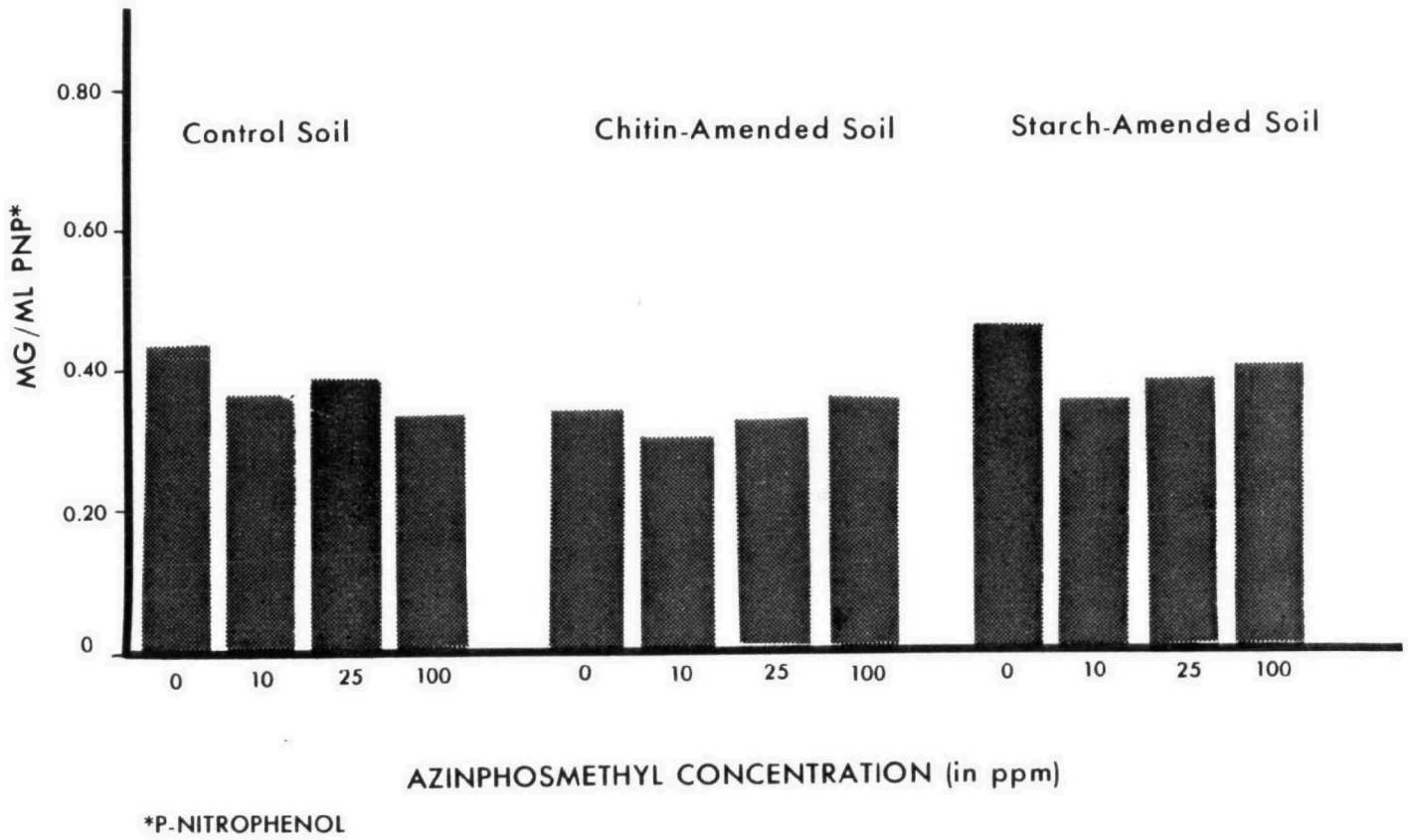


Figure 23. Effect of Guthion on Phosphatase Activity in Three Soil Types (Control, Chitin-Amended, Starch-Amended).

Table 10. Residual Guthion Concentrations in Soil from Dehydrogenase and Phosphatase Activity Trials

Exposure Time: 17 Days

Soil Type	Initial Conc.	Final Conc.	Percent Recovered	Half Life (Days)	Mean Half-Life (Days)
Natural Soil	0	0	1.923		
	10	0.1923	1.923	2.982	
	25	0.5824	2.330	3.134	
	100	6.083	6.083	4.209	3.44
Chitin-Amended Soil	0	0			
	10	0.1873	1.873	2.962	
	25	0.7405	2.962	3.348	
	100	1.878	1.878	2.964	3.09
Starch-Amended Soil	0	0			
	10	0.1907	1.907	2.976	
	25	0.7488	2.995	3.359	
	100	3.759	3.759	3.591	3.31

of concentration compare closely. The half-life ($T_{\frac{1}{2}}$ = 3 days) for the pesticide in our study was longer than half-life values noted in field application studies ($T_{\frac{1}{2}}$ = 2 days). However, this value was shorter than values given by Yaron (1974) of 5 days in upland, agricultural soils.

Initial Carbon Metabolism Studies--

The carbon metabolism microcosms was designed to test for the rate of N-acetyl-D-glucosamine (NAG) utilization, as well as for relative respiration rates, by using NAG (glucosamine-^{14}C). The objective was to obtain a more definitive picture of substrate utilization, i.e., NAG, as affected by pesticide treatment, compared with earlier studies using chitin, and to determine more precise fluctuations in the microbial biomass due to pesticide addition based on $^{14}CO_2$ production.

As noted in Figs. 24 and 25, both $^{14}CO_2$ respiration and ^{14}C-glucosamine rates increased with Guthion addition. Variation in evolution of $^{14}CO_2$ with time by treatment proved to be highly significant ($P < 0.01$). An immediate response to the tagged NAG was detected for static microcosms containing Guthion. A somewhat delayed response was noted for static microcosms without the pesticide. Evolution of $^{14}CO_2$ for all microcosms leveled off after 48 hours and remained invariant for a period of 24 days. The $^{14}CO_2$ evolved during these experiments accounted for between 27 to 42% of the added ^{14}C.

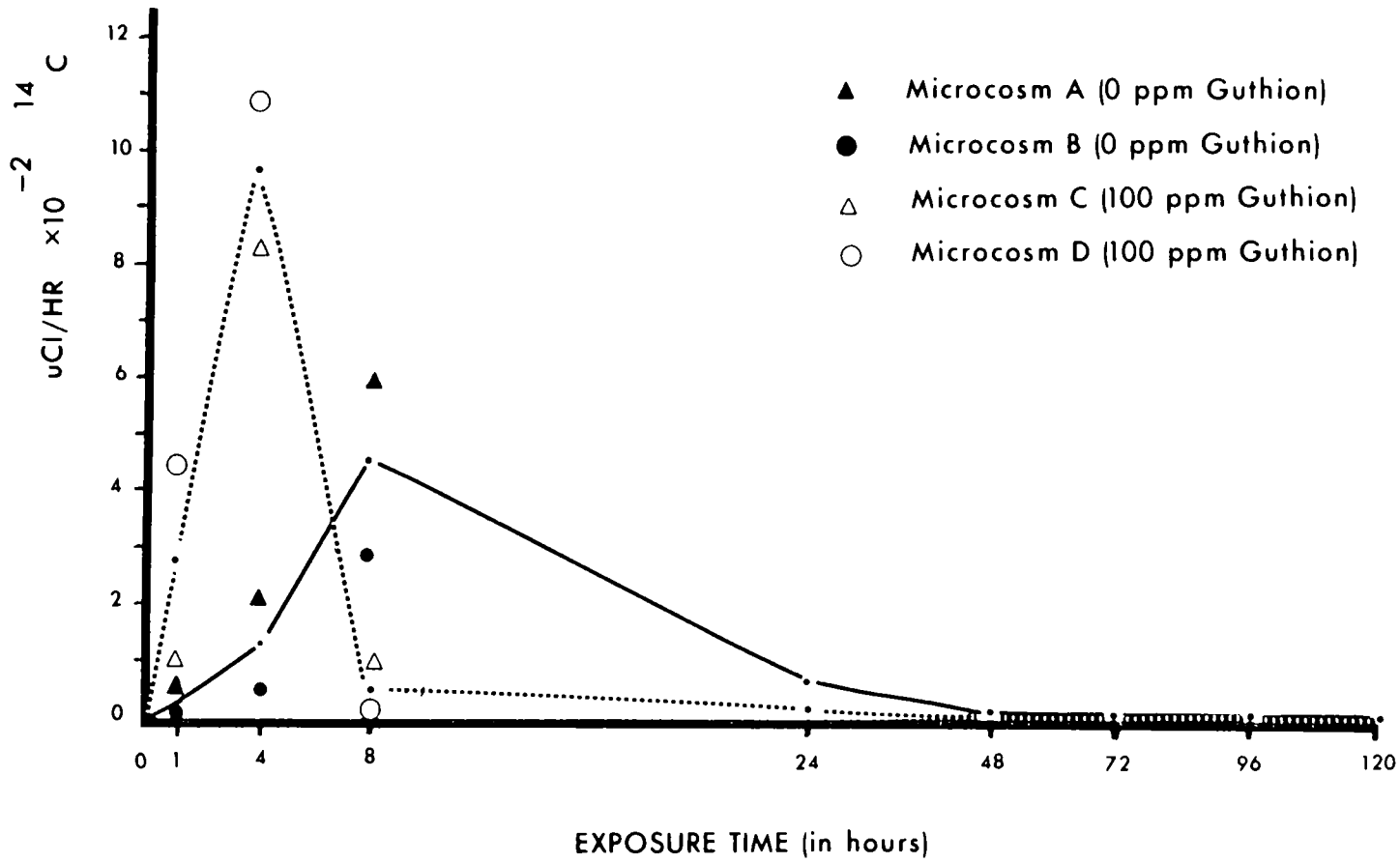


Figure 24. Effect of Guthion on $^{14}CO_2$ Release in Carbon Metabolism Microcosm.

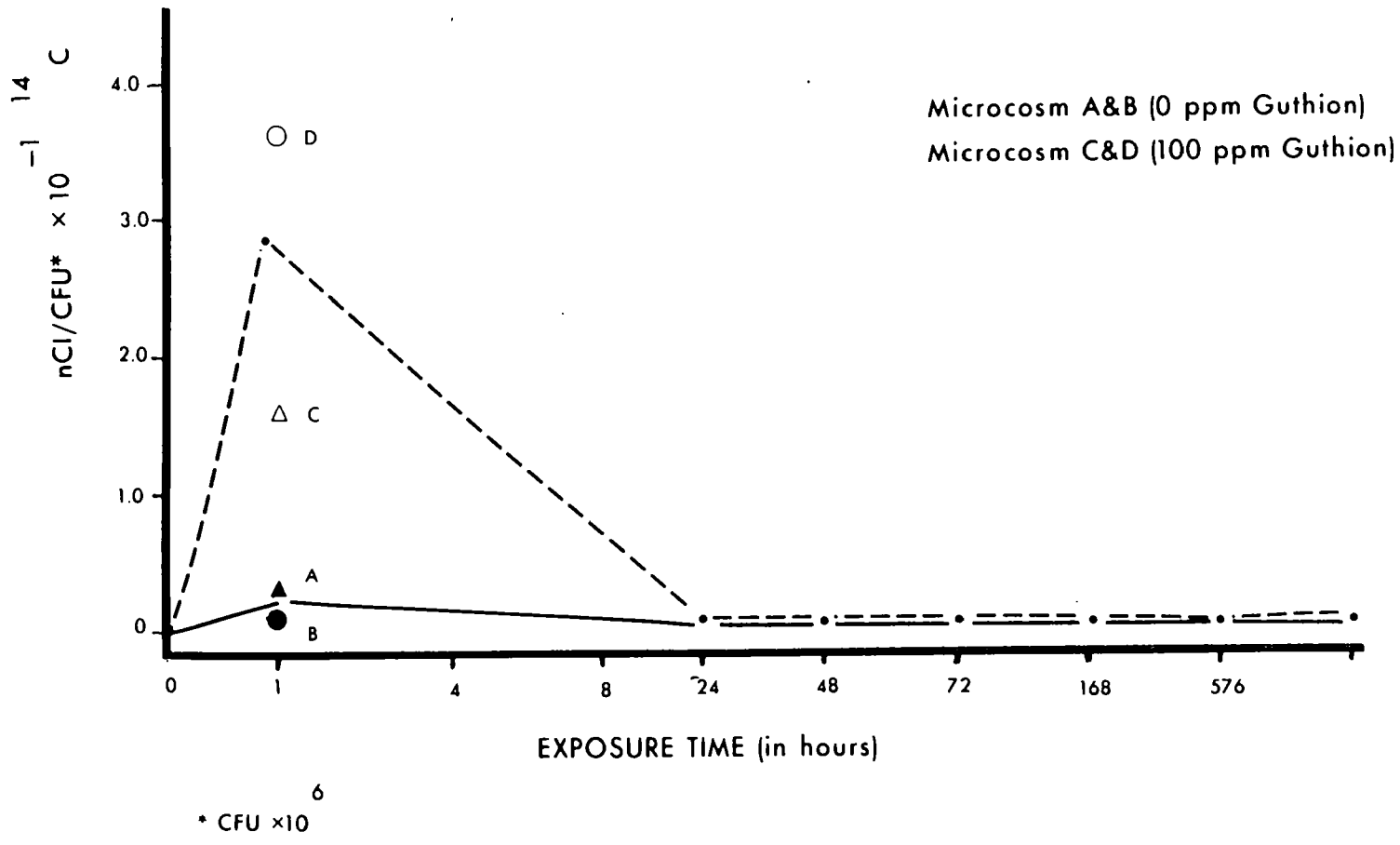


Figure 25. Effect of Guthion on ^{14}C-Glucosamine Uptake in Carbon Metabolism Microcosm.

Earlier, Bartha et al. (1967) reported organophosphates cause an initial increase in total CO_2 production in upland soil, the magnitude of which is directly related to concentration applied.

As data in Fig. 26 suggest, Guthion has a positive and rapid action on NAG utilization, corresponding to increases in microbial activity concentrations and $^{14}CO_2$ evolution. This increase in microbial activity correlates to the response noted earlier in chitin-amended continuous flow-through microcosms. Thus, a stimulatory effect by Guthion on chitin and its breakdown products is postulated. This stimulatory effect by Guthion is augmented by benthic invertebrate mortality resulting in additional chitin input into the total substrate pool. Chitin, an energy-rich substrate readily colonized by the extant microbial community, we feel provides an absorptive medium for the pesticide. Faust (1977), in his extensive review of organic pollutants in aquatic systems, noted the effect of organic matter content in controlling pesticide adsorption. Presence of Guthion has a positive effect on chitin utilization, which may trigger a more complete and rapid mineralization of the xenobiotic.

Determination of Pesticide Half-Life--

The breakdown products of many organophosphates have been reported to have an inhibitory effect on microbial respiration. However, this was not apparent for Guthion for the 24-day study period. Table 11 lists the residual pesticide concentrations in soil from the carbon metabolism microcosm study. A half-life of 7-7½ days was determined. A longer $T_{\frac{1}{2}}$, for these studies, reflected in part the utilization of NAG rather than chitin as an energy rich substrate. For a 24 day test period, over 90% of the initial pesticide added was degraded to various breakdown products, possibly anthranilic acid and

Table 11. Residual Guthion Concentrations in Soil from Carbon Metabolism Microcosm Study.

Microcosm	Initial Conc. (ppm)	Final Conc. (ppm)	Percent Recovery	Half Life ($T_{\frac{1}{2}}$) Days
A	0	0		
B	0	0		
C	100 ppm	9.467	9.467	7.057
D	100 ppm	10.960	10.960	7.524

Exposure Time: 24 days
pH: 7.0 - 7.2
Temperature: 30 C

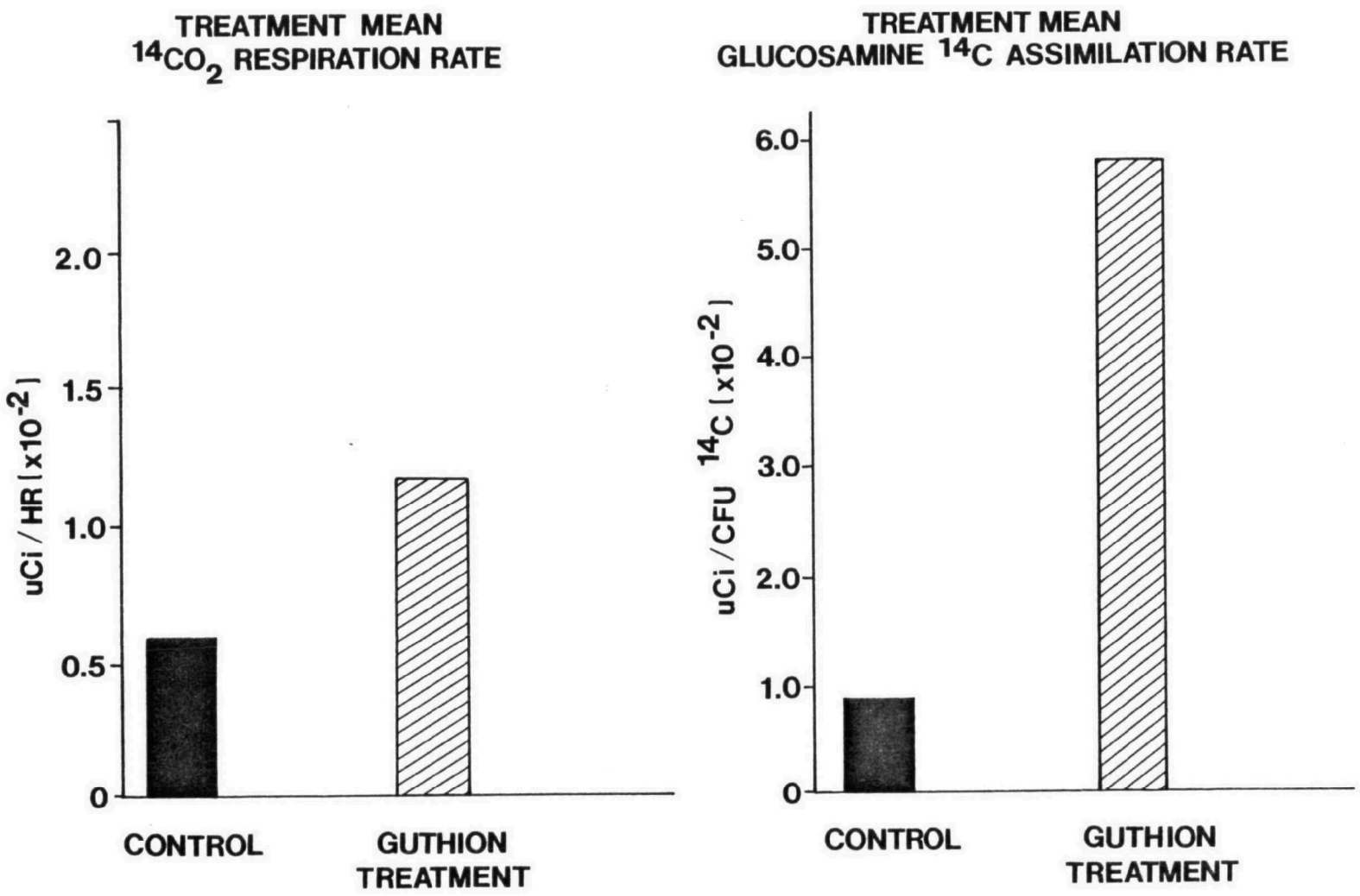


Figure 26. Mean Treatment Effect of Guthion in Carbon Metabolism Microcosms.

benzazimide (Liang 1972). Present data suggest that these and other possible breakdown products are not toxic to microbial respiration. Nevertheless, continuing studies, that amend flooded soils with these compounds are needed to present a thorough picture of the total spectrum of effects of Guthion on the composite soil environment.

Our field studies support observations of other investigators on effect of organophosphate pesticides. Sethunathan (1973) noted a more rapid degradation of parathion in soils under flooded conditions compared with those under non-flooded conditions. Guthion is similar in action on the microbial community in flooded soils as are other organophosphates previously tested in upland, agricultural soils (Atlas et al. 1977). Rapid increases in bacterial concentrations coincide with rapid degradation of the pesticide. Higher pesticide concentrations correlate with higher bacterial concentrations. No significant variations in phosphatase and dehydrogenase activity are noted as a result of pesticide addition. These aspects, correlated with data from microcosm studies, are of significance in the selection and use of pesticides on upland, agricultural soils adjacent to highly productive estuarine systems.

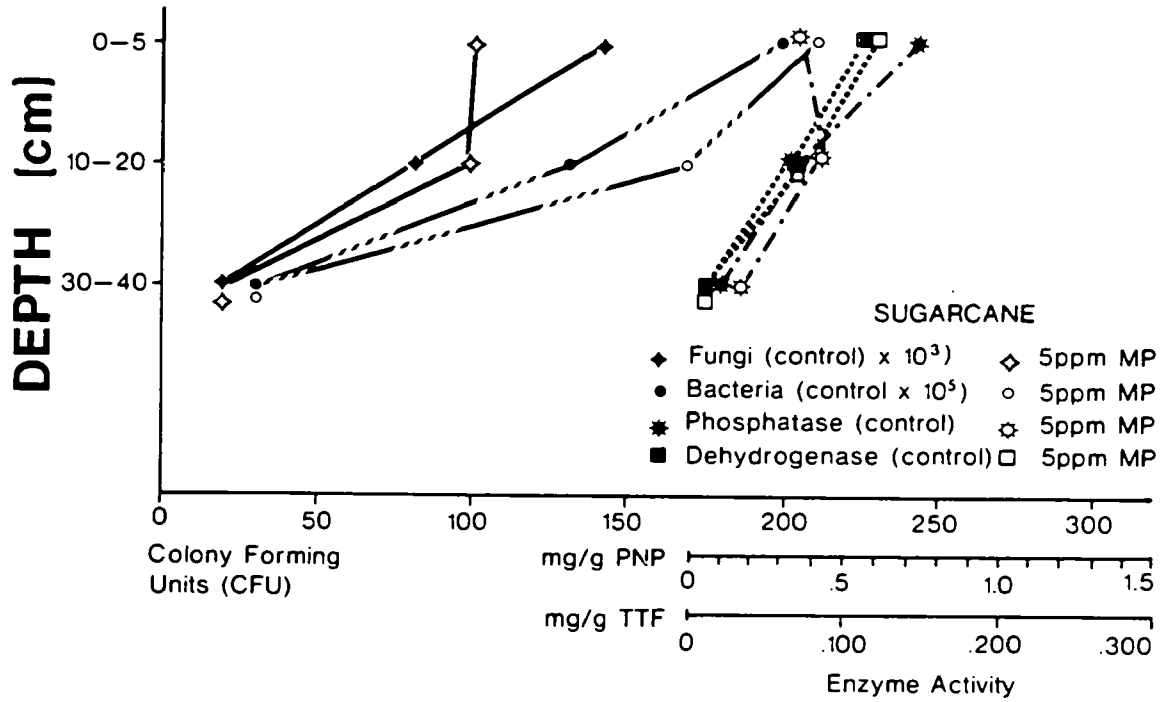
Studies with Guthion, Methyl Parathion and Kepone®

Microbial/Enzymatic Correlations: Guthion and Methyl Parathion--

In field tests with Guthion and methyl parathion, enzyme activity, phosphatase, and dehydrogenase correlated with increases in microbial activity. Results of field tests for methyl parathion are given in Fig. 27. Soil phosphatase and dehydrogenase activities were examined in terms of bacterial and fungal populations with depth and the particular soil environment. High correlations were noted between phosphatase and microbial activity as well as between dehydrogenase and microbial activity. These variations in population/enzyme profiles provided indications of the impact of land management practices on impounded freshwater swamp-forest habitat compared with pristine sites. Organic material accumulation from litter-fall on dry and submerged swamp forest floors were reflected by highly stratified bacterial and fungal populations. Xenobiotic addition reflected shifts in these stratifications, indicating microbial stress and response. Addition of methyl parathion was evidenced by increases in both bacterial and fungal populations, along with enhanced enzyme activity. Subsequent microcosm studies showed these biomass increases to be highly significant.

Continuous Flow Studies (Part 2)--

Microbial and enzymatic responses in control and Guthion-fortified microcosms over a 20-day period are shown in Fig. 28A. Fig. 28A represents the control set while Fig. 28B shows the effect of pesticide addition. Guthion was added to the test microcosms after 13 days. In general, enzyme activity was correlated with increases in microbial activity, with an order of magnitude change in fungal biomass. Similar responses in both fungal and bacteria levels (Fig. 29) were noted in different tests using methyl parathion.



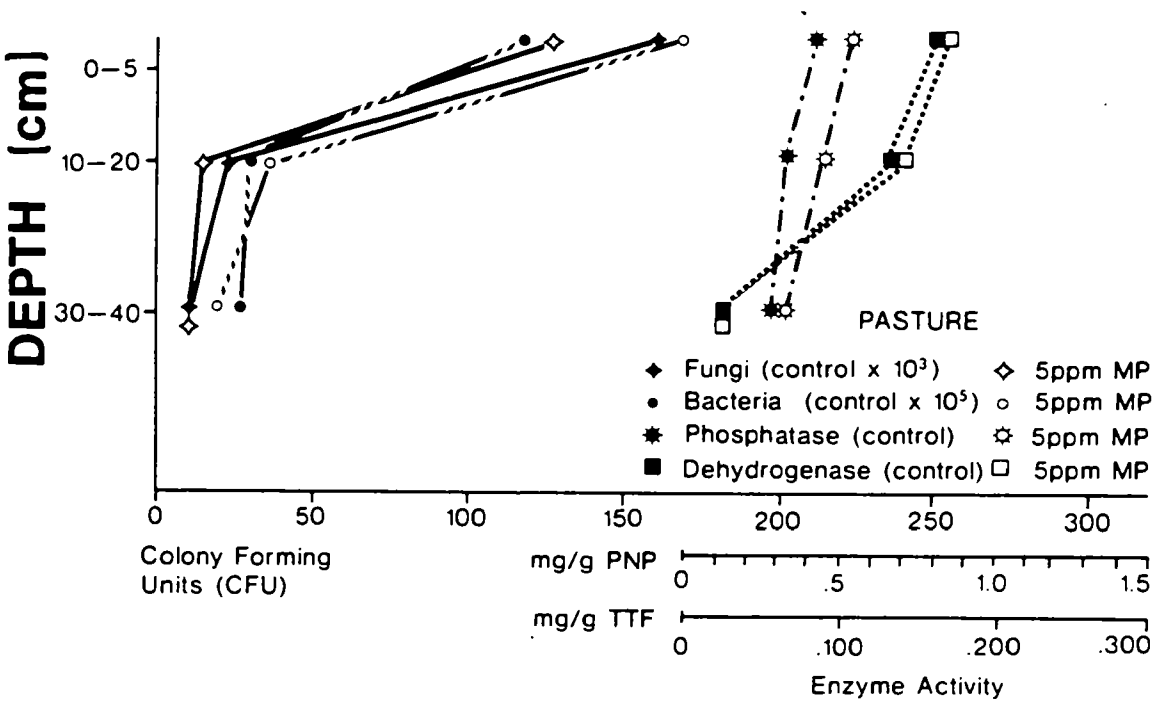
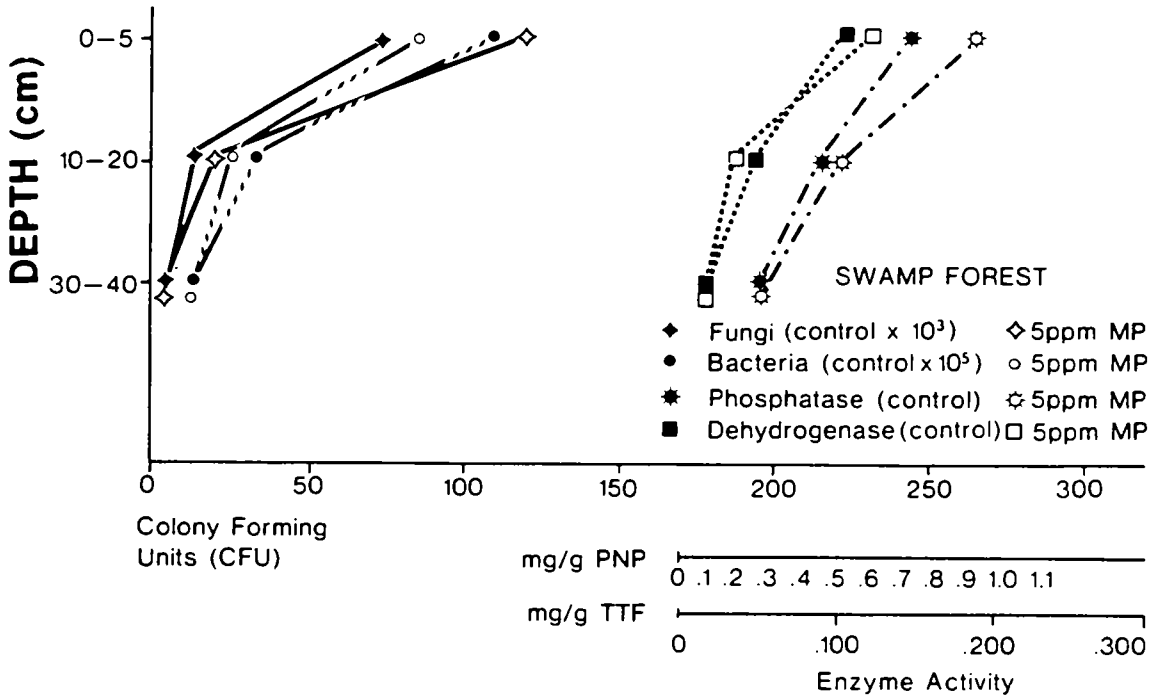


Figure 27. Effect of Methyl Parathion on In Situ Microbial and Enzymatic Activity.



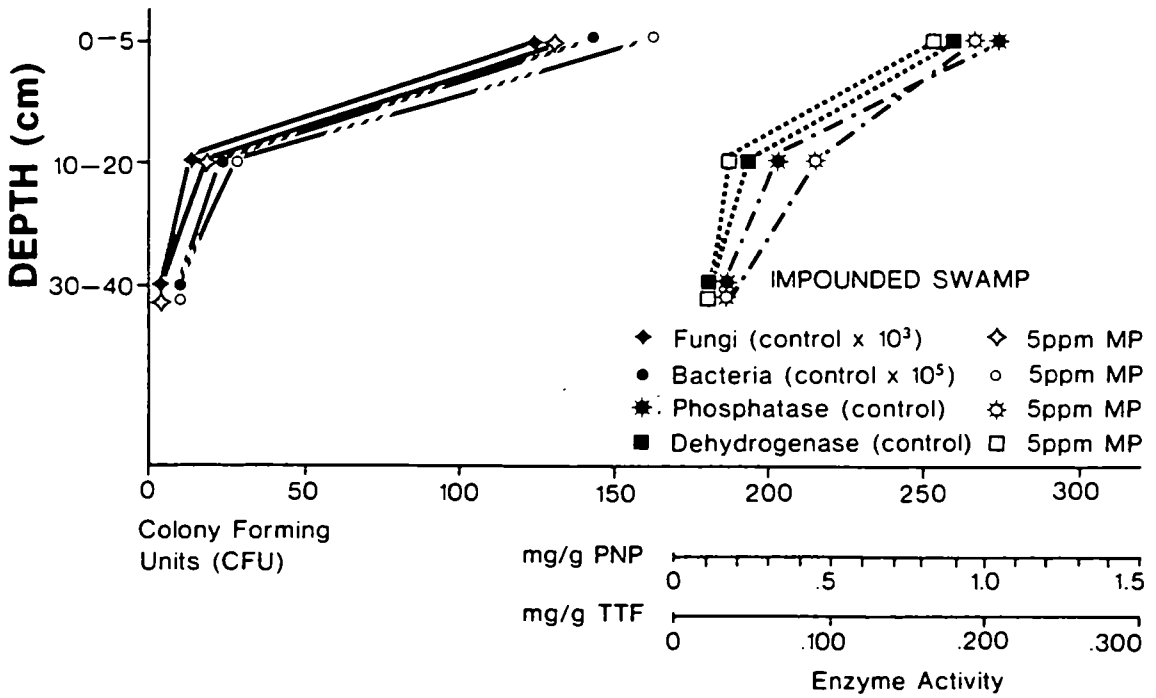


Figure 27 (cont.) Effect of Methyl Parathion on In Situ Microbial and Enzymatic Activity.

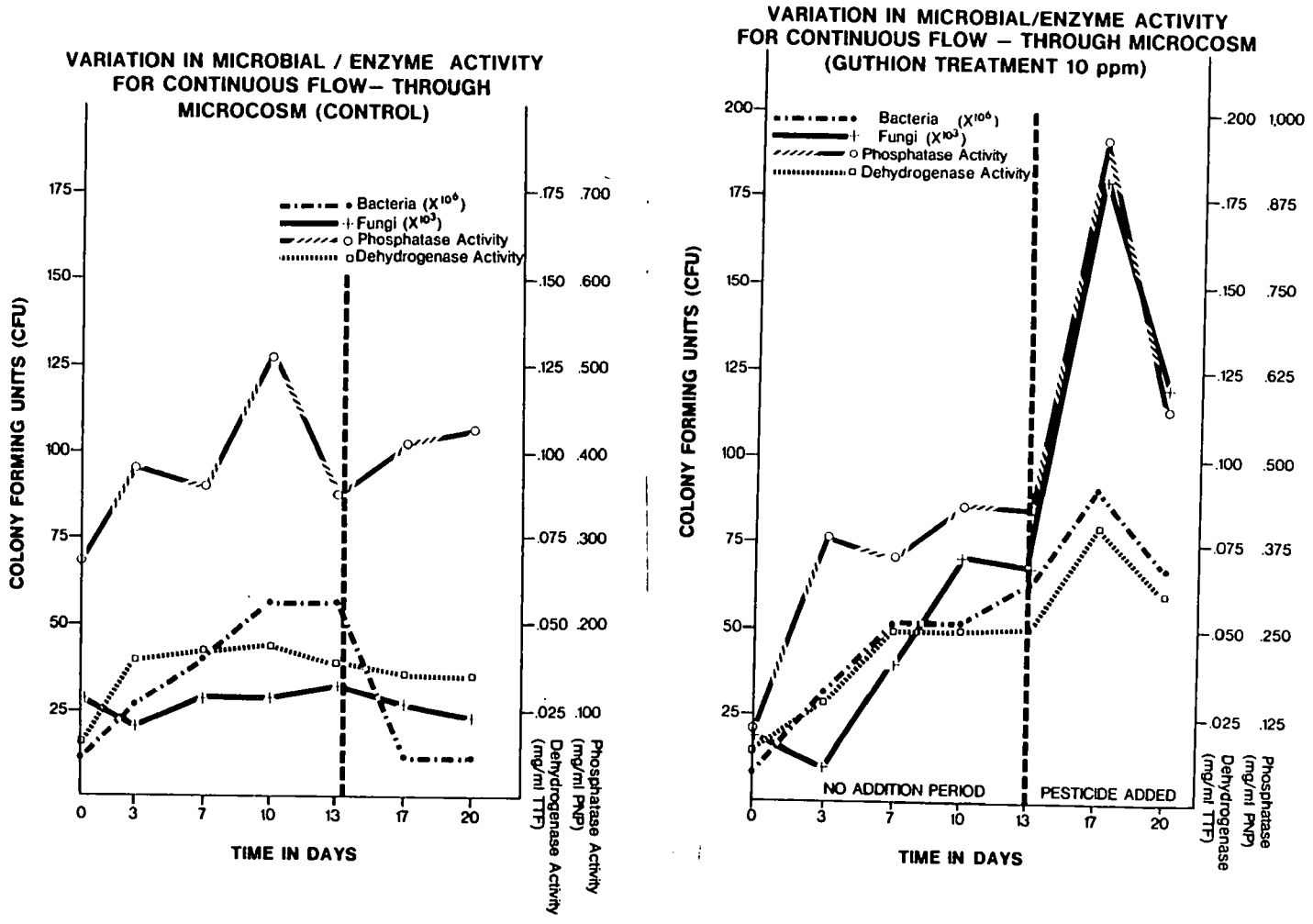


Figure 28. Variation in Microbial/Enzyme Activity in Continuous Flow Microcosm (Control and Guthion-Treated).

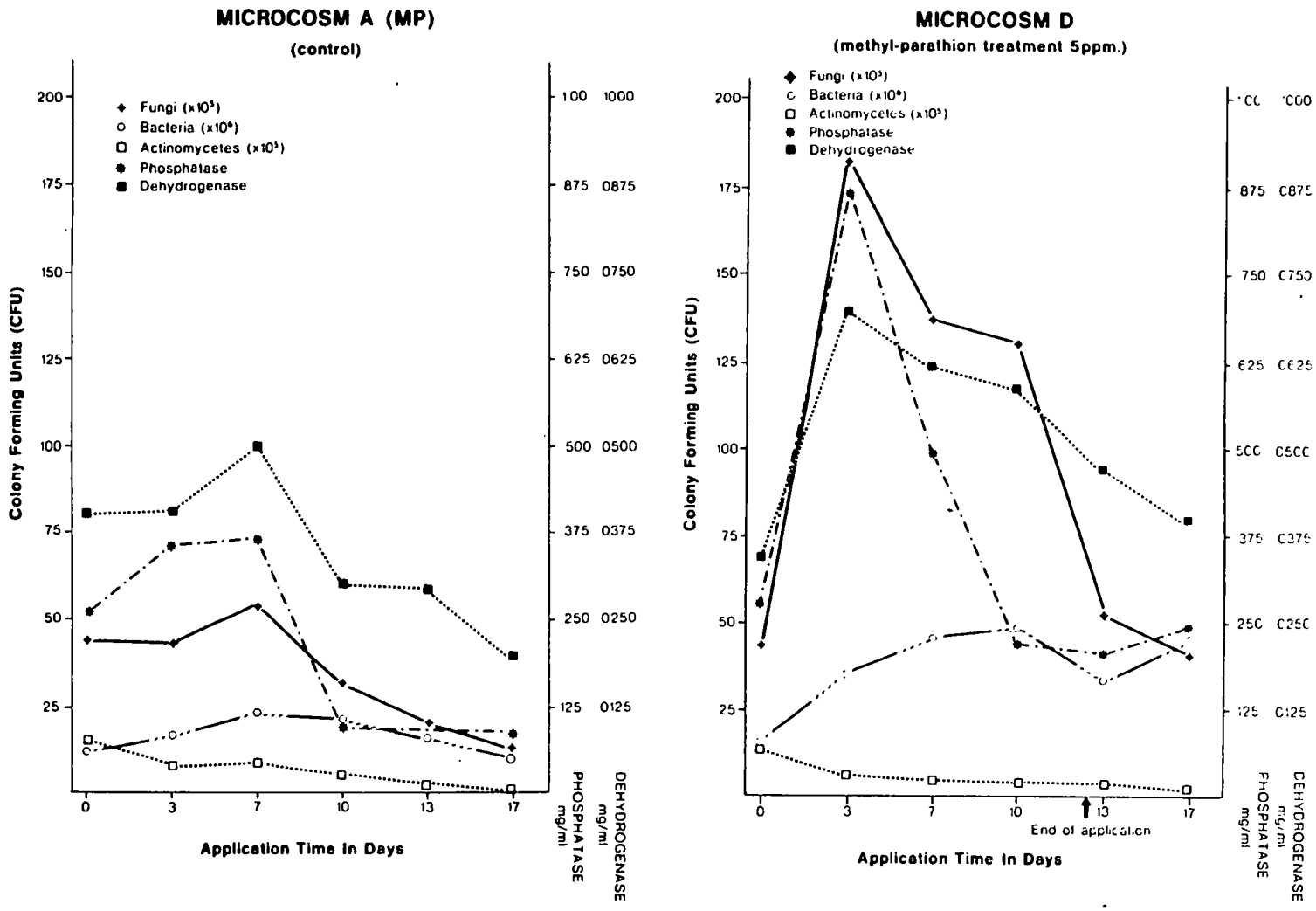


Figure 29. Variation in Microbial/Enzyme Activity in Continuous Flow Microcosm (Control and Methyl Parathion-Treated).

Bollag (1972), in his discussion on the biochemical transformation of pesticides by soil fungi, noted that bacteria and fungi will react to xenobiotic compounds differently; bacteria will often attach and degrade a compound to its mineral components whereas fungi will induce minor chemical changes. Cognizant of the high fungal biomass found in both sugarcane and impounded swamp soils, significant degradation and utilization of organophosphates such as Guthion by fungi, may be a contributing factor in the relatively short half-life, i.e., 7-7½ d, of Guthion in soils.

The mean treatment variations for the various test parameters are given in Table 12. Analyses of data for the dependent variables, bacteria and fungi showed significant variation between the control and test microcosms. Using analyses of variance revealed significant ($P < 0.01$) variation in biomass numbers. The Duncan's multiple range test of variations in treatment means showed significant variations at an alpha level of 0.05. Significant variation in phosphatase level by respective treatment was not noted, in contrast to the significant variation in phosphatase activity observed over time of application. Highly significant variations ($P < 0.01$) in dehydrogenase activity were noted by treatment and time. Duncan's multiple range tests for dehydrogenase indicated significant variation between the control and experimental microcosms at an alpha level of 0.05.

Table 12. Mean Treatment Variations and Enzyme Response for Total Heterotrophs in Continuous Flow Microcosms.

	Treatment I (control)	Treatment II (Guthion)
Filamentous Fungi (x 10^3	24.5	58.8
Yeasts (x 10^3)	4.3	2.0
Bacteria (x 10^6)	34.5	50.6
Actinomycetes (x 10^6)	4.1	5.1
Phosphatase (mg/ml PNP)*	0.416	0.437
Dehydrogenase (mg/ml TTF)**	0.036	0.048

*PNP - p nitrophenol.

**TTF - triphenyltetrazolium formazin.

Continuous-Flow Studies (Part 3)
and Carbon Metabolism Studies (Part 2)--

Microbial response to addition of methyl parathion (5 ppm) and Kepone® (0.5 ppm) in control and test static carbon metabolism microcosms is shown in Figs. 30 and 31. Data are given for fungi and bacteria, expressed as the log of colony forming units (CFU), for both compounds. Statistical analysis indicated significant variations in both microbial groups with a single application of methyl parathion. After incubation for 10 days, microbial concentrations returned to initial levels, with no significant changes noted subsequently. No significant total treatment variation was seen in the static carbon metabolism Kepone® microcosms with either bacteria or fungi, although bacterial CFU were higher in the control microcosm at day three and fungal CFU were higher in control microcosms at day seven. Nevertheless, in general, control microcosm chambers had higher CFU than did experimental units.

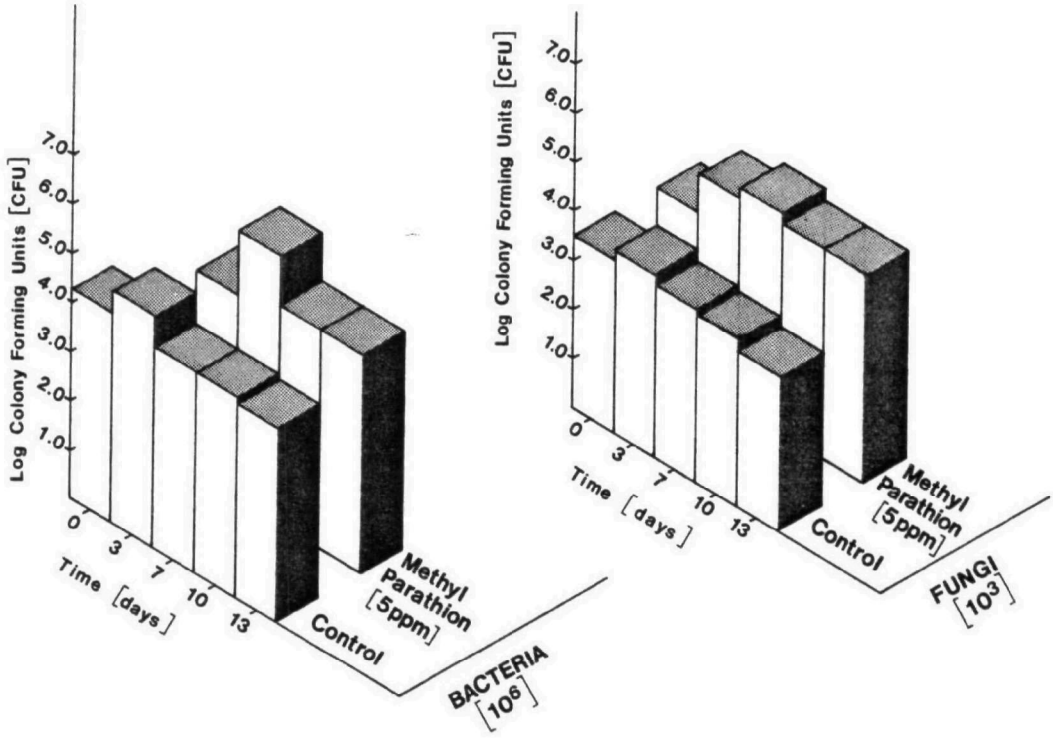


Figure 30. Microbial Response to Methyl Parathion (5 ppm) in Static Carbon Metabolism Microcosm.

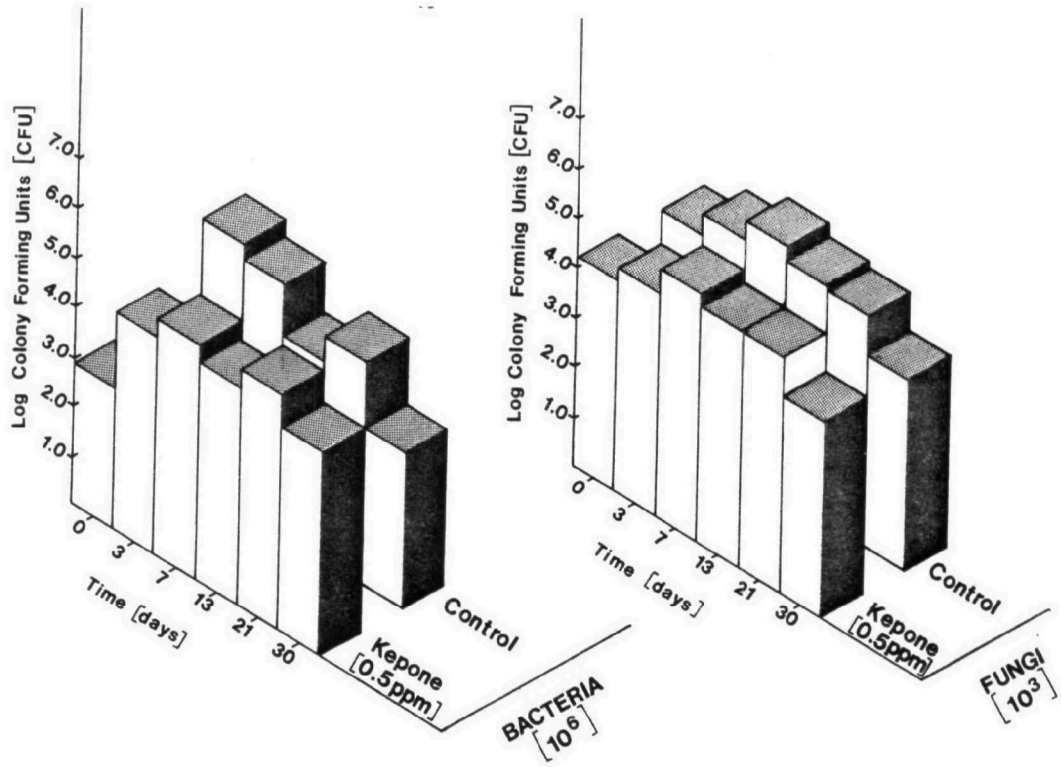


Figure 31. Microbial Response to Kepone (0.5 ppm) in Static Carbon Metabolism Microcosm.

Fluctuations in bacterial and fungal biomass, expressed as log CFU/ml, in control and xenobiotic-amended continuous flow microcosms are shown in Figs. 32 and 33. Significant increases in both bacterial and fungal log CFU occurred within the first three days of continuous application of methyl parathion. Xenobiotic input was terminated after 7 days with no significant variation in bacterial biomass after 10 days. However, fungal populations declined more rapidly after 10 days, with the control microcosms having the greatest decrease after day 17.

Microbial response to continuous application of Kepone® (Fig. 33) was similar to responses noted in static microcosm tests (Fig. 31). Significance by treatment variations due to Kepone® addition was not evident between control and test microcosm units. Fungal log CFU in the control unit were higher at 7 days than in the kepone-amended microcosm. Bacterial log CFU for both control and test microcosms were comparable, although the control units were somewhat higher. Controls for both methyl parathion and Kepone® were not significantly different in terms of bacterial and fungal log CFU (Fig. 30), in contrast, fungal log CFU were higher for Kepone®. It appears that variations in microbial populations in the microcosm information provide

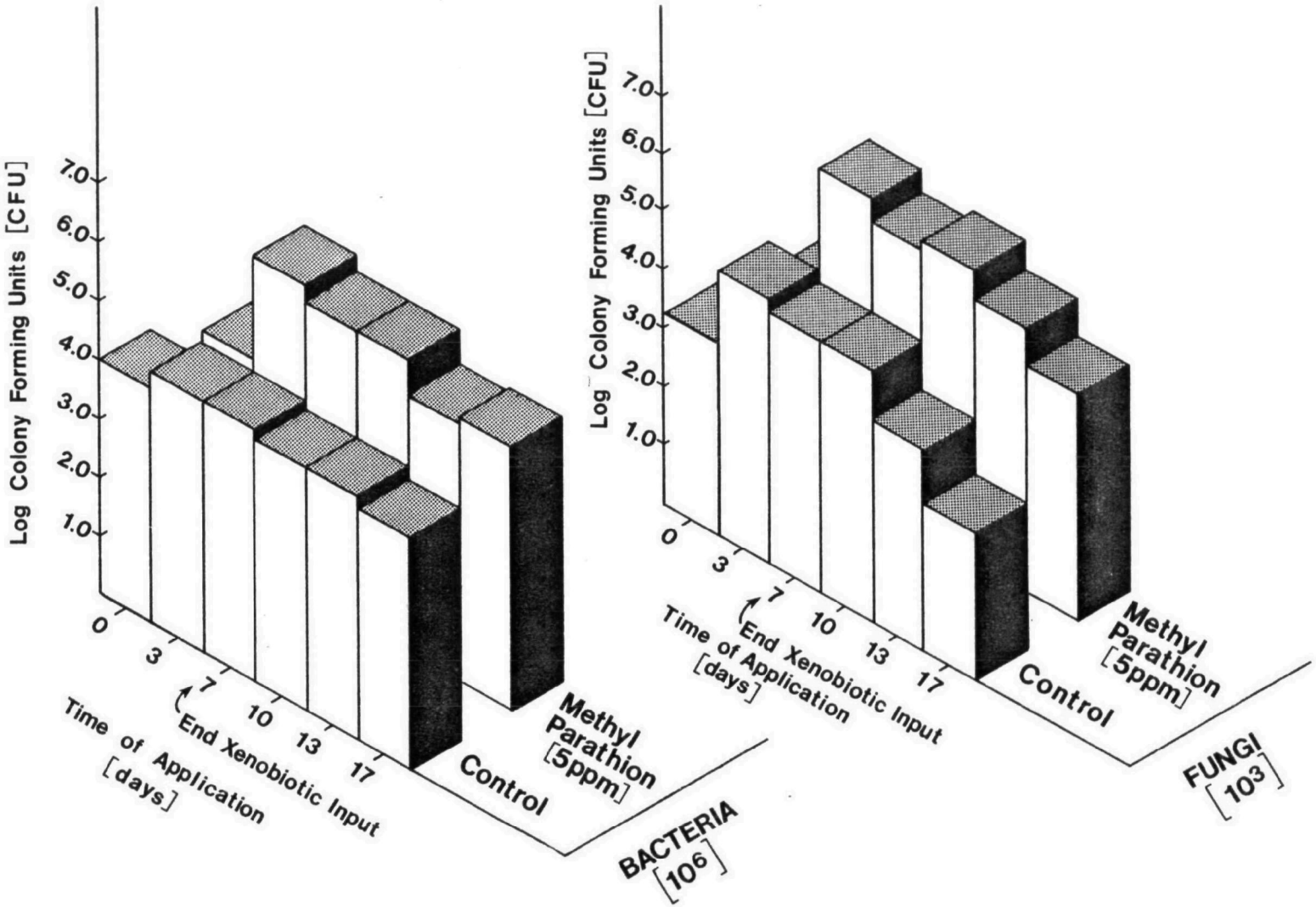


Figure 32. Microbial Response to Methyl Parathion (5 ppm) in Flow Through Microcosms.

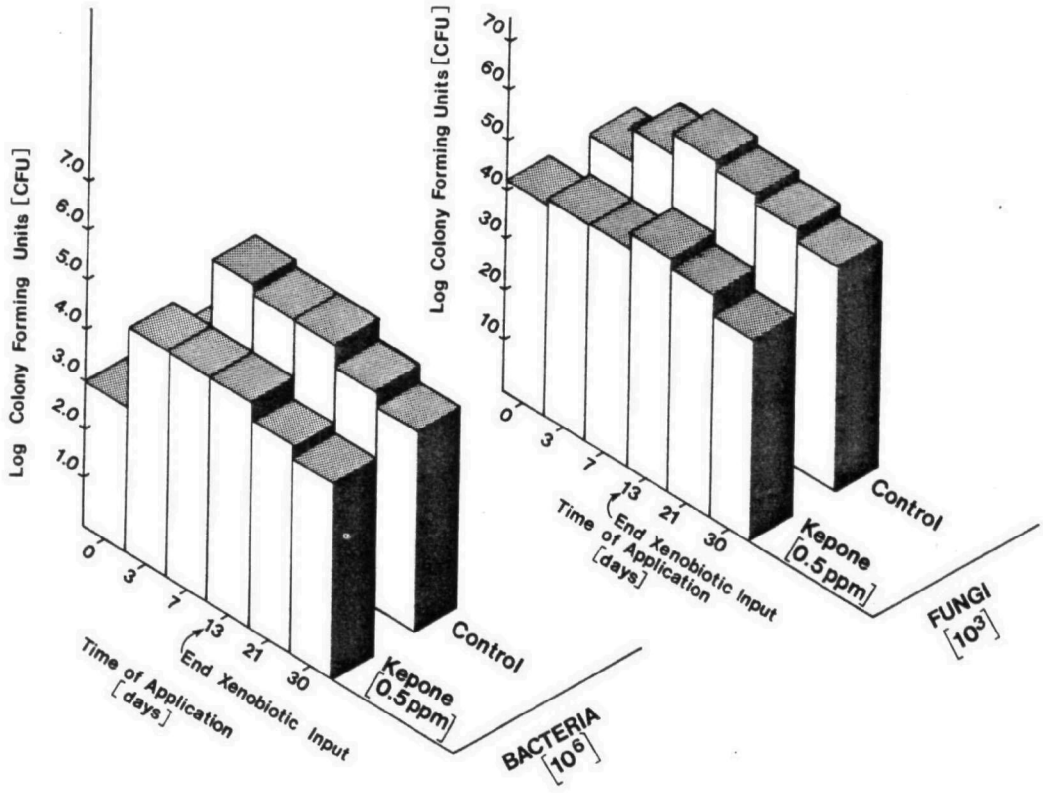


Figure 33. Microbial Response to Kepone (0.5 ppm) in Flow-Through Microcosm.

information on xenobiotic selectivity and interaction with the total microenvironmental community, indications of possible mortality substrate contributions from higher trophic levels and indications of compound recalcitrance and fate.

ATP Response--

Estimates of viable biomass, as provided by adenosine triphosphate (ATP) concentrations, for control and test static carbon metabolism and flow-through microcosms are shown in Figs. 34 and 35, respectively. Log ATP estimates for static microcosms (Fig. 34) provided indications of significant increases in microbial biomass for methyl parathion, in contrast to Kepone® addition. These observations were further confirmed in flow-through microcosms (Fig. 35), wherein a significant increase in microbial ATP was noted following application of parathion. ATP levels remained consistently high for the test microcosm, declining rapidly after day 13. ATP concentrations for Kepone® were statistically invariant with both control and test microcosms having variable high and low data points throughout the growth period.

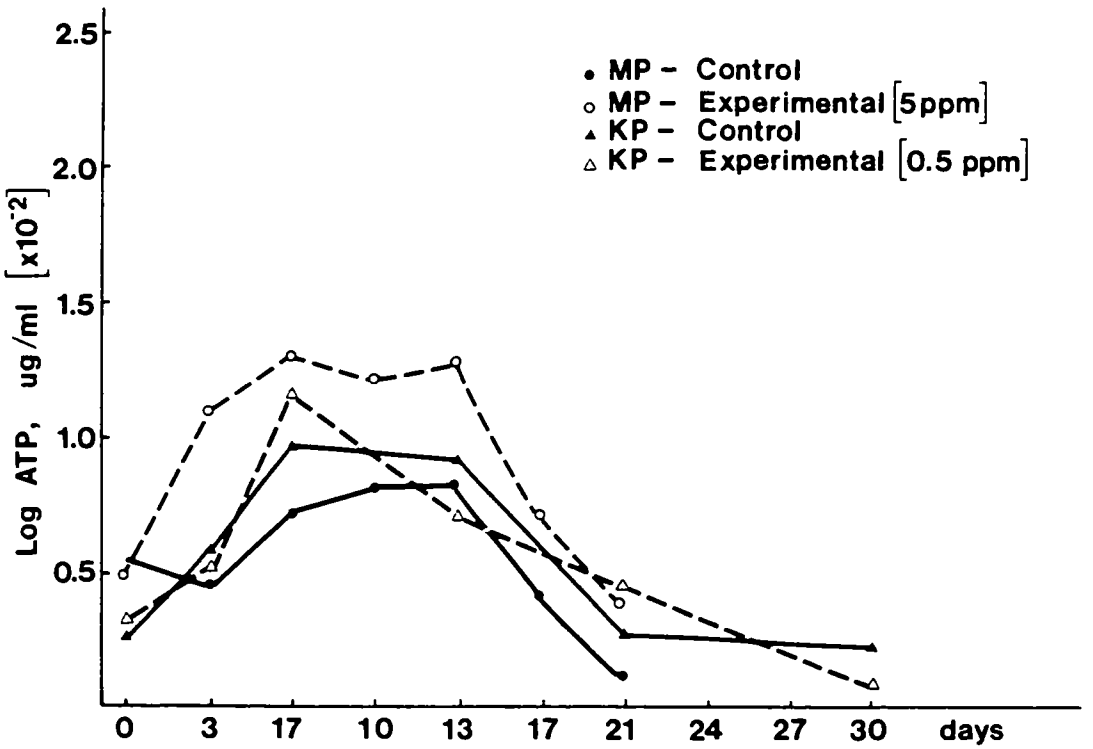


Figure 34. Response of ATP to Addition of Methyl Parathion and Kepone in Static Microcosms.

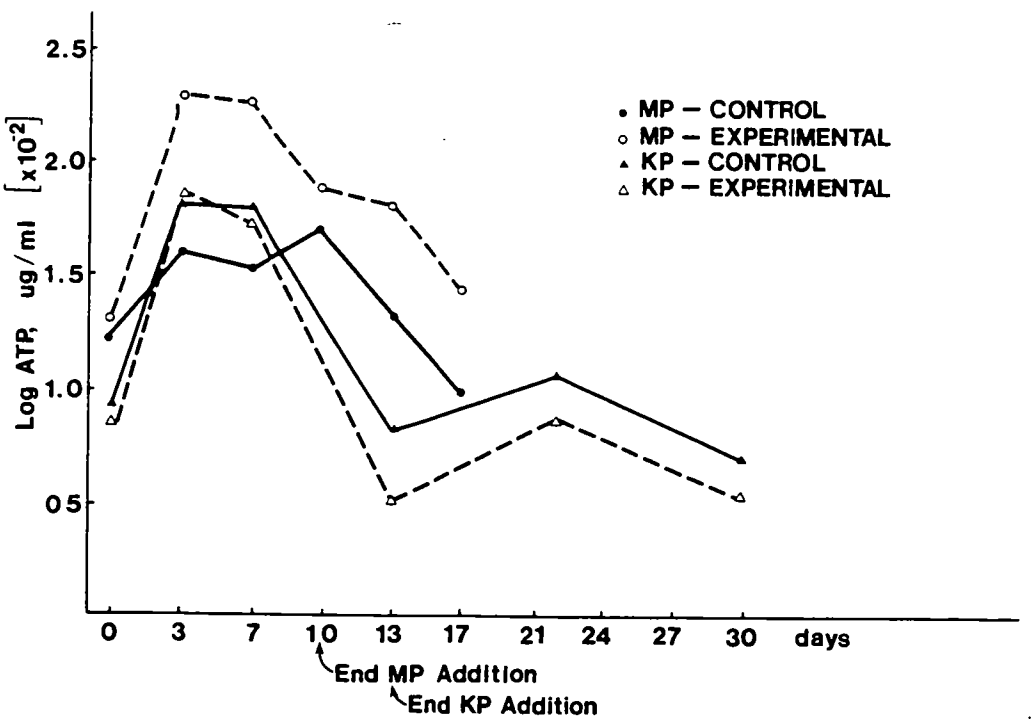


Figure 35. Response of ATP to Addition of Methyl Parathion and Kepone in Flow-Through Microcosms.

ATP concentrations, in continuous flow through microcosms (Fig. 35), showed comparable responses for methyl parathion. ATP levels were significantly higher for methyl parathion microcosm units, with peaks occurred within three days of continuous application. ATP levels dropped by day 10 except for a transitory peak in the control microcosms at this time. ATP levels dropped rapidly for both control and test units after ten days. Concentrations in Kepone® units again were not significantly different from those of the control. However, after day three, Kepone®-amended microcosms had somewhat lower ATP levels compared to those of the control. A moderate increase in ATP concentrations on day 21 for both control and test microcosms is difficult to explain. This may be due to a containerization effect of the microcosms or to subsequent degradation of the lignocellulose component present in the sediment. Probably, Kepone® itself was not solely responsible for this ATP increase, although a slight increase in $^{14}CO_2$ release in static microcosms was noted during this time.

Further Enzyme Responses--

A notable decrease in phosphatase activity was seen between Kepone®-amended and control static microcosm units (Fig. 36). Phosphatase levels, initially higher than that of controls for Kepone®, steadily dropped after day three. In contrast, phosphatase levels for methyl parathion again were significantly higher than controls for Kepone®, and steadily dropped after day

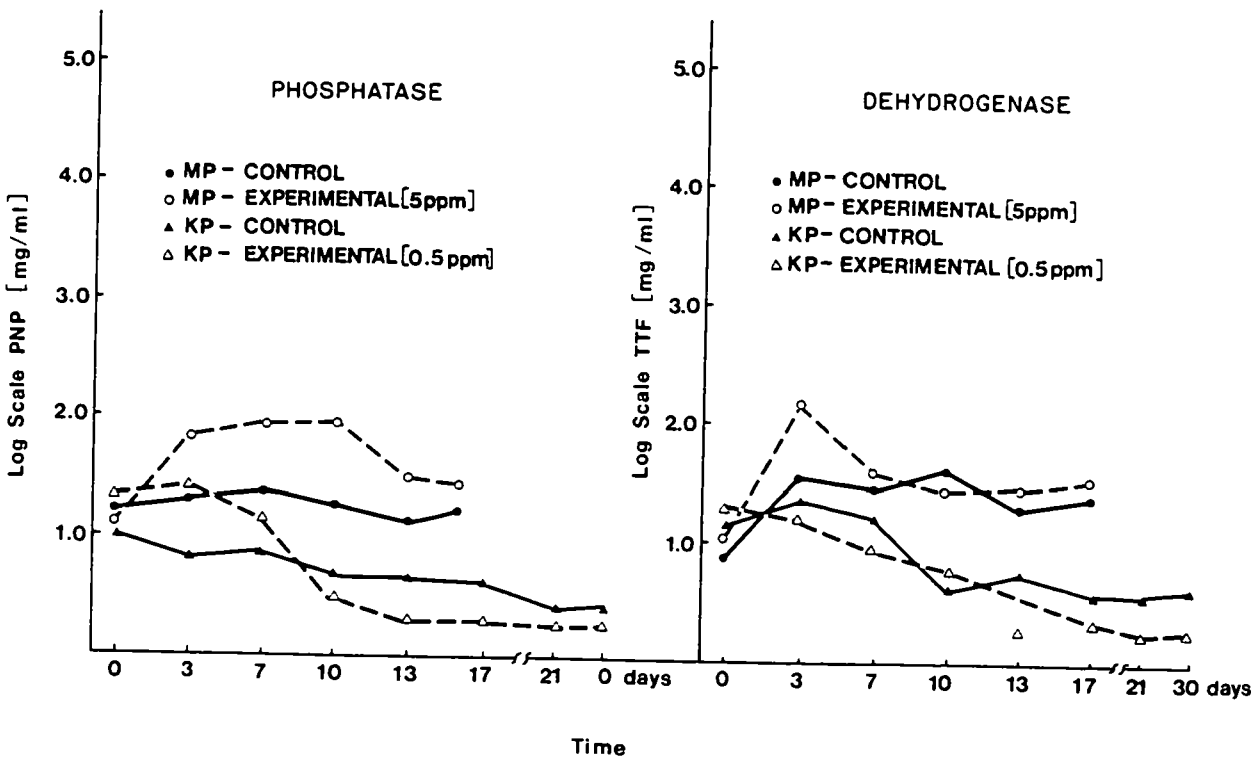


Figure 36. Response of Phosphatase and Dehydrogenase to Addition of Methyl Parathion and Kepone in Static Microcosms.

three. Levels of dehydrogenase for methyl parathion were higher at day three but subsequently dropped to, or below, control levels. Hence, no statistically significant methyl parathion by treatment variation was discerned. Dehydrogenase levels for Kepone®, although somewhat lower than controls, were not statistically significant.

Significant increases in both phosphatase and dehydrogenase activity in flow-through microcosms were noted for continuous methyl parathion addition (Fig. 37). These increases coincided with those for total microbial CFU, as noted previously for methyl parathion. Dehydrogenase levels for Kepone® were somewhat below control values, but were not statistically significant.

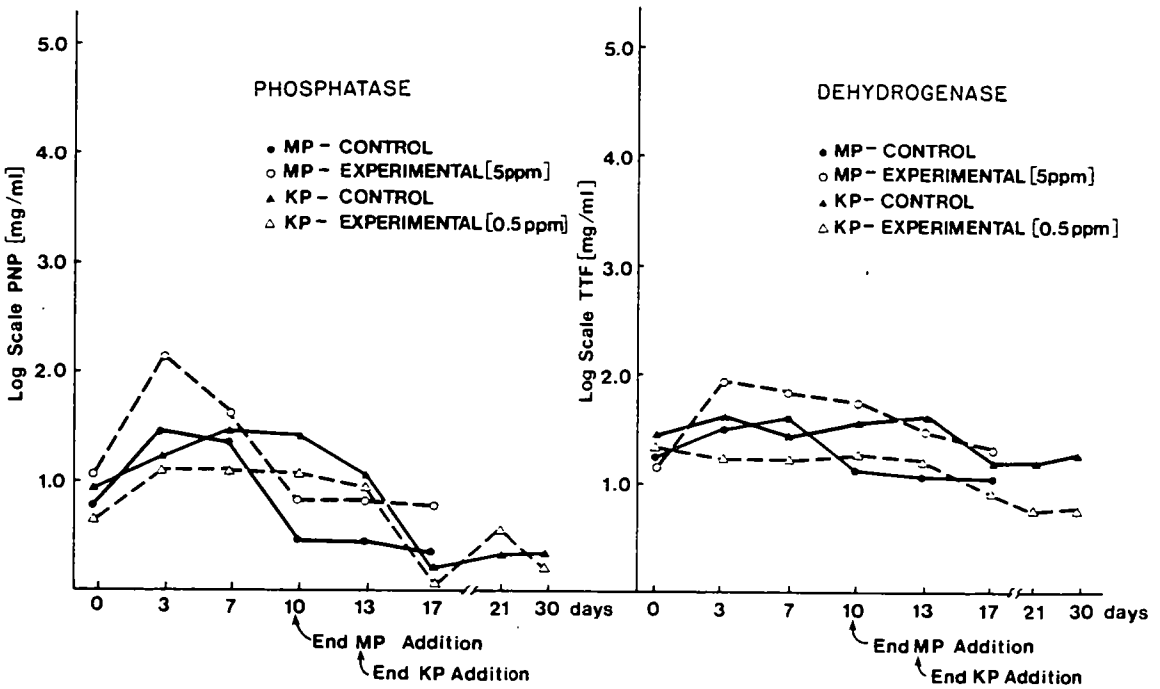


Figure 37. Response of Phosphatase and Dehydrogenase to Addition of Methyl Parathion and Kepone in Flow-Through Microcosms.

Radiotracer Analysis--

Residual levels of ^{14}C-methyl parathion and ^{14}C-Kepone® for replicate microcosm series, based on $^{14}CO_2$ mineralization of the parent molecule, is shown in Fig. 38. After 13 days under microcosm conditions outlined previously, a significant loss of methyl parathion was evident. Rapid biotransformation and degradation of the parent molecule occurred with the first week of application. This initial degradation period correlated with increasing CFU, phosphatase, dehydrogenase, and ATP levels, as mentioned previously. Fig. 39 shows maximal ^{14}C-assimilation of both parent molecules, or their major metabolites, within the CFU, occurring by day seven for methyl parathion. A sharp decrease is noted in ^{14}C-assimilation by day 13, along with similar decreases for $^{14}CO_2$ levels.

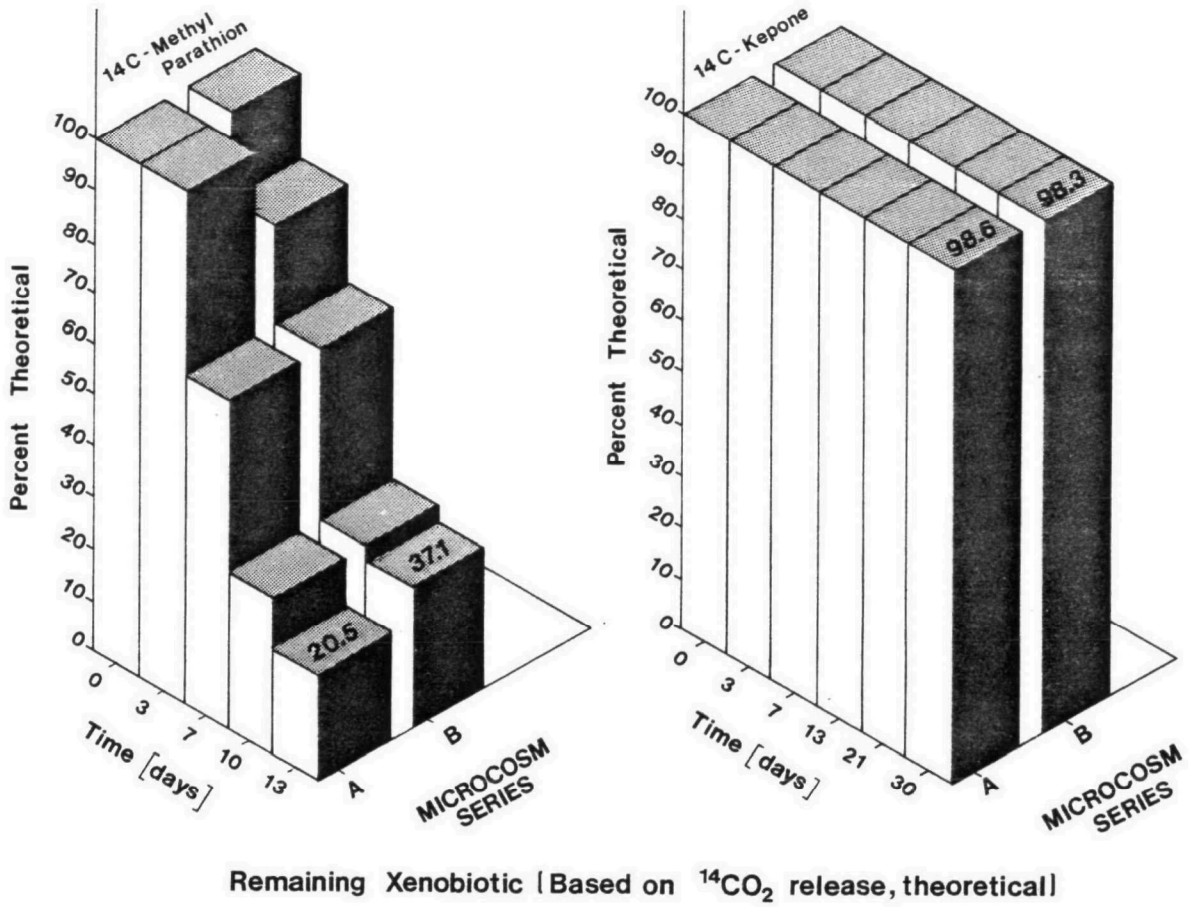


Figure 38. Residual levels of ^{14}C-Methyl Parathion and ^{14}C-Kepone in Static Microcosms.

Extremely low levels of $^{14}CO_2$ were detected for Kepone® during the 30 day incubation time period (Fig. 37). Over 98% of the original Kepone®, or possibly major metabolites, remained after this period. Although it is possible that the $^{14}CO_2$ detected could be small quantities of Kepone®, follow-up sterile microcosm tests with ^{14}C-Kepone® resulted in no $^{14}CO_2$ detection. ^{14}C-assimilation of Kepone® gradually increased during this period, raising the possibility of its being actively assimilated by the microbial component, however, with minimal utilization.

In carbon metabolism studies with ^{14}C-μL-methyl parathion and chitin (Fig. 40) a more rapid evolution of $^{14}CO_2$ occurred in chitin-supplemented microcosms compared with control microcosms. Although rates of ^{14}C uptake were approximately equal for CFUs for both control and chitin-supplemented microcosms, the total μCi of ^{14}C assimilated was greater for chitin-supplemented microcosms due to a significant increase in CFU. Methyl parathion degradation was further induced by the presence of this energy rich substrate.

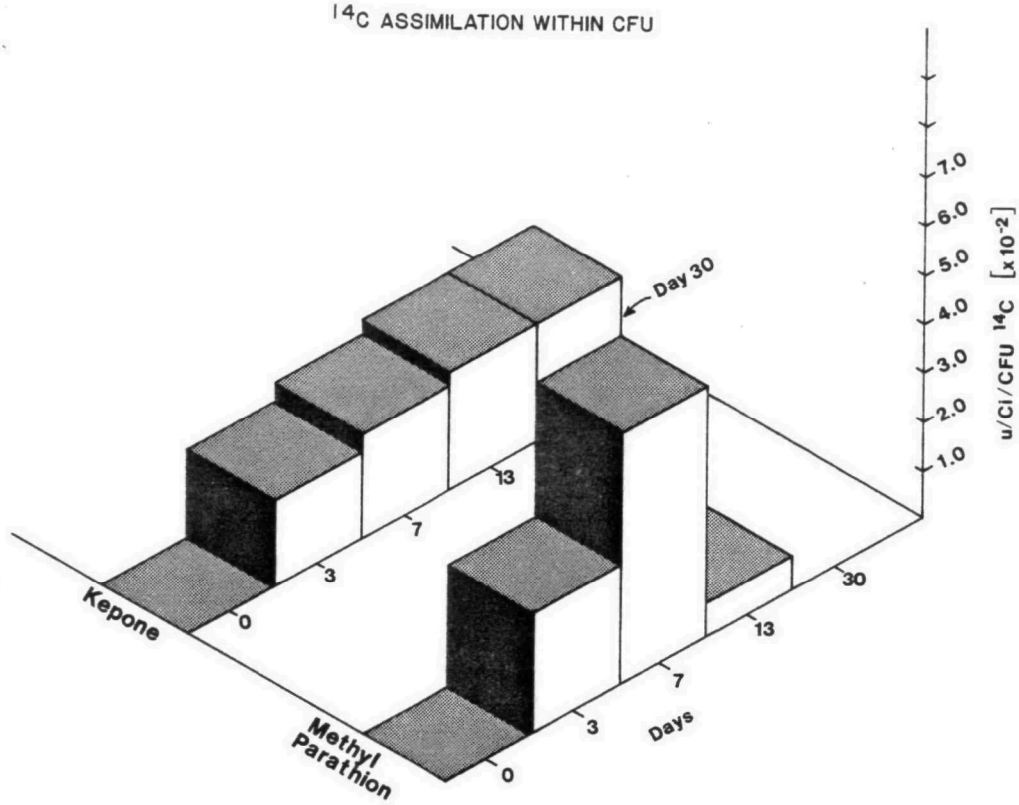


Figure 39. ^{14}C Assimilation Within Colony Forming Units (CFU) With Methyl Parathion and Kepone in Static Microcosms.

Particular attention in our research has been given to the structural polysaccharide chitin, probably the most abundant biodegradable polymer in marine and estuarine environments. Turnover of this energy and nutrient-rich material in estuarine environments is a critical basic transformation process in the functioning of trophic food webs (Fig. 41). Analyses of microbial ecology and chitinoclast populations requires careful study of chitinoclastic processes and substrate mineralization as affected by a variety of xenobiotics.

In earlier sections of our report, data were presented on interactions between a labelled breakdown product of chitin, N-acetyl-D-glucosamine (glucosamine-^{14}C), and the organophosphates, azinphosmethyl (Guthion) and methyl parathion. In these studies, significant increases in chitinoclastic populations, were indicative of rapid substrate transformation and utilization, coinciding with disappearance of the xenobiotic in question. The compound N-acetyl-D-glucosamine (NAG) is a key pathway substrate in enzymatic hydrolysis of chitin (Hood 1973) and appears to be an ecologically significant material in in situ chitin transformation (Hood and Meyers 1977a, b). Interaction between NAG and a variety of xenobiotics as will be shown in

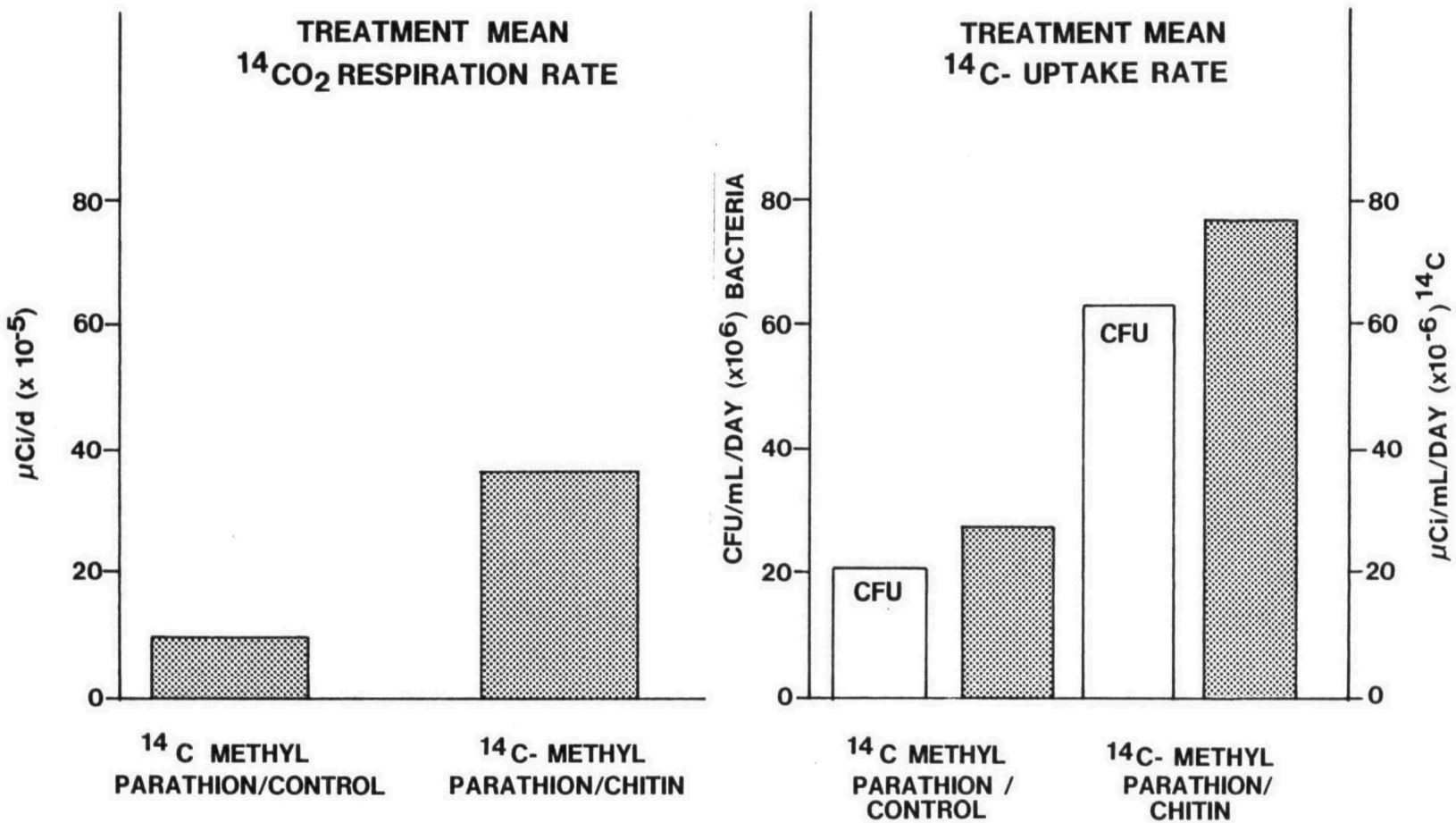


Figure 40. Effect of Chitin on $^{14}CO_2$ and ^{14}C Uptake in Methyl Parathion Carbon Metabolism Microcosms.

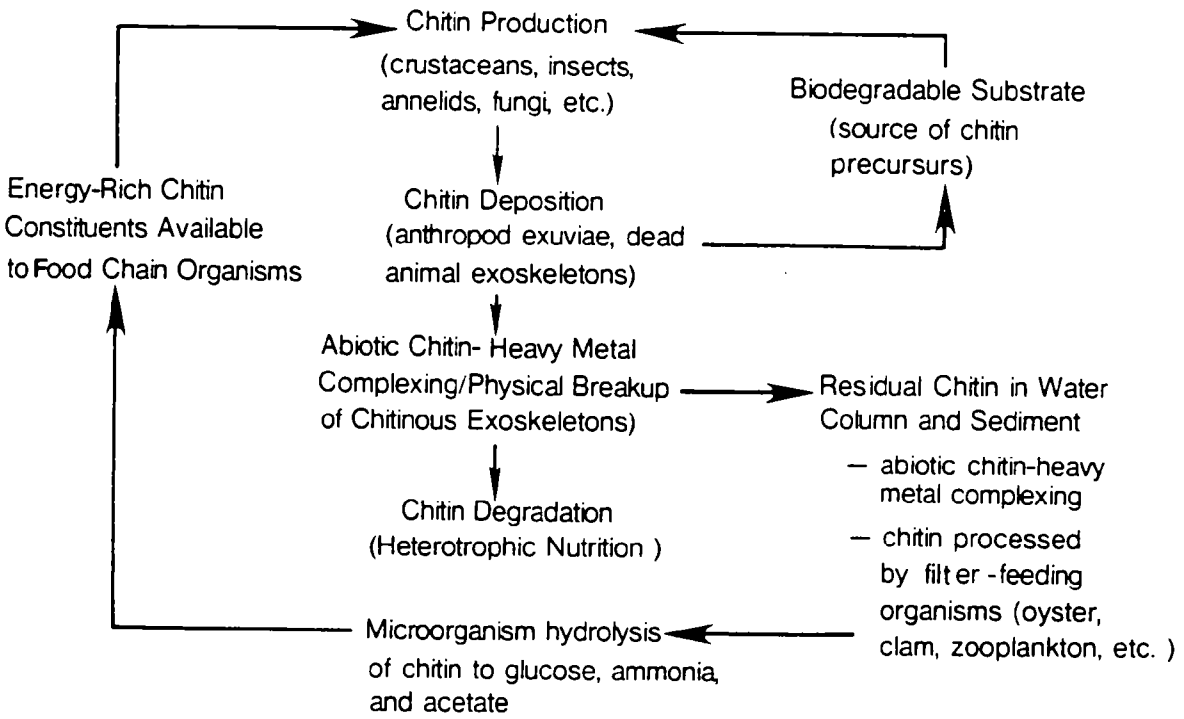


Figure 41. Chitin Turnover in an Estuary.

subsequent research with methyl parathion, Kepone® and selected phenols, provides an extremely sensitive monitoring "tool" that can be effectively utilized in microcosm systems.

In subsequent investigations, ^{14}C-μL-methyl parathion and ^{14}C-Kepone® were introduced into both continuous flow and static chitin-amended microcosms at concentration levels noted in previous work (5.0 ppm and 0.5 ppm, respectively). Figs. 42 and 43 present microbial population responses of continuous flow studies for methyl parathion and Kepone®, respectively. In the methyl parathion run, significant increases in bacterial, fungal and total chitinoclasts were noted in experimental microcosms for days 3 and 7 compared with controls. Microbial population levels for Kepone®-amended microcosms were lower than controls for days 3, 7, and 13, with fungal and chitinoclastic groups showing significance ($p < .05$).

Similar responses for phosphatase, dehydrogenase and microbial ATP were noted for both Kepone® and methyl parathion as evidenced in earlier investigations with chitin, except that dehydrogenase and microbial ATP levels were significantly higher in methyl parathion/chitin microcosms. Fig. 44 shows residual levels of ^{14}C-methyl parathion and ^{14}C Kepone® for comparable microcosm series based on $^{14}CO_2$ release. Chitin produced no effect in compound disappearance in the Kepone® study, whereas in the methyl parathion study, chitin contributed to both compound assimilation and utilization.

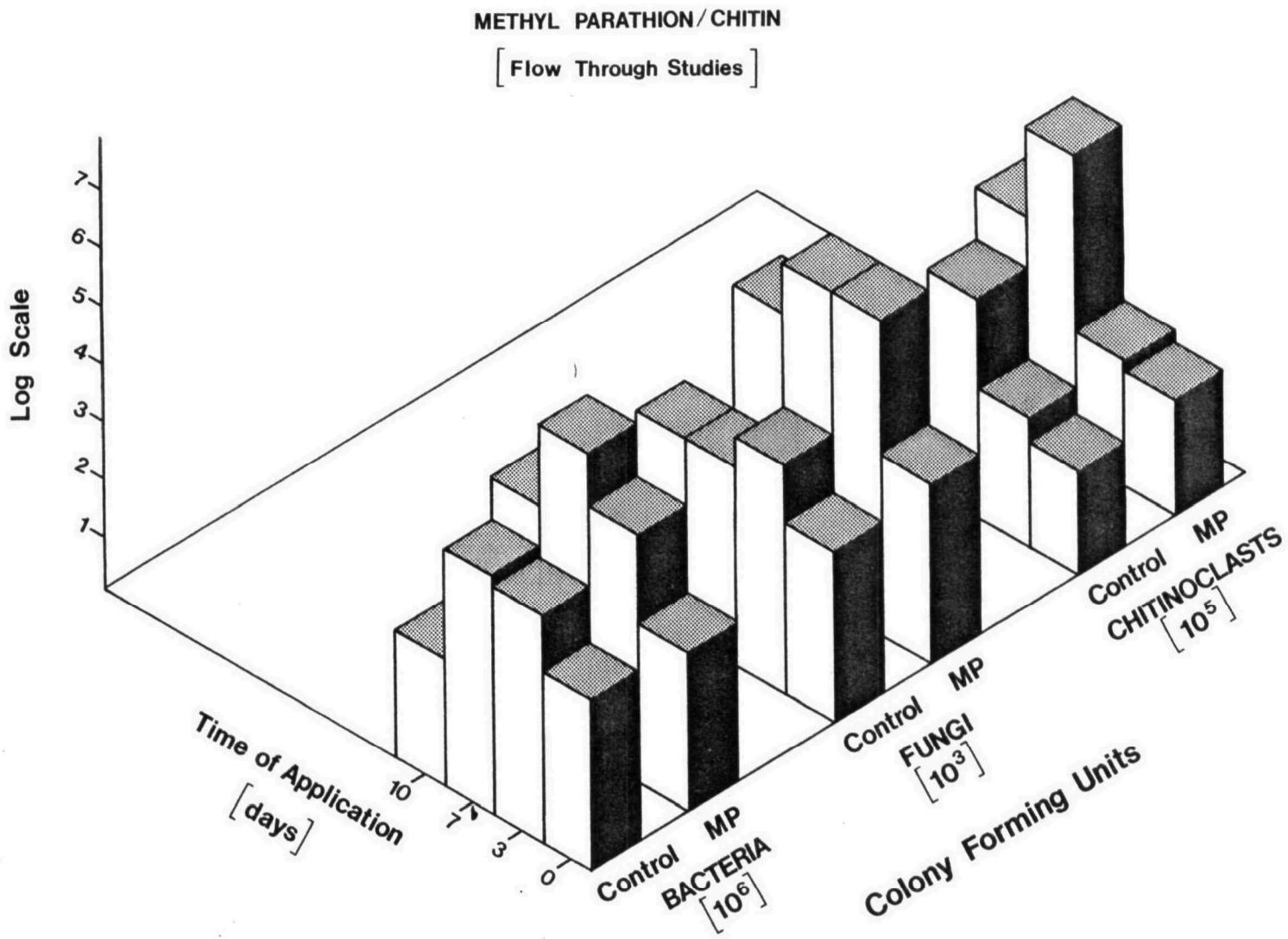


Figure 42. Microbial Response to Methyl Parathion in Chitin-Amended Flow Through Microcosms.

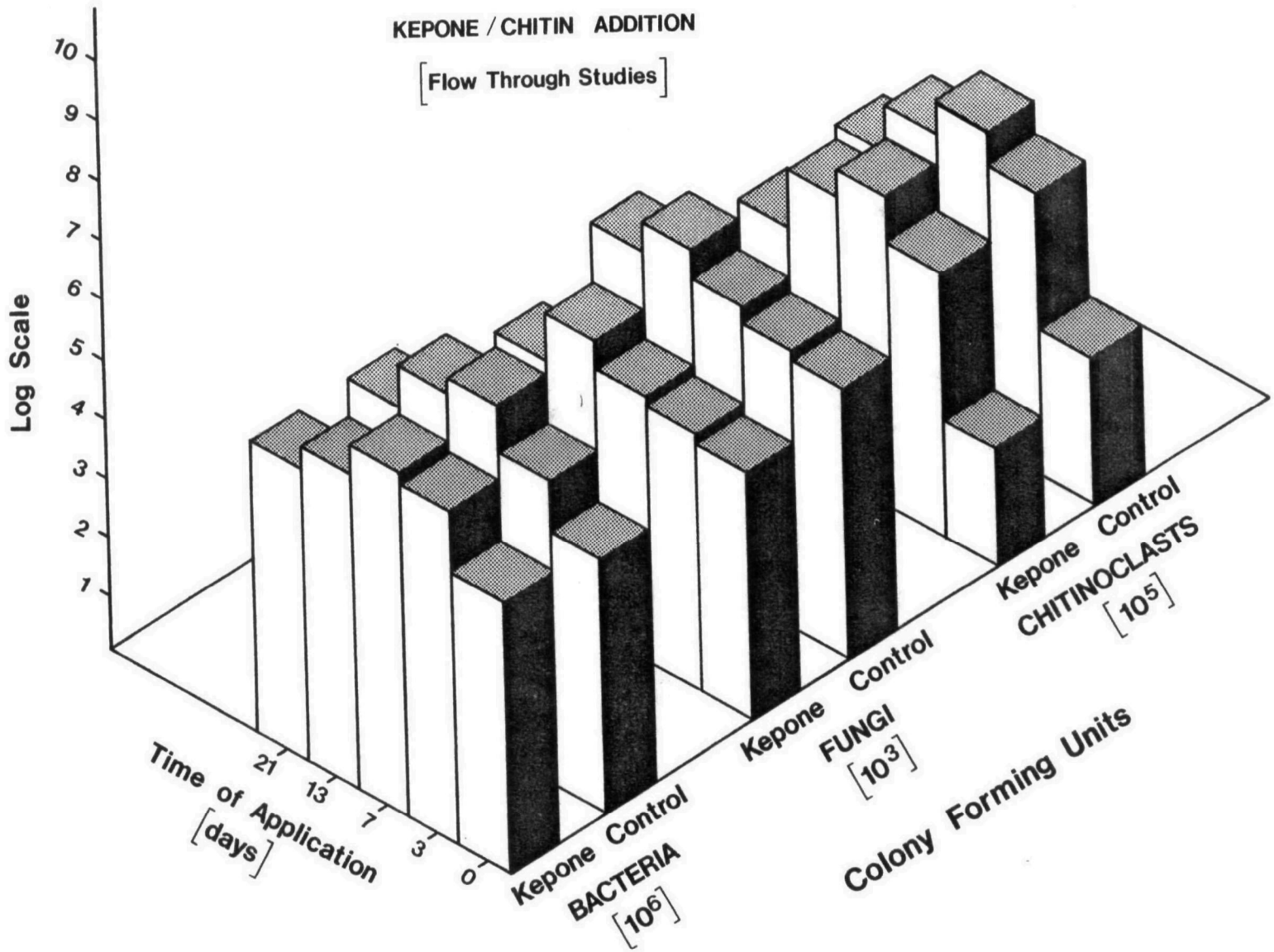


Figure 43. Microbial Response to Kepone in Chitin-Amended Flow Through Microcosms.

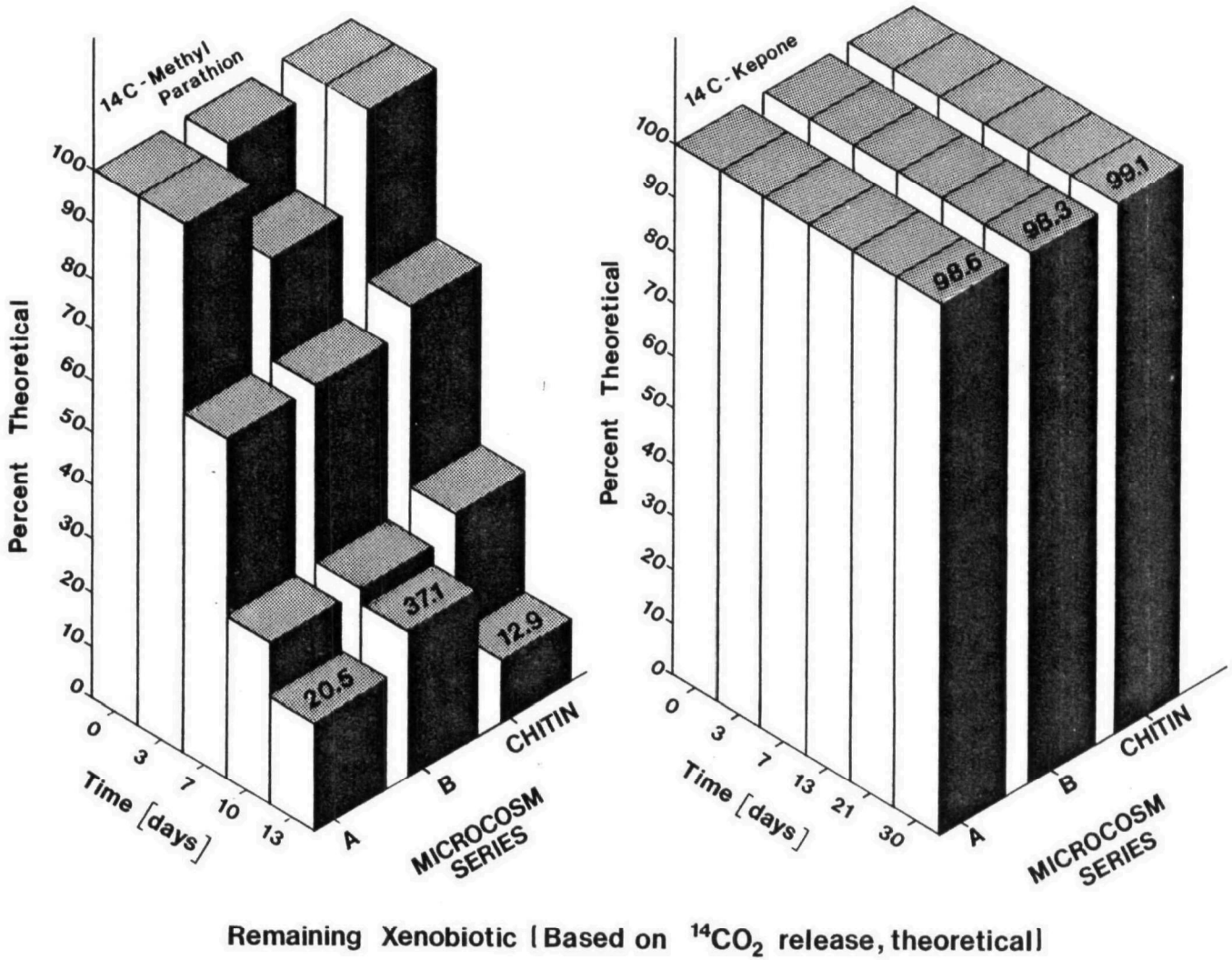


Figure 44. Residual Levels of ^{14}C-Methyl Parathion and ^{14}C-Kepone in Carbon Metabolism Microcosms.

Understanding of the substrate and enzymatic events in chitin breakdown is critical in terms of discerning effects on pathway flow and points of maximal compound utilization. These involve particle size reduction, followed by decalcification and deproteinization of the chitinous matrix. Effects can be direct, i.e., on the process itself, or indrect, i.e., via inhibition or reduction of activities of the chitinoclast biomass. Investigators have noted that adsorption effects (surface phenomena) are especially important in chitinoclastic processes and related colonization activities. Analyses of chitinase elaboration as a major activity factor and its relationship to xenobiotics should provide relevant data on environmentally-significant surface phenomena. Xenobiotics may physically block active sites on the chitin substrate, thus inhibiting inception of breakdown processes. The products N-acetylglucosamine and glucosamine resulting from activity of chitinolytic enzymes can be monitored to study various physiological determinants intrinsic to the composition of the microbial community and transformation of ecologically significant substrates such as chitin.

Axenic Flask Studies--

Growth responses of representative microcosm isolates are shown in Table 13. In all instances with methyl parathion, a moderate to high response (> 30% change) was noted in overall ATP levels. This correlated with rapid assimilation of the parent molecule in mixed enrichment media as designated and a notable total biotransformation. A low response was evident for isolates No. 1, 2, and 3 for methyl parathion in minimal salts media, whereas a negative response occurred for the bacterial isolate No. 4 along with a moderate response in the chitin medium. The most appreciable loss of methyl parathion occurred with fungal Isolate No. 1: 38% after seven days in Martin's media. However, when methyl parathion was the sole carbon source, only low responses were noted. These results are comparable to those of Rao and Sethunathan (1974), using enrichment culture techniques, in which the fungus Penicillium waksmani, tolerated high levels of parathion and actively converted parathion to aminoparathion. Our observations also indicate the important role that fungal species may play in sequential biotransformation of a xenobiotic. Munnecke and Hsieh (1976) alluded to similar findings using specifically adapted mixed microbial cultures for detoxification of parathion.

All representative microcosm isolates grown in the presence of Kepone® showed minimal sensitivity to the compound at a concentration of 100 μg/l. Relatively low levels of ^{14}C were assimilated by each isolate during the seven-day incubation period. Total ^{14}C biotransformation was not noted for any of the Kepone® microcosm isolates. However, the actinomycete isolate from the methyl parathion microcosm induced a noticeable total ^{14}C biotransformation, suggesting possible xenobiotic biotransformation by this particular organism. Bourquin et al. (1977) mentioned the possible interference of methyl parathion degradation by Kepone®. While species specific responses to xenobiotic input are noted in axenic culture, extrapolation of such data to provide information on the metabolic integrity of an aquatic system may present an incomplete picture.

Table 13. Microcosm-Isolated Species Level Response to Tagged Xenobiotic Addition.

Isolate No.	Microcosm Source	^{14}C-MP + Enrichment	^{14}C-KP + Enrichment	ATP Response	^{14}C Assimilation	Total ^{14}C Biotransformation
1 - Fungal	MP	MS		+	+	+
		MAR		+++	+++	+++
		CHT		+++	+++	+++
2 - Yeast	MP	MS		+	+	+
		MAR		+++	+++	+++
		CHT		+++	++	++
3 - Actino.	MP	MS		+	+	+
		JEN		++	++	++
		CHT		+++	+++	+++
4 - Bact.	MP	MS		-	-	-
		JEN		+++	+++	+++
		CHT		++	++	++
5 - Fungal	KP		MS	-	-	-
			MAR	+++	+	-
			CHT	+++	+	-
6 - Yeast	KP		MS	-	+	-
			MAR	+++	+	-
			CHT	+++	+	-
7 - Actino.	KP		MS	-	-	-
			JEN	+++	+	-
			CHT	+++	+	-

Table 13. (Continued)

Isolate No.	Microcosm Source	^{14}C-MP + Enrichment	^{14}C-KP + Enrichment	ATP Response	^{14}C Assimilation	Total ^{14}C Biotransformation
8 - Bact.	KP		MS	-	-	-
			JEN	+++	+	-
			CHT	+++	+	-
1 - Fungal	MP		MS	-	-	-
			MAR	+++	+	-
			CHT	+++	-	-
3 - Actino.	MP		MS	-	-	-
			JEN	++	+	+
			CHT	+++	+	+

MP - methyl parathion (500 μg/ℓ)
KP - Kepone® (100 μg/ℓ)
MS - minimal salts
MAR - Martin's agar medium*
JEN - Jensen's agar medium*
CHT - Chitin agar*

- negative response
+ low response (<10%)
++ moderate response (<30%)
+++ high response (>30%)
incubation time - 7 days
incubation temperature - 30 C

*see p. 38

Gas Chromatography Analysis--

Residual sediment samples analyzed for methyl parathion via GC techniques showed close correlations between laboratory and in situ rates at experimental plots. The latter ranged from 3.7 to 5.2 days for pesticide half-lives. Microcosm half-life values for methyl parathion® were 3.3 days (mean treatment determination). Field applications of Kepone® were not conducted. However, GC analysis of microcosm sediments showed appreciable, accumulation levels of Kepone® in flow-through systems and negligible loss of the compound in static microcosm systems, i.e., 92.4% remaining.

CONCLUSIONS

Our studies, using a combination of microbial and enzymatic approaches, and environmental correlations, support the value of the benchtop microcosm as an analytical tool. Rates of utilization and breakdown product generation can be effectively monitored. Furthermore, varying rates of utilization and biotransformation of two dissimilar chemical toxicants, i.e., methyl parathion and Kepone®, can be discerned within the microcosm. The rapid disappearance of methyl parathion coincides closely with in situ results. ATP and other enzymatic tests provide sensitive indications of initial degradation of the parent compound, and can be used as early indices for fate analysis. Axenic flask studies have further confirmed these findings. Control microcosm units have repeatedly reflected a comparable microbial, biomass found in control field sites and also have exhibited comparable microbial diversity and specific enzyme levels. Exposed microcosms, statistically identical to control units, provided reproducible information on initial environmental effects, as well as data on environmental fate of the target pesticides used in this study. Variations in microbial biomass, microbial diversity and specific enzyme level responses were more apparent and precise in exposed microcosms than in exposed field sites. However, these variations on a broader scale were indeed noted in field exposed plots. The relative half-life, $T_{\frac{1}{2}}$, of target compounds were of the same order of magnitude in both microcosm and field studies. Multivariant statistical analysis of all parameters analyzed in field plots provided a correlation matrix similar, if not at times identical, to comparable multivariant studies of microcosm parameters. The concept of the microcosm itself is validated and verified in that it can provide a straightforward, reproducible, and accurate means of simulating an ecosystem to generate analytical data on stress and impact of abiotic and biotic factors.

The importance of monitoring chitinoclasts (chitin-decomposing microorganisms) and chitin substrate cycling in terms of energy flow in estuarine food webs is clearly evident. Use of the microcosm in analyzing the effect of xenobiotics on chitin transformation provides a valuable "tool" in discerning early trophic level responses as well as overall environmental impact by target toxic substances.

It is equally important in validation of the microcosm approach to establish whether recalcitrant compounds, such as Kepone®, behave similarly in the laboratory microenvironment. A rapid biodegradation rate for such a recalcitrant would indicate a containerization effect attributable to either

incorrect parameter assessment or to the microcosm design itself. As shown in these studies, Kepone® was recalcitrant, with minimal $^{14}CO_2$ expiration. ATP and specific enzyme systems, i.e., phosphatase and dehydrogenase, supported this finding, together with lack of significant variations in both bacterial and fungal components of the microbial biomass.

Certainly, statistical tests, including analysis of variance and multiple regression techniques, are essential methods to be employed in evaluating data generated in our studies. Types of data correlations evaluated are summarized on the following page. Along with the above tests, Duncan's multiple range test have been used to elucidate microbial/enzymatic responses of treatment variations. In our conceptual approach of aquatic microcosm techniques, a logical and economical subsequent step would be mathematical model interpretation of microcosm results. The systems developed represent prototype models that closely approximate important critical parameters of the target environment.

The approaches and experimental data given here should allow development of a quantitative baseline of major microbial groups and related enzyme activities for interconnected ecosystems within an ecologically important drainage basin. Development of information on enzyme/microorganism interactions is relevant to understanding determinants of xenobiotic impact on productivity in aquatic regions such as the Barataria Basin. Furthermore, total microbial biomass and species diversity may serve as valid indices of biodegradable substrate turnover and productivity, and should provide information on microhabitat features as affected by the addition of a variety of toxic substances.

Microcosms provide a means for replacement of possible sensitive microorganisms by tolerant species, as well as monitoring sequential biodegradation processes among important indigenous microbial populations. Fungal and actinomycete groups appear to be particularly significant in this regard. The approaches and experimental data given here should permit further development of a quantitative "baseline" of major microbial groups together with relevant enzyme activities for evaluation of ecosystems within larger aquatic regions. With careful consideration given to those physicochemical and microbial processes needed to simulate environmental conditions, microcosm approaches reported here can generate valuable and relevant information on xenobiotic activities in wetland and aquatic ecosystems.

DATA CORRELATIONS

*OVERALL			.9208	.9478	Compound
	Phosphatase	Dehydrogenase	.9375	.8948 .9140	
	ATP	$^{14}CO_2$ ^{14}C Assimilation			
Microbial Biomass			.9153	.9965	MP
	Phosphatase	Dehydrogenase	.9371	.9072 .7571	MP K
	$^{14}CO_2$	^{14}C Assimilation			
*SPECIFIC Bacteria			.9564 .8778	.9535 .9010	MP K
	Phosphatase	Dehydrogenase	.7434 --	.9061 .5203	MP K
	$^{14}CO_2$	^{14}C Assimilation			
Fungi			.9043	.8798	Guthion
	Phosphatase	Dehydrogenase	.9146	.8741	Guthion
	$^{14}CO_2$	^{14}C NAG Assimilation			
Chitinoclasts			.8840 .9126	.8788	MP MP
	Phosphatase	Dehydrogenase			
	ATP				
$^{14}CO_2$			.8462 .8814	.9148 .7214	MP K
	Phosphatase	Dehydrogenase	.9649 .8372		MP
	ATP				
^{14}C Assimilation			.9967 .7185	.7264 .6367	MP K
	BAC	Fungi	.9182 .6556		MP K
	CHT				
ATP					

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SECTION 4

IN SITU EFFECTS OF GUTHION ON BACKSWAMP AQUATIC INVERTEBRATES AND SHALLOW WATER LEAF LITTER DECOMPOSITION

J. W. Day Jr. and F. H. Sklar

INTRODUCTION

Substitute chemicals, like Guthion, move throughout the environment whereby their total impact cannot be evaluated by tests that deal with only one species or a single component of the particular environment. The application of Guthion in this portion of the overall study was *in situ*, and experiments were designed to answer two major questions:

a. How are the aquatic invertebrate communities affected by Guthion? The invertebrate biota was chosen for a number of reasons: 1) they are important as fish food items and are considered to be ecologically significant members of the swamp food web; 2) they occupy all trophic levels; 3) their biomass and activities in general reflect the environmental conditions. Furthermore, the limited motility of the benthos makes it less difficult to observe real population changes, based on ease of sampling.

b. What is the effect of Guthion on swamp litter decomposition rates? Nutrient recycling, export of inorganic and organic materials, and incorporation of the latter into sediments, are critical resultants of wetland decomposition processes that could be adversely affected by increasing use of pesticides on agricultural lands adjacent to swamps and marshes. To our knowledge, the effects of pesticides on leaf litter decomposition rates in wetland habitats have not been examined, although a number of terrestrial studies have been conducted (Neary and Merriam 1978; Gottschalk and Shure 1979) with variable results.

Insecticides accounted for nearly half of the 340 x 10^6 Kg of pesticides used in the USA in 1964 (Thompson and Edwards 1974). Of this volume, 31.8 x 10^6 Kg were organophosphates (Brown 1978). By 1974, the use of organophosphates had increased to 84 x 10^6 Kg. Currently, between 1.61-4.27 (x 10^4)Kg/yr of the organophosphate Guthion is applied to nearly 17,000 hecatres of sugarcane in the Barataria Basin hydrologic unit (Hopkinson 1978; Dozier 1978). The majority of these farmlands are adjacent to freshwater swamps and marshes, thus the need for the tests described here to evaluate the impact of Guthion runoff on the biota of these latter localities.

Guthion is not as tightly bound to soil particles as organochlorine insecticides such as endrin. When a mixture of Guthion and aldrin are added to a 60 cm column of soil, Guthion readily percolates through the column but aldrin does not (Lichtenstein et al. 1966). Because of their greater water solubilities and higher mobility in soils, the organophosphates are not readily volatilized into the atmosphere and are more likely to enter aquatic environments via runoff (Westlake and Gunther 1966). Percent runoff can vary from 8.6% for a 2.8 cm/hr rainfall, to 66% for a 11.2 cm/hr rainfall in the upper Barataria Basin (Hopkinson 1978). Based on the Hopkinson runoff model (Hopkinson 1978), maximal Guthion concentration in the runoff after a 1" rain (2.5 cm/hr) would be 328 ppb if Guthion were applied at the usual dose of 0.85 lbs/ acre (Dozier 1978). As rainfall increases, the insecticide is diluted but the percent runoff increases. Assuming all of the applied Guthion dissolves in rainwater, then the maximal concentration in the runoff after a 4" rainfall, i.e., 10.2 cm/hr, would be 616 ppb.

When endrin was used on sugarcane, the runoff rate was 5 ppb in runoff and 1 ppb in groundwater, and was considered to be safe to aquatic life (Willis and Hamilton 1973; Pionke and Chesters 1973; Lauer et al. 1966). At that time, the majority of the fish kills in the Barataria basin were attributed to pesticides (U.S. Army Corps of Engineers 1974). Endrin was postulated to be the major factor in the extensive fish kills in the lower Mississippi River in 1960 and 1963, and its runoff is now considered to represent an environmental hazard (Brown 1978).

The toxicity of Guthion for fish is less than that of endrin, but greater than endrin for crustaceans, nematodes, and other invertebrates (Brown 1978; Torgeson 1972). Guthion could, therefore, pose a more indirect hazard to fish. This chemical, soluble in water at 24 ppm (at 25C) is a non-systemic phosphorodithioate organophosphate with high mammalian toxicity, i.e., the acute oral LD_{50} for rats is 15 mg/Kg (Eto 1974). Laboratory measured toxicities to aquatic fauna range from a high of 0.20 ppb for the cladocerans (Daphnia) to a low of 3,300 ppb for channel catfish (Table 14).

Destruction of any food web component can affect an entire ecosystem. Attempts to restock insecticide-contaminated rivers with fish often are unsuccessful because the fish food organisms do not return (Gray 1974). Subtle biological changes can occur over long periods of time which could induce permanent ecological changes. For example, methyl parathion affects prey selection of gulf killifish (Farr 1978) and sediment turnover rates of lugworms (Bourquin et al. 1979). Malathion produces spinal deformations in juvenile bluegills at 10 ppb (Eaton 1970). DDT affects avian eggshell thickness (Anderson and Hickey 1972; Bitman et al. 1970). Mosquito fish accumulate parathion and produce lethal excreta (Mulla et al. 1966). These types of changes affect all trophic levels.

Table 14. Toxicity of Guthion (ppb) to aquatic fauna

Organism		LD (ppb)	Exposure (in Hrs)	Reference
Brown Shrimp		1	48	Brown 1978
Amphipoda (Gammarus lacustus)		0.25	48	Sanders 1969
Cladoceran (Daphnia magna)		0.20	48	Sanders and Cope 1966
Oyster Eggs (Crassostrea virginica)		620	48	Davis and Hidu 1969
Clam eggs (Mercenaria mercenaria)		860	48	
Stovefly (Pternarays california)		1.50	96	Sanders and Cope 1968
Tadpole (Bufo woodhousii fowleri)		0.13	96	Sanders 1970
Crawfish (Procambarus clarkii)		100	96	Baker 1974
Fish	Rainbow trout	14	96	
	Coho salmon	17	96	
	Yellow perch	13	96	
	Bluegill sunfish	22	96	Macek and McAllister 1970
	Largemouth bass	5	96	
	Channel catfish	3,300	96	
	Fathead minnow	240	96	
	Carp	700	96	
	Mosquito fish	78	96	Carter and Graves 1973
	Stickleback	4.80	96	Katz 1961
	Sheepshead minnow	3	72	Coppage 1972
	Pinfish	10	24	Coppage and Mathews 1974
	Spot	20	24	

EXPERIMENTAL

Short term effects of a single exposure to Guthion. Test Series I:

In the fall of 1977, four 48 cm diameter plastic enclosures were placed in the open area of an impounded swamp. Guthion, dissolved in acetone, was used to inoculate three enclosures; an equivalent quantity of acetone alone was added to the control enclosure. Concentrations of Guthion in the treatment enclosures were 1 ppm, 10 ppm and 25 ppm. Samples of water, mud, and floating vegetation were taken after 1, 5, and 8 days for analysis of Guthion. Mud and water samples were stored in dry ice. The floating vegetation (Lemna spp.) and associated invertebrates were sampled by scooping water and vegetation with a 15 cm diameter corer (Paterson and Fernando 1971) and slowly pouring the liquid through 4-6 layers of cheesecloth. The animal/duckweed mixture retained in the cheesecloth was stored in a 10% formalin rose bengal solution to aid sorting by coloring the invertebrates a bright red (Masen and Yevich 1967). Material retained by a 500 μ sieve was sorted under a dissecting microscope at 2.5 x magnification.

A settling experiment was conducted to demonstrate that the fauna living in the floating duckweed do not remain there after death. Thus, any species retained in the cheesecloth will represent living specimens unaffected by short-term exposure to Guthion. Four 1-gallon plastic jugs were filled with swamp water, duckweed and invertebrates, and two of the jugs treated with 5 mg of Guthion in 5 ml of acetone while the other two were treated with 5 ml of acetone alone. Containers were kept at room temperature (18-24 C) for 48 hr. Differences between number of animals in the floating vegetation, and on the bottom and sides of the jugs were recorded for contaminated and control containers. The additional interfaces supplied by the walls of the containers decreased the number of animals found in the floating vegetation by 9% (Fig. 45). The lethal dose of Guthion decreased the number of animals in the floating vegetation by 100%, all sank to the bottom soon after death. Animals appeared unaffected by the 1 ppt acetone solution.

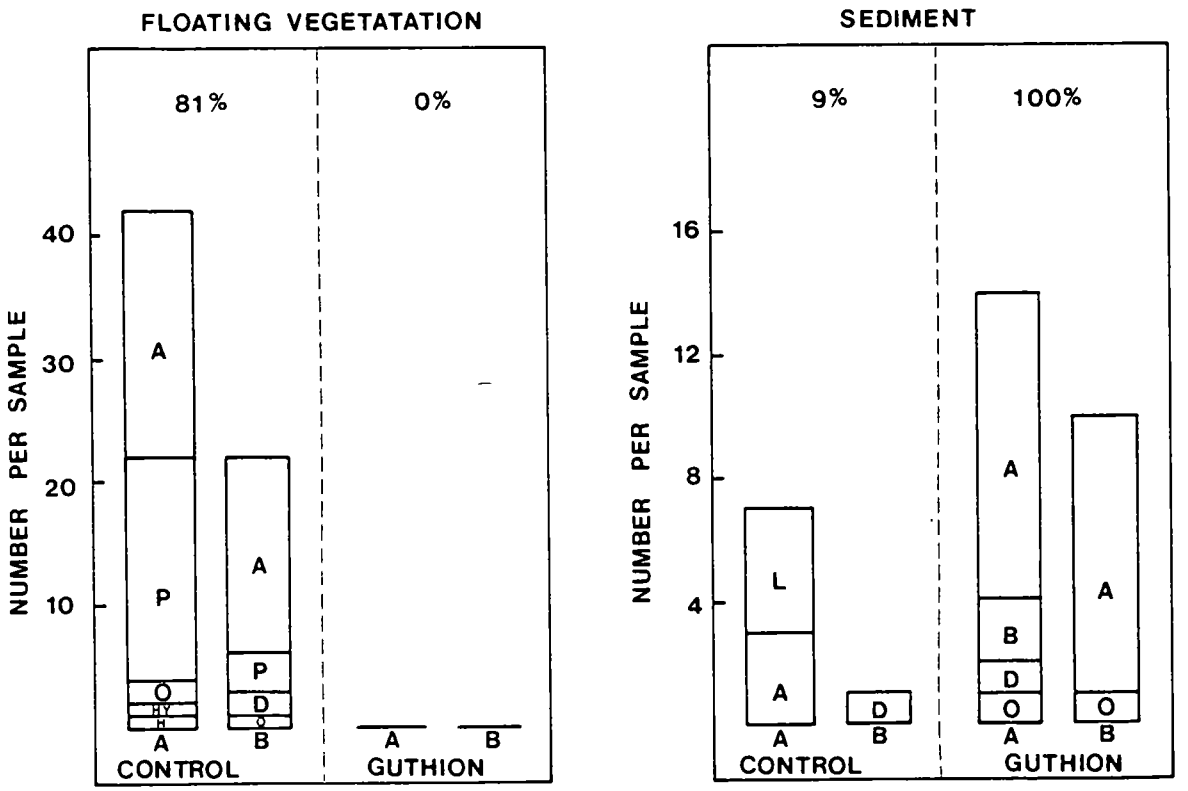


Figure 45. Effects of Guthion on Settling of Biota of Floating Vegetation (F.V.).

The data from Test Series 1 begin with Figure 46. The amount of dissolved Guthion in each of the swamp enclosures decreased very rapidly. After 1 day, the 25 ppm Guthion concentration in the water, decreased to 1.5 ppm, a 94% reduction. The reduction rates after 8 days for the 25 ppm, 10 ppm and 1 ppm enclosures were 97.6%, 96% and 95%, respectively. As the Guthion concentrations in the water decreased, concentrations in the

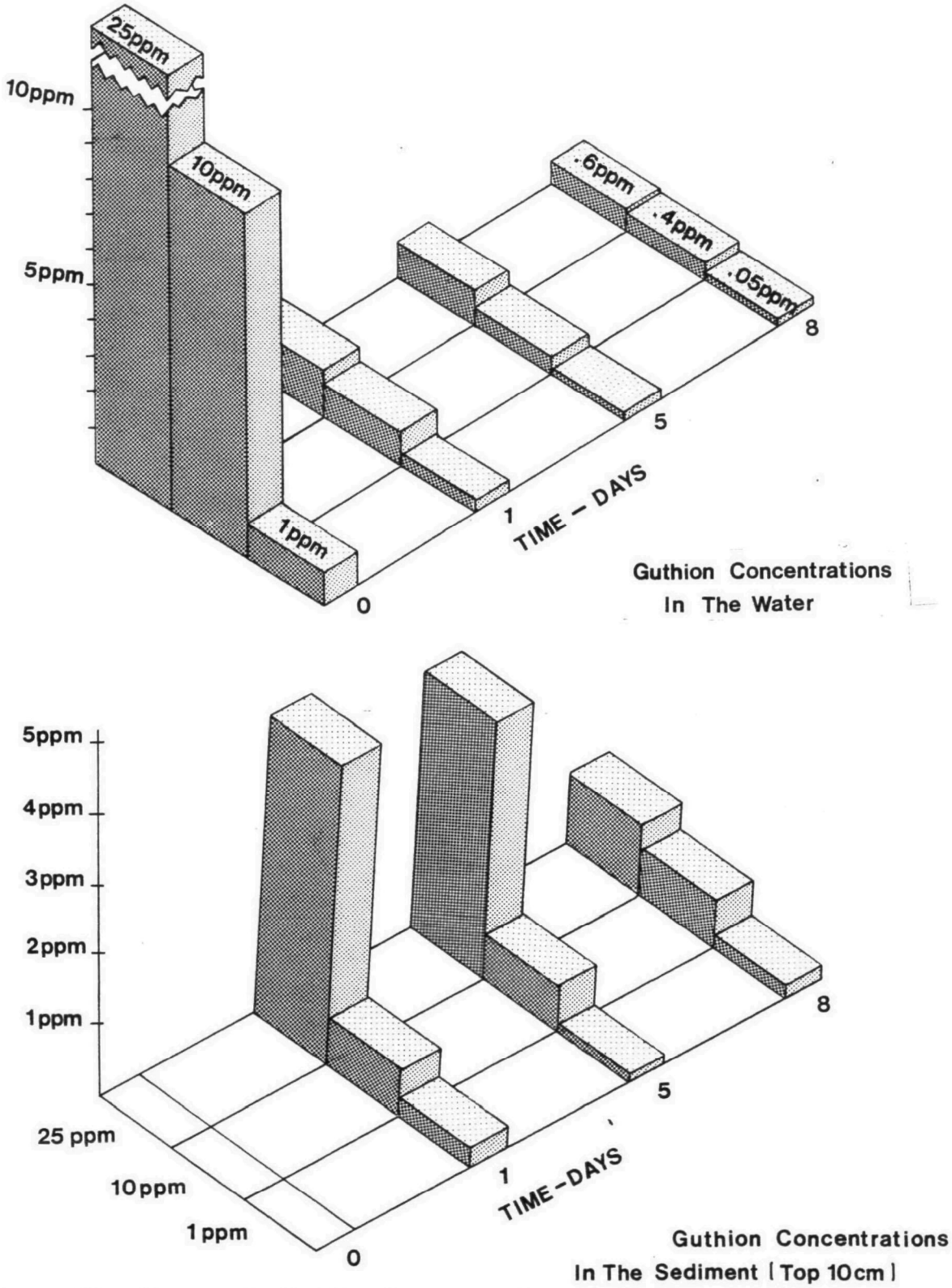
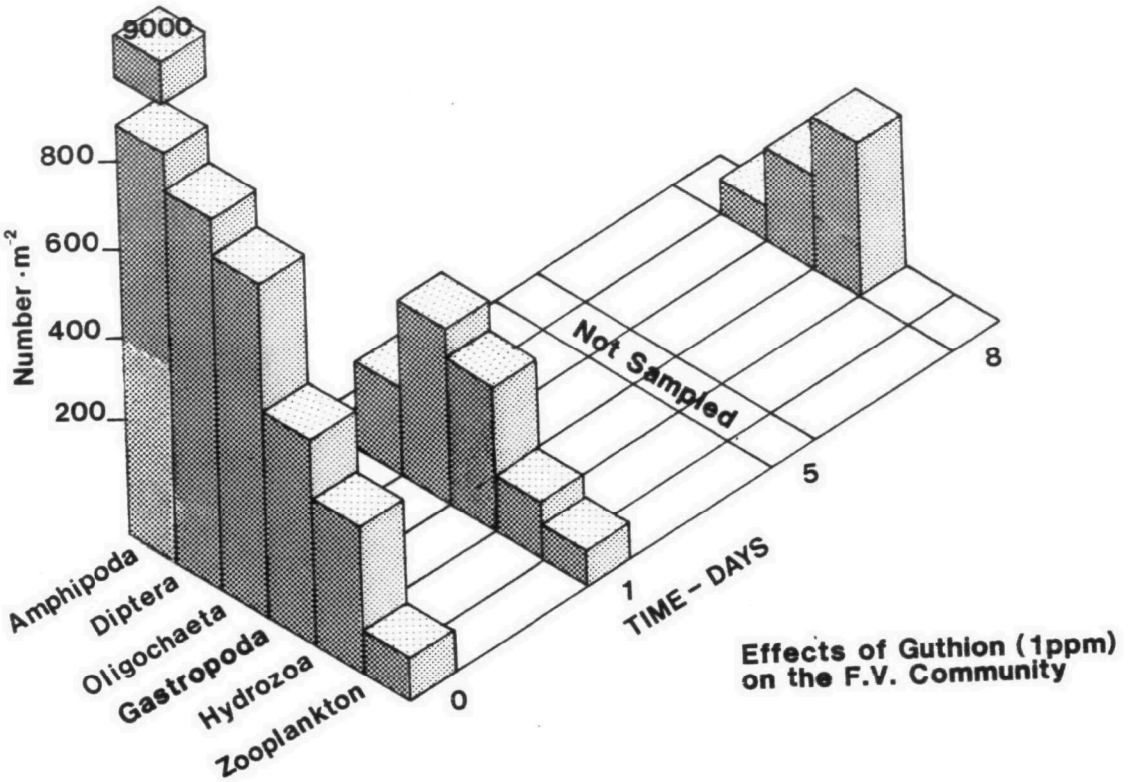


Figure 46. Changes in Guthion Concentrations in Sediment and Water.



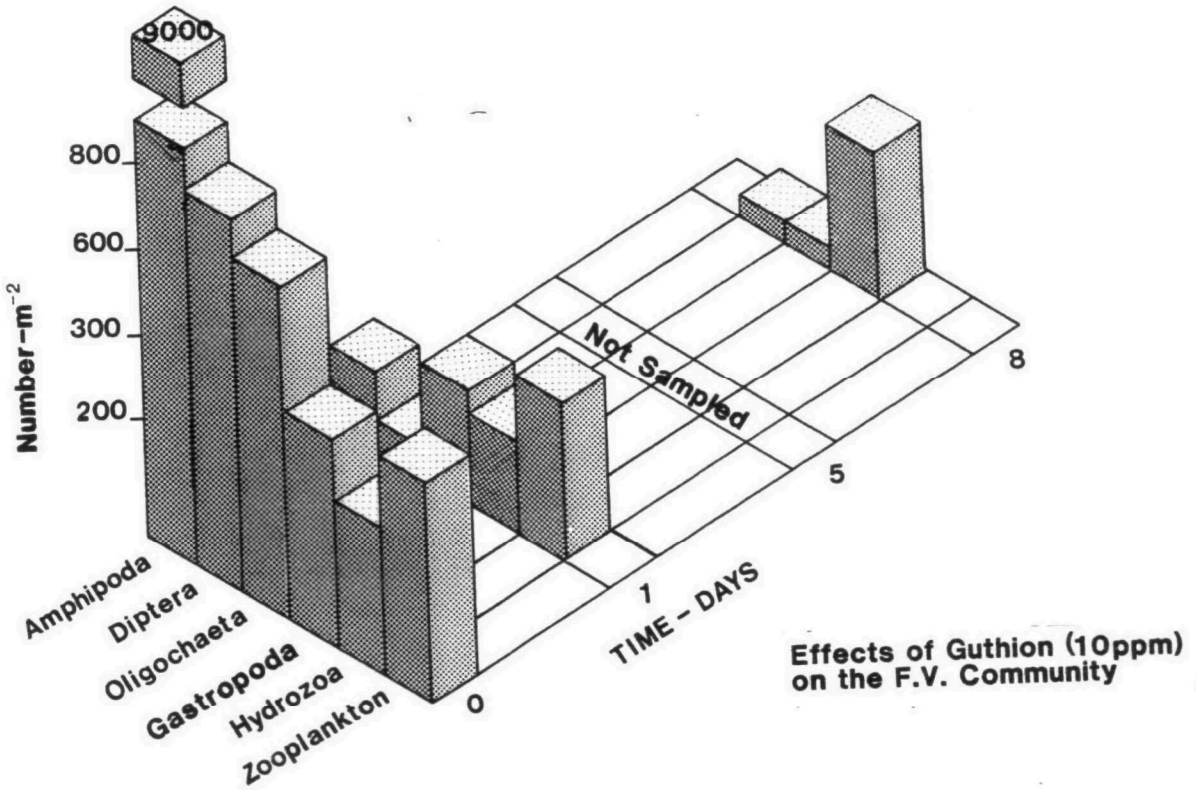


Figure 47. Effects of Guthion (1 and 10 ppm) on the Biota in Floating Vegetation (F.V.).

sediment increased (Fig. 47). Guthion was probably rapidly sorbed to sediments, unless added at concentrations above solubility. As much as 20% of the initial concentration was found in the top 10 cm of sediment 24 hr later. In this sediment Guthion disappeared slowly; seven days after the first appearance of Guthion in the sediment, the concentration in the sediment of the 25 ppm, 10 ppm and 1 ppm enclosures was 1 ppm, 0.56 ppm and 0.10 ppm, respectively. The effects of this sediment Guthion on benthic populations was part of another experimental design (Test Series II). The floating vegetation community was immediately affected by the presence of Guthion (Figs. 47 and 48).

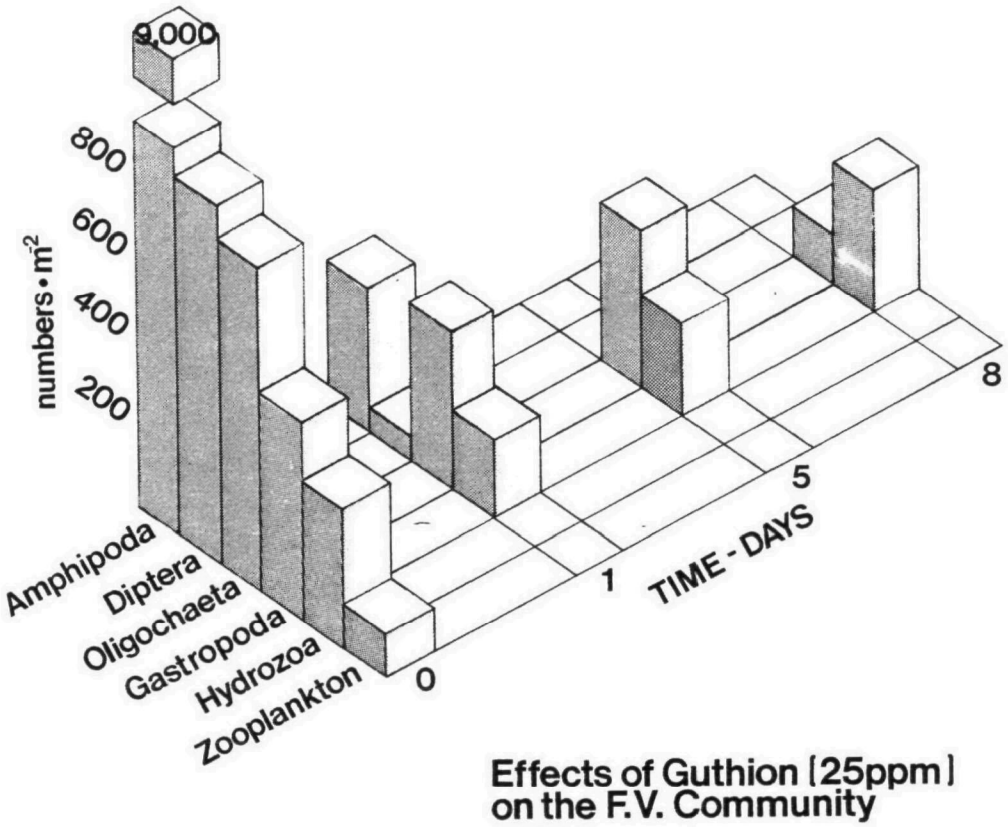


Figure 48. Effects of Guthion (25 ppm) on the Biota in Floating Vegetation (F.V.)

The control enclosure increased in total population, from 11,544/m^2 to 18,584/m^2 in 8 days (Fig. 49). The populations in 1 ppm, 10 ppm, and 25 ppm enclosures decreased to 680/m^2, 737/m^2 and 441/m^2, respectively, in 8 days. The large population of amphipods (i.e., Hyallela azteca) was eradicated after 8 days at all concentrations tested. Hydrozoans and zooplankton tolerated 1 ppm Guthion for the first day; however, by day 8, hydrozoans or zooplankton were absent. The diptera population (i.e. 1,400/m^2) was eradicated after 5 days at 25 ppm Guthion, but was still found after 8 days

at 10 ppm and 1 ppm Guthion. Gastropods and oligochaetes were the most abundant animals after 8 days.

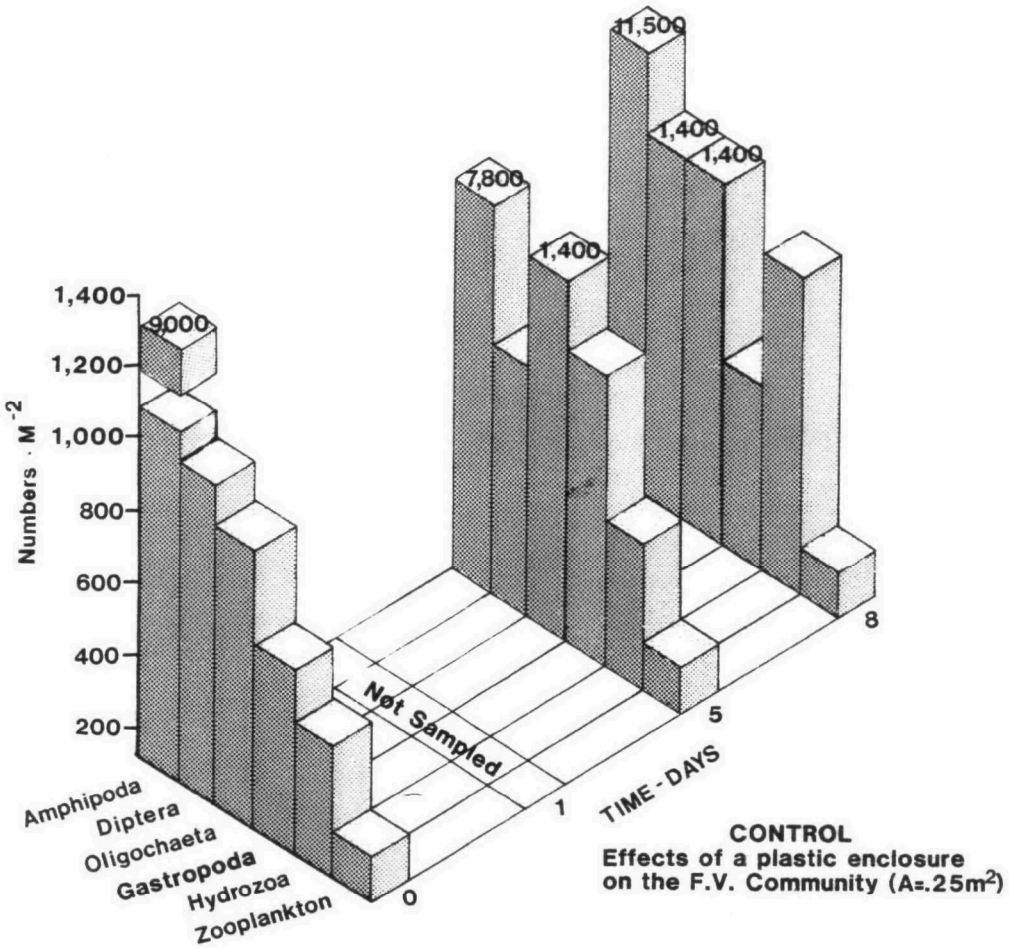


Figure 49. Effects of Plastic Enclosure on the Biota in Floating Vegetation (F.V.).

Long term effects of a single exposure to Guthion. Test Series II:

The benthic habitat was sampled with a 20-cm-diameter stovepipe corer, manually forced into the sediment. All contained materials were removed with a long handle ladle to a depth of 20-25 cm and poured into a 500 u sieve. Samples were preserved in a 10% formalin-rose bengal solution. According to Weber (1973), a corer, is the only quantitative device suitable for sampling shallow-water benthic habitats. Estimates of standing stock comparable to those obtained with an Ekman grab can be obtained with a corer, but with less effort because of the smaller volume of sediment that requires sorting (Flannagan 1970; Kajak 1971).

The benthic population within the 48-cm-diameter polypropylene enclosures was sampled with the stovepipe corer 16 days after inoculation with Guthion during summer 1977. Concentrations of Guthion in the enclosures were 0.01 ppm, 0.1 ppm, 1 ppm, and 10 ppm. An equivalent quantity of acetone alone was added to the control enclosure. The long period between inoculation and sampling allowed for decomposition of any invertebrates killed by the Guthion. A dissecting microscope at 2.5 magnification was used for sorting.

In spring 1978, another long-term experiment was designed to study the rate of recovery of the invertebrate community after a lethal dose (i.e., 25 ppm) of Guthion. A study to determine effects of a sublethal continuous exposure of Guthion was conducted at the same time (outlined in Test Series III). A series of 34, 1-m high, 20-cm diameter galvanized steel stovepipes were used as enclosures. Twelve of the enclosures were treated with 25 ppm Guthion and twelve with a equivalent quantity of acetone. Every 6-7 days all the floating vegetation from the enclosures, from each treatment, was removed with a ladle, stored on cheesecloth, and that retained by a 500 μ sieve was preserved in 10% formalin-rose bengal solution. The sampling schedule is given in Table 15. Sorting was done with a dissecting microscope at 2.5 magnification.

Another long-term experiment, May through September 1979, was designed to study the effects of Guthion on leaf litter decomposition rates. Tupelo and ash leaves were dried at 30 C for 7 days; 5 grams of leaves were placed in 20 x 20 cm nylon bait bags with a 1.6 mm square mesh. Fifty-four of these litter bags were soaked for 24 hrs. in each of the following concentrations of Guthion-treated water, 100 ppb, 1 ppm and 100 ppm. Fifty-four bags were soaked in an equivalent amount of acetone-treated (i.e., 2 ppm) water. Treatments were spaced a minimum of 30 M apart in a tupelo stand within the impounded swamp. Bags were hung from nylon cord and spaced approximately 60 cm apart. Six samples of nine replicates were collected from each treatment through the course of the experiment. Amphipods, were particularly reduced; the control enclosure had 350/0.0314 m^2, the 0.01 ppm Guthion enclosure, only 5 animals/0.0314 m^2. Freshwater clams were present in all treatments except for the 0.01 ppm enclosure. Aquatic worms (Plesiopora) were highest in the 0.01 and 0.1 ppm enclosures, but completely absent from the 1 and 10 ppm enclosures. Although nematodes were not counted, their absence was noted from all treatments. The control enclosure had one less taxa than the surrounding swamp and the density of invertebrates was very similar.

Table 15. Sampling Schedule for the Test Series II (April-June, 1978) and III (June, 1978)

	Period of Exposure (in Days)									
Treatment	1	2	8	15	22	30	36	43	50	57
Control	Acetone Inoculated	S	S	S		S		S		S
25 ppm	Acetone & Guthion-Inoculated	S	S	S		S		S		S
10 ppb	Acetone & Guthion-Inoculated	S	R	S&R	R	S&R	R	S&R	R	S
Natural Swamp		S		S		S		S		S

S= Sample the floating vegetation; R= re-inoculation with Guthion.

The invertebrate population from the pesticide treatments sampled 16 days after inoculation averaged only 9% as dense as the control or natural swamp community. The results of this experiment (Table 16) indicate smaller numbers of amphipods, dipterans, and nematodes in the Guthion enclosures.

Table 16: Effects of Guthion on Back-Swamp Invertebrates (Sediment plus Floating Vegetation) Number Per Sample (20-cm diameter corer)

TAXA	Natural Swamp	Control	.01 ppm	0.1 ppm	1 ppm	10 ppm
Amphipoda	335	350	5	6	9	7
Heterodonta	3	2		2	1	5
Odonata	1					
Diptera	3	7	2			
Plesiopora	4	9	26	37		
Nematoda	Present	Present	Absent	Absent	Absent	Absent
Basommatophora		1	8	8		
Isopoda				2		1
Collembala			1			
Hemiptera	1					
Ephemeroptera			1			
Total	347	369	43	55	10	13

The results of the spring 1978 experiment to measure recovery rates are given in Tables 17 and 18. The population of invertebrates living in the

duckweed from control and treatment enclosures decreased with time. The experiment could not be continued beyond May 1978 due to death of the enclosed floating vegetation. After one week, the invertebrate density was reduced by 99% and 82% for the 25 ppm enclosures and 82% for the control enclosure. After one month, the invertebrate density for all enclosures averaged only 3% of the density recorded at the start of the experiment.

Table 17. Test Series II. Use of Stovepipe Enclosures (25 ppm Guthion)

Taxa	Day-	0		6		13		28	
	Date-	4/19/78		4/26/78		5/3/78		5/18/78	
		A	B	A	B	A	B	A	B
		Number per .03 m^2							
Amphipoda		9	1					1	
Oligochaeta		520	420	4	6				
Diptera		19	16			6	2	1	1
Basommatophora		126	42	2					
Coleoptera		3	1		1	10	13	14	4
Odonata			1						
Hemiptera					1	11	11	3	4
Collembola						4	1	2	8
Lepidoptera									3
Tricladida			2		1				
Nematoda		P	P	A	A	A	A	A	A
Zooplankton		P	P	A	A	A	A	A	A
Totals		677	483	6	9	31	27	21	20

*Note P = Present; A = Absent

Table 18. Test Series II - Use of Stovepipe Enclosures (Control)

TAXA	Day-	0		6		13		28	
	Date-	4/19/78		4/26/78		5/3/78		5/18/78	
		A	B	A	B	A	B	A	B
		Number per .03 m^2							
Amphipada		4	12			1	1	1	
Plesiopora		300	340	16	1	1			
Diptera		145	104	89	30	25	21	1	
Basommatophora		2	15	10	1				
Rhynchobdellida			1						
Ephemeroptera			2						
Coleoptera			2	1	1	3	1	5	5
Neuroptera			1						
Hemiptera		1	1	8	8	27	12		2
Odonata				5		1		1	1
Acari				1				2	
Lepidoptera					1				
Collembola						3	2	2	2
Araneida							1	1	1
Cyprindontiformes		1	1						
Zooplankton*		P	P	P	P	A	A	A	A
Totals		453	479	130	42	61	38	13	11

*Note P = Present; A = Absent

Leaf litter weight loss, due to decomposition during the summer of 1979, was not affected by Guthion (Fig. 50). The 1 ppm Guthion-treated leaves appears to have a greater decomposition rate, however, the rate of weight loss for all treatments, including the control, was not significantly different throughout the course of the entire experiment. On the average, 43% of the leaf litter decomposed in 127 days. This represents a 16.9 mg dry wt. loss/day during the summer months. No invertebrates were found in any of the litter bags.

Effects of continuous exposure to Guthion (0.01 ppm). Test Series III:

Ten stovepipe enclosures were re-inoculated with Guthion each week over a four week period, to a concentration of 10 ppb. The original design called for an eight week experiment (Table 19) but death of the floating vegetation invalidated further sampling. The sampling technique was the same as that used in the previous recovery rate experiment of Test Series II.

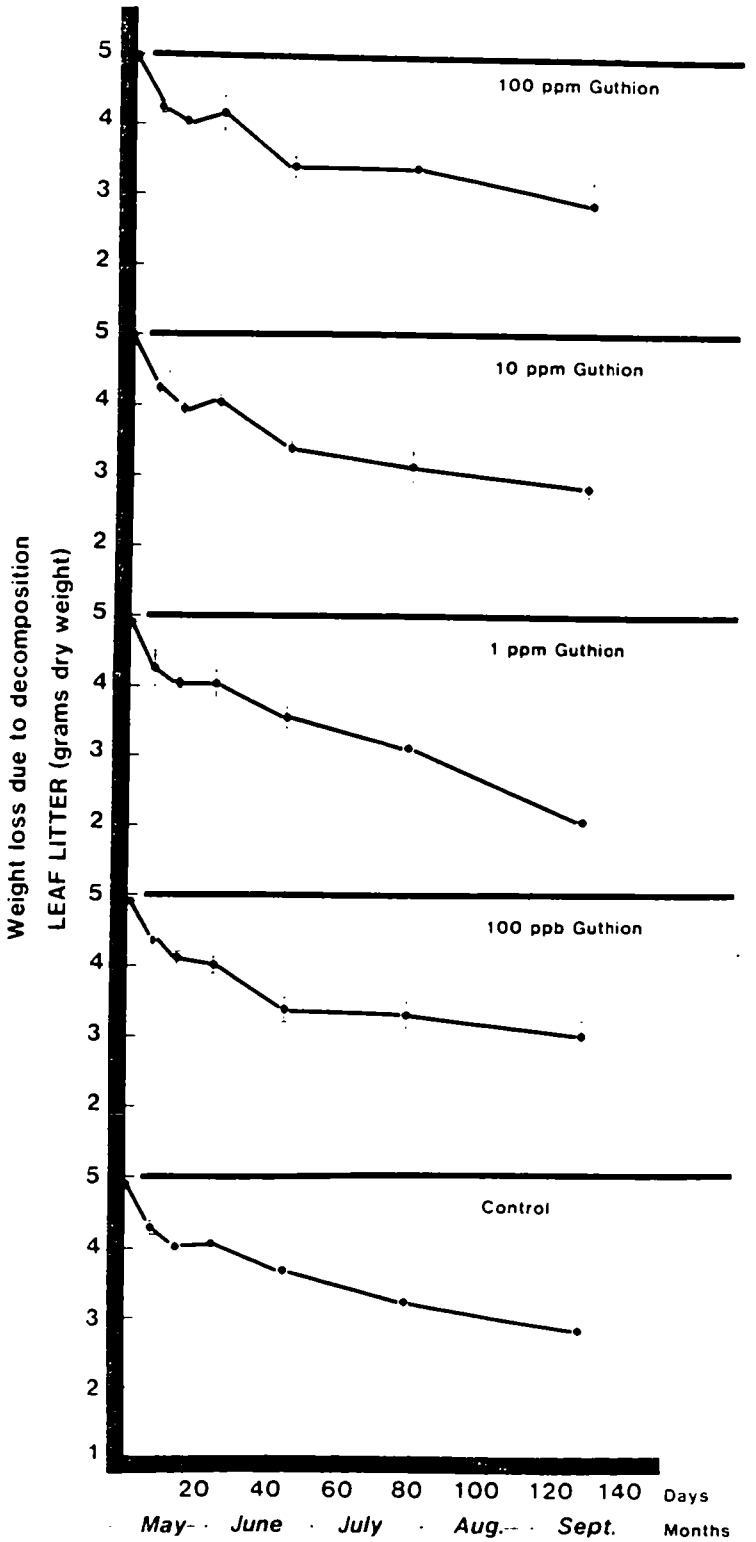


Figure 50. Effects of Guthion on Leaf, Little Weight Loss Due to Decomposition.

The results of the experiment are given in Appendix A.

The invertebrate population living in the floating vegetation and not enclosed in a 20 cm diameter stovepipe, ranged during the 4 week experiment from 517 individuals/0.03 m^2 to 1765 /0.03 m^2 (Table A-1). Concurrently the population in the control enclosures steadily decreased from 466 individuals/0.03 m^2 to 12 /0.03 m^2 (Table A-2). The number of taxa decreased from 11 to 8. The population in the 0.01 ppm enclosures (Table A-3) decreased only slightly more than that in the control enclosures. Density decreased from an original average of 631 individuals/0.03 m^2 to a final population of only 9 /0.03 m^2. The number of taxa decreased from 13 to 6.

Aquatic worm density was most affected; a population of 300-500 individuals/0.03 m^2 was reduced to zero in both control and treatment enclosures.

A summary of all three treatments is given in Table 19. The average density for the 0.01 ppm Guthion treatment was slightly higher than that for the control, but was not significantly different. However, both were significantly different from the natural swamp situation. The diversity was statistically the same for all three situations, although the 0.01 ppm Guthion treatment averaged one less taxon than the natural swamp. The distribution of invertebrate density, i.e., evenness, was the same for all treatments.

Short term comparison of Guthion with Furadan, Methyl Parathion and Azodrin. Test Series IV:

In October, 1978, four insecticides were tested on floating vegetation invertebrate communities contained within 20-cm diameter stovepipe enclosures over a 48 hr period. The insecticides were: Guthion, Furadan (a carbonate used on rice), methyl parathion (an organophosphate used on soybeans) and Azodrin (a systemic organophosphate used on cotton.) Each pesticide was tested at two concentrations; 1 ppb and 10 ppb. Three to five replicate enclosures were used for each treatment. Control enclosures were not treated with acetone. Stovepipe enclosures were placed randomly in the impounded swamp in October, 1978, and the entire floating vegetation, plus water column from each enclosure, was separated by a 500 μ sieve 72 hr later. Samples were stored in 10% formalin-rose bengal and sorted at 2.5 x under a dissecting microscope.

The results from this experiment are tabulated in Appendix B. Replicates varied greatly, with coefficients of variation ranging from 4.6 to 78%. While the density of invertebrates in the floating vegetation in the surrounding swamp did not change during the course of the experiment, densities in the enclosures were greatly reduced. Those in the control enclosures were less than the natural swamp, i.e., 68/0.016 m^2 and 225 individuals/0.016 m^2, respectively. The average density of the insecticide enclosures ranged from a low of 17/0.016 m^2 with 10 ppb methyl parathion to a high of 34/0.016 m^2 at 1 ppb Furadan. The dominant taxon in the control

and natural swamp treatments were Amphipoda and Oligochaeta. Azodrin treatments were completely devoid of oligochaets and very few (maximum 4/0.016m^2) were found in the other three insecticide treatments. Amphipoda populations ranged from 4 individuals/.016 m^2 at 10 ppb Guthion to 27/0.016 m^2 at 1 ppb methyl parathion. The average density of Amphipoda in the control and natural swamp samples was 33/0.016 m^2 and 56/0.016 m^2, respectively. The population of damselflies and dragonflies (i.e. Odonata) appeared unaffected.

Table 19. Average density, diversity and evenness of animals found in the floating vegetation after 30 days of continuous exposure

	DENSITY (Number per .03 m^2)				
	X	S.E.	MIN	MAX	
Natural Swamp n = 6	992	260	386	1850	
Control n = 8	153	70	11	479	
.01 ppm n = 6	225	130	8	700	
	DIVERSITY (p. lnp)				
	X	S.E.	MIN	MAX	Number of Taxa
Natural Swamp n = 6	1.29	.09	.92	1.5	14
Control n = 8	1.13	.11	.73	1.7	15
.01 ppm n = 6	1.2	.13	.77	1.6	13
	EVENNESS Diversity / (Maximum Diversity)				
	X	S.E.	MIN	MAX	
Natural Swamp n = 6	.61	.04	.47	.72	
Control n = 8	.61	.07	.38	.89	
.01 ppm n = 6	.67	.10	.33	.90	

The average density and evenness of each treatment are shown graphically in Fig. 51. No differences were noted among the four insecticides. Methyl parathion at 10 ppb, had the lowest density of invertebrates and Furadan at 1 ppb had the highest. The evenness of the invertebrate population distribution was highest in the insecticide treatments and lowest in the natural swamp.

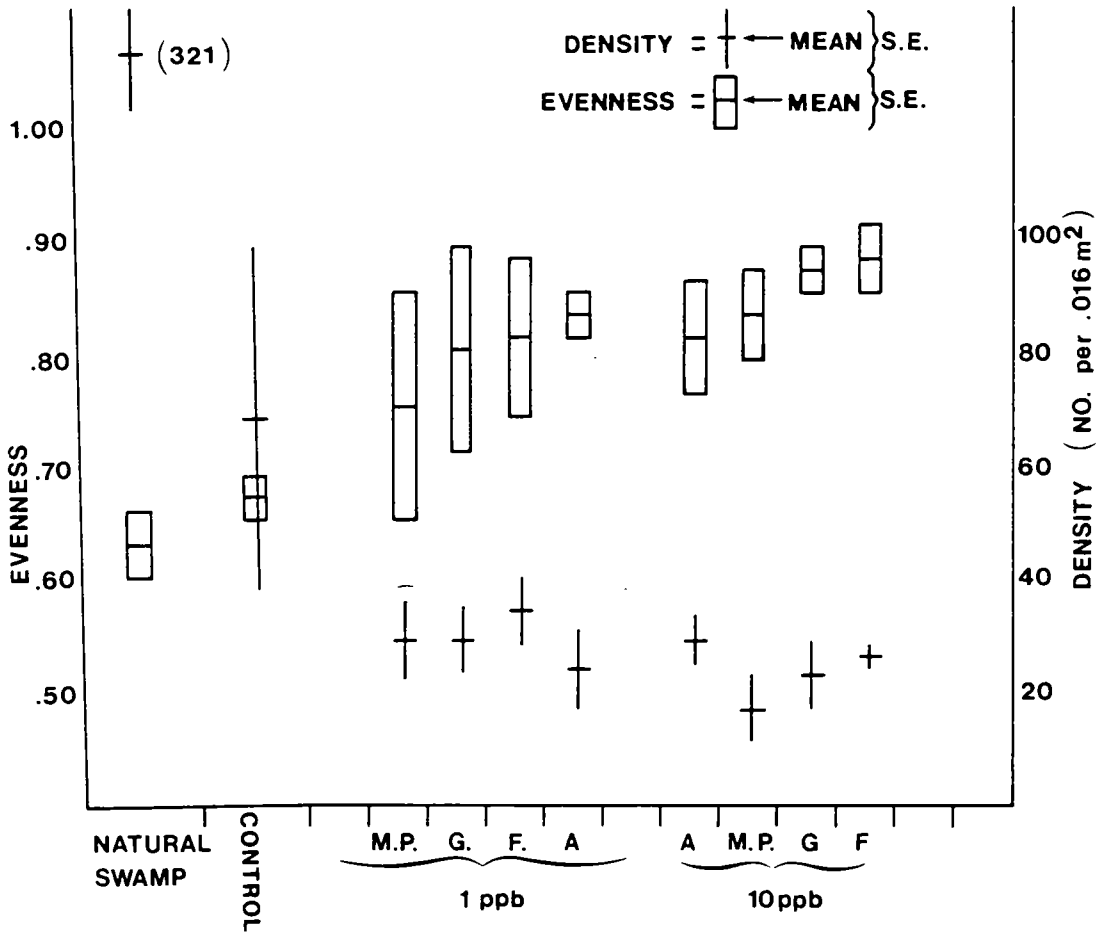


Figure 51. Response of Swamp Invertebrate of Methyl Parathion (MP), Guthion (G), Furadon (F), and Azodin (A).

CONCLUSIONS

Containment of a parcel of swamp water, by a 20 cm stovepipe for periods greater than 2 weeks, has an adverse effect upon the community contained within. The effect of small field enclosures can mask effects due to the insecticides. Floating vegetation within the 20 cm diameter stovepipe enclosures was dead after 30 days. Thus, the recovery rate of the community to 25 ppm Guthion (Test Series II) could not be evaluated. Similarly, conclusions cannot be drawn as to the effects of continuous exposure since density of the control group and treatment group decreased at approximately the same rate. Conclusions can only be made from those experiments where the control replicates were found to be unaffected by enclosure. They include the experiments that used the larger 48 cm enclosures and all short-term experiments.

The complex community of swamp invertebrates is composed of crawfish (Procambarus), amphipods (Hyallela azteca), aquatic worms (Haplotaxida), snails (Basommatophora), diptera larvae (Chironomidae, Ceratopogonidae, Culicidae), springtails (Collembola), aquatic caterpillars (Synclita accidentalis), various beetles (Haliplidae, Dytiscidae, Noteridae, Hydrophilidae, Helodidae, Curculionidae), predacous Odonata (Anisoptera, Zygoptera), Hemiptera (Corinadae, Notenectidae, Naucoridae, Belastomatidae Mesovelliidae), nematodes, zooplankton (Cladocera), flatworms (Tricladida), leeches, and spiders. These contribute to the stability, integrity and function of swamp ecosystems by transferring and regulating the flow of energy and materials. Guthion can, at concentrations as low as 1 - 10 ppb, alter the density and distribution of the community in the water column and in the flooded soil. Crustaceans and insects were the most sensitive to Guthion in the water, while molluscs and annelids were the most tolerant. The aquatic worm Aulophorus spp. and the Basommatophora snails tolerated 25 ppm Guthion for short periods and 0.1 ppm for long periods. This is in agreement with the work of Harman (1974) who found snails generally unaffected by insecticides. In fact, snail populations can increase after insecticide treatment (Pimentel 1971). Annelids also exhibit natural resistance to insecticides (Ferguson 1969; Davey 1963). Our data presented here show the resistance of the annelids to Guthion over short periods with decreasing tolerance over extended time. Brown (1978) reported that Guthion was harmless to the terrestrial worm Eisenia spp. at 5.6 Kg/ha. The death of the floating vegetation and annelids in the control enclosures of Test Series III and IV suggests a sensitivity to material or conditions other than Guthion. Brinkhurst and Cook (1974) noted that aquatic worms are influenced more by the quality and quantity of organic matter than by the chemical parameters of the water or sediment. According to Anderson et al. (1965), when organic matter is high and there is a scarcity of fauna other than worms, the existence of toxic substances is suggested.

The worms were not resistant to all of the organophosphates tested. The 10 ppb methyl parathion treatment and all monocrotophos treatments of Test Series IV were completely devoid of oligochaetes. Parathion has been reported to have no effect on earthworms at 8 lb/acre in England, although toxic at 8 Kg/ha in Germany (Brown 1978). The fact that monocrotophos is a

systemic could contribute to its toxicity. Other organophosphorus systemics have been found to eliminate earthworms when applied at normal agricultural rates (Edwards et al. 1967).

The population density of amphipods (H. azteca) was reduced more than any other group of invertebrates by treatment with Guthion. In Test Series I, a concentration of 1 ppm reduced the population from 9,000/m^2 to zero in 24 hr. In Test Series II a concentration of 0.01 ppm reduced the population from approximately 11,000/m^2 to 160/m^2 over a period of 16 days. The short term results of test Series III and IV showed the same trends. The amphipod population was reduced by approximately 50% in the presence of 1 ppb Guthion, methyl parathion, Furadan, or Azodrin. Farlow (1976) found H. azteca to be very susceptible to field application of Dimilin and recommended its use as an indicator organism to determine the impact of mosquito control chemicals.

Swamp amphipods in floating mats of Lemma spp. can reach densities of 60,000/m^2 (Sklar unpublished data). Densities of such magnitude imply that amphipods play a dominate role in the cycling of materials, and their reduction due to insecticide runoff could have significant ecological consequences. For example, in a stream contaminated with BHC, an organochloride used on sheep, a thick layer of benthic algae Cladophora and Spirogyra, developed due to the destruction of the herbivorous Gammarus amphipods (Hynes 1961). In terrestial systems, removal of the dominate pest herbivores results in the elimination of most predators and parasites, culminating in an even greater infestation of some other undesirable pest species (Pimentel 1961; Brown 1978). Hulbert et al. (1972) found that the organophosphorus chlorpyrifos applied to shallow freshwater ponds created an ecological imbalance favorable to the very insects it was meant to control. We need to know what ecological changes will result from low level contamination of amphipod-rich wetlands.

Aquatic insect populations were very resistant to short-term exposure to organophosphates. After 8 days at 10 ppm Guthion, a population of diptera larvae still remained. In Test Series IV, the total number of insects was as high in the 1 ppb treatment enclosures as in the unenclosed swamp (see below).

Natural Swamp	- 28	Methyl Parathion	- 22
Control	- 20	Carbofuran	- 26
Guthion	- 30	Monocrotophos	- 34

The total number of insect orders was highest in the 10 ppb Azodrin treatment and included one species of Ephemeroptera (i.e., mayflies). The mayflies have generally been considered to be very sensitive to pollutants. Their appearance in organophosphate-treated water in Test Series II and IV, and in the data of Roback (1974), indicate that as a generality, this is not true. Roback found that the majority of aquatic insects are not good "indicator organisms" because of their ability to tolerate a broad range of water chemistry and physical conditions. However, our data indicate that

their ability to tolerate Guthion decreases with time. Not a single insect species was found at 100 ppb after 16 days in Test Series II.

Toxicity of an insecticide depends on its concentration and its distribution through the water column. The more insoluble the chemical the more rapidly it is lost from the water (Thompson and Edwards 1974). Beynon et al. (1971), however, reported that even the comparatively soluble organophosphorus insecticide, chlorfenvinphos, was rapidly deposited on bottom sediments. Guthion was found to decrease very rapidly from the water, much of it appearing in the sediments within 24 hr. Edwards et al. (1964) demonstrated that the susceptibility of diptera larvae to TDE was due to the close association of these animals with the organic sediments on which the insecticide was absorbed. The effect of Guthion on the benthos was demonstrated in Test Series II. Nematodes were not found in any of the Guthion treatments, even at concentrations as low as 10 ppb. The natural nematode density was not calculated, only the presence or absence of the species retained by a 500 μ sieve. As a result, the effectiveness of Guthion as a nematicide in cypress tupelo swamp sediments is unknown. The sensitivity of nematodes to Guthion is not surprising since other organophosphates (i.e., Parathion, Furadan, Dasonit) have been found to decrease nematode populations and are sometimes used as soil nematicides (Brown 1978; Torgeson 1972).

The other benthic invertebrate, the clam (Sphaerium spp.), was unaffected by Guthion at the concentrations tested, i.e., 10 ppb to 10 ppm. This too is not surprising since mortality caused by pesticides in mussels and clams has not been demonstrated (Brown 1978; Fuller 1974) even though these animals can concentrate insecticides at levels greatly in excess of natural concentrations in water (Miller et al. 1968). Mainly, the immature stages are affected by organophosphates. The LD_{50} of Guthion for the larvae of the hard clam, Mercenaria mercenaria, is 860 ppb, and 620 ppb for the eggs of the american oyster, Crassostrea virginica (Davis and Hidu 1969). The ecology of swamp bivalves might be affected by Guthion as a result of its ability to interfer with progeny and not its direct effect upon adults.

Guthion did not affect the rate of ash-tupelo litter decomposition. All treatment results were fit into a daily linear regression model with the following results:

	Untreated	0.1 ppm	1 ppm	10 ppm	100 ppm
Correlation Coef. (r)	0.93	0.87	0.94	0.90	0.89
Daily wt. loss (mg)	14.1	12.4	15.0	14.1	13.3

No trends were observed and significant differences were not obtained. It has been noted that, in general, organophosphate insecticides do not affect bacterial populations at practical application levels (Brown 1978).

Parathion applied at 10 lb/acre had no effect on the numbers of soil fungi or bacteria (Eno 1958). In some instances, the microbial activity is stimulated by applications of organophosphates (Tu 1970). There is a need to expand this type of experiment to other wetland types, especially in areas where microarthropods and annelids play an important role in comminuting dead plant material, making it more available for bacterial decomposition. When soil microarthropods were eliminated by naphthalene, the leaf litter decomposition rate was reduced by as much as 25% (Brown 1978). In the current study, the sediment is relatively devoid of micro and macrofauna that could aid in decomposition. Other swamp and marsh habitats have significant numbers of arthropod decomposers (Sklar and Conner 1979). Before it can be said conclusively that Guthion does not affect aquatic decomposition processes, more litter decomposition experiments must be conducted in other major wetland soil types.

The aforementioned can be summarized below:

Guthion added to backswamp aquatic environments is rapidly removed from the water column, much of it appearing in the sediments within 24 hr. Guthion concentrations, ranging from 0.1 ppm to 100 ppm has no effect upon the microbial degradation rate of ash-tupelo litterfall in the aquatic environments of a permanently flooded swamp. The density and distribution of backswamp aquatic invertebrates from a permanently flooded cypress-tupelo swamp is altered by contact with the insecticide Guthion at concentrations ranging from 0.01 ppm to 25 ppm.

Annelids and molluscs are the most tolerant backswamp aquatic organisms. They can survive high dosages of Guthion (i.e., 1-25 ppm) for periods greater than one week when the insecticide is injected into habitat enclosures and allowed to degrade "naturally". *In situ* survivability of these animals to long term exposure is still unknown. The dominant backswamp invertebrate, the amphipod, *Hyallela azteca*, is the most sensitive aquatic organism. Large populations (e.g., 9,000/sq. m) can be reduced to zero in 24 hr. at Guthion concentrations greater than 1 ppm.

Aquatic insect populations were able to tolerate a broad range of insecticides (i.e., Guthion, methyl parathion, carbofuran and monocrotophors) and dosages (i.e., 0.01-10 ppm) for short periods of time. Their natural genetic variability coupled with their high rate of reproduction, give aquatic insects, like their terrestrial counterparts, the ability to develop resistant populations.

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CONCLUSIONS

The procedural methodologies developed in this multidisciplinary tiered approach to analysis of the environmental effect and fate of selected xenobiotics in agriculturally affected wetlands provides a useful "tool" for further evaluation of other EPA priority-pollutant classes of compounds, i.e., phenols, phthalate esters, chlorobenzenes. In addition, information is contributed with regard to modifying current agricultural practices and to furthering development of coastal zone management strategies. The continuing formulation of valid predictive models will generate needed information on the fate (and effects) of a variety of toxic chemicals in fresh, brackish, and saline ecosystems. Furthermore, data should be obtained on microbial and related physicochemical transformation processes in situ and under laboratory conditions that affect the environmental fate of toxic chemicals.

In the projected evaluation of other EPA priority pollutants, the combination of field and laboratory methodology, the latter employing a variety of microbial, benthic, enzymatic, and physicochemical transformation activities, is the preferred approach. This allows for on-going formulation of a valid computer data base program for statistical and numerical model analysis to standardize laboratory methods and to establish a ranking system for other classes of toxicants. In continuing these evaluations of xenobiotic impact in economically and biologically important wetland systems, we feel additional information should be garnered in the following areas:

(1) The further identification of relevant abiotic processes, particularly Eh/pH, which complement biotic transformations of "target" compounds.

(2) The role of ecologically-significant metabolizable substrates, i.e., chitin, which provide an active microbial and enzymatic pool for both acclimation to, and subsequent enzymatic adaptation of, "target" xenobiotics.

(3) The ultimate bioavailability of selected xenobiotics to the aforementioned microbial pool and estimation of minimal, or threshold levels, at which biotransformation processes can proceed, and over what duration.

Biotransformation test protocols must include an evaluation of sorption reactions affecting rate(s) of microbial transformations. In addition, there needs to be a monitoring of microbial/benthos at varying Eh, pH, and salinity levels for comparative purposes.

Particular attention should be given to the structural polysaccharide chitin, among the most prevalent biodegradable polymer in marine and estuarine environments. It is recognized that turnover of chitin is a critical key transformation process in the functioning of trophic food webs. The role of chitinoclastic microorganisms in bioconversion of this carbon/nitrogen-containing substrate needs careful examination. Analyses of microbial ecology and the extant chitinoclast population requires careful study of chitinolytic processes on fate of a variety of toxic substrates. Similarly, correlations between the latter compounds and chitin mineralization warrants thorough investigation.

Analyses of chitinase elaboration as a major activity factor and its relationship to toxic substances should reveal noteworthy data on environmentally-significant surface phenomena or adsorptive effects. Toxic substances may physically block active sites on the chitin substrate, thus inhibiting initiation of essential breakdown processes. Products resulting from such activities, i.e., N-acetyl-glucosamine and glucosamine, all enter into the various physiological determinants of the microbial community. Correlations between these processes and toxic compound fate are germane to elucidation of the effect of environmental and chemical perturbances on transformation of such ecologically significant substrates as chitin.

The physicochemical experiments conducted during the course of this investigation have provided particularly useful information on mobilization or immobilization processes occurring in soil environments. Implications as to the development, migration, and permanency of aerobic/anaerobic layers in both wetland flooded soils and adjacent agricultural soils with regard to pesticide environmental fate were presented. In recognization of the importance of oxidation-reduction conditions on the environmental chemistry of pesticide compounds, agricultural practices near sensitive wetland systems should be examined in terms of such physicochemical processes. Possible modifications should be designed to consider the following in overall agricultural practices:

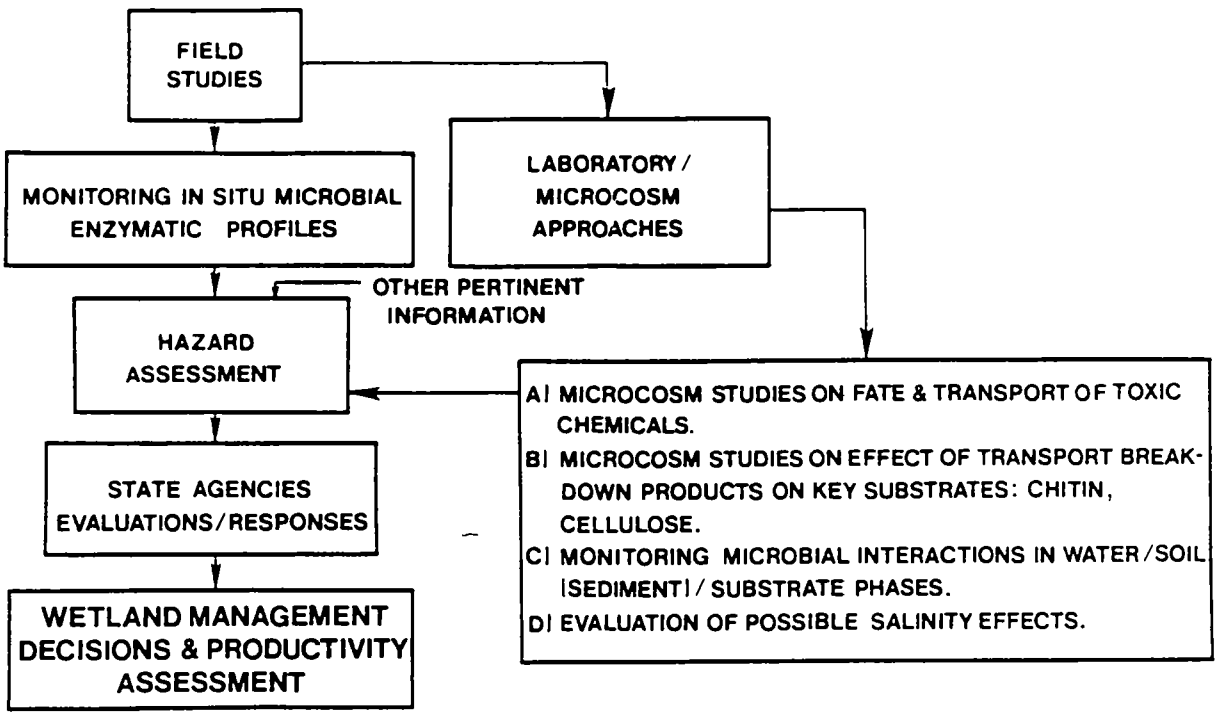
(1) The implementation of draining vs. non-draining practices, particularly in rice growing regions, to take advantage of oxidation-reduction variations to regulate pesticide residence time.

(2) The monitoring of pH effects based on individual crop needs as well as pesticide environmental fate.

(3) The careful analysis of wetland soils adjacent to agricultural areas, with regard to their various physical and chemical properties to ascertain accumulation effects and residence time phenomena of pesticides.

Additional research is needed on mobility and degradation of toxic compounds, especially related to runoff activities. Incorporation of such data into appropriate data information bases, in an effort to further refine EPA's agricultural runoff model, is anticipated.

The overall purpose of these investigations has been to incorporate experimental data and overall experimental design, primarily developed for federal and state monitoring and management strategies, into environmental impact and hazard assessment of toxic chemicals in waterways and wetland systems. More information is needed on aquatic soil/sediment processes that are critical in productivity and food web dynamics. In continuing to pursue these aforementioned objectives, the overall conceptual approaches involved, and the ultimate use of information obtained, is illustrated below.



The implementation of effective and optimal management practices for maintaining agricultural productivity must include an economic analysis of cost and energy use. Nevertheless, such practices should be deleterious adjacent to highly productive wetland areas. Development of economically efficient strategies for managing and monitoring agricultural pollution need to be based on use of the best agricultural and wetland environmental science and technology. Procedural wetland research efforts, such as presented here, must continue to resolve both compatability as well as possible conflicts between hydrologically-linked habitats within a major aquatic ecosystem.

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APPENDIX

Table A-1. Test Series III: Various Taxa of Animals in Natural Swamp*

TAXA	4/19/78		4/26/78		5/3/78		5/18/78	
	A	B	A	B	A	B	A	B
	Individuals/0.03 m^2							
Amphipoda	37	481			352	258	157	334
Oligochaeta	290	345			524	600	101	154
Diptera	69	88			658	561	77	90
Basommatophora		40			274	223	19	35
Tricladida					3	3	5	3
Ephemeroptera						1		
Coleoptera	5	5			7			
Hemiptera	3				8	14	13	19
Odonata	3	11			20	15	11	13
Collembala					1			
Lepidoptera	3				3	3	3	
Homoptera						1		
Rhynochobdellida		1						
Araneida		1						
Zooplankton	P**	P			P	P	P	P
Total	410	981			1850	1679	386	648

* Sampled with F.V. Scoop; ** P = Present

Table A-2. Test Series III: Various Taxa of Animals Present (Control)

TAXA	4/19/78		4/26/78		5/3/78		5/18/78	
	A	B	A	B	A	B	A	B
				Individuals/0.03 m^2				
Amphipoda	4	12			1	1	1	
Oligochaeta	300	340	16	1	1			
Diptera	145	104	89	30	25	21	1	
Basommatophora	2	15	10	1				
Rhynchobdellida		1						
Ephemeroptera		2						
Coleoptera		2	1	1	3	1	5	5
Neuroptera		1						
Hemiptera	1	1	8	8	27	12		2
Odonata			5		1		1	1
Acari			1				2	
Lepidoptera				1				
Collembala					3	2	2	2
Araneida						1	1	1
Cyprindontiformes	1	1						
Zooplankton*	P	P	P	P	A	A	A	A
Totals	453	479	130	42	61	38	13	11

*P = Present; A = Absent

Table A-3. Test Series III: Various Taxa of Animal Present (0.01 ppm Guthion)

TAXA	4/19/78		4/26/78		5/3/78		5/18/78	
	A	B	A	B	A	B	A	B
				Individuals/0.03 m^2				
Amphipoda	3	28			1	4		
Oligochaeta	556	400				9		
Diptera	46	39			3	22	1	1
Basommatophora	67	79						
Tricladida	2							
Ephemeroptera	5	2						
Coleoptera	2	4			4	2	1	3
Hemiptera	3	5			13	6	1	4
Odonata	8	1						
Collembala		1			1	3	4	
Araneida	2	1					1	
Homoptera		1			1	1	1	
Lepidoptera					1			
Zooplankton*	P	P			A	A	A	A
Totals	700	561			24	47	9	8

* P = Present; A = Absent

APPENDIX B

Results of Test Series IV Expressed
as density per unit area
($\bar{H}$ = Average Diversity. $\bar{V}$ = Average evenness.
C.V. = Coefficient of Variation.)

Control

TAXA (order)	A	B	C	$\bar{X}$	S.D.
		Individuals/0.016 m^2			
Amphipoda	25	53	21	33	17
Oligochaeta	3	48	2	18	26
Diptera	1	3	1	2	1
Basommatophora	7	16	7	10	5
Odonata	2	7	2	4	3
Lepitoptera	1			--	--
Coleoptera	1			--	--
Hemiptera		1		--	--
Cyprinodentiformes	1	1	1	1	0
Zooplankton		Yes		--	--
TOTAL	41	129	34	68	53
$-\sum p_i \ln p_i$ (Diversity) $\bar{H}$ = 1.29	1.3	1.3	1.27	C.V. = 78% S.E. = 30.6	
H/H_{max} (Evenness) $\bar{V}$ = .67	.63	.67	.71		

Natural Swamp - 10/16/78

TAXA (order)	A	B	C	$\bar{X}$	S.D.
		Individuals/0.0113 m^2			
Oligochaeta	128	101	98	109	17
Amphipoda	59	57	51	56	4
Basommatophora	32	20	24	25	6
Odonata	5	1	1	2	2
Tricladida	44	25	22	30	12
Hemiptera	1			--	--
Hydroida		1		--	--
Diptera		5	6	4	3
Nematomorpha			1	--	--
Lepidoptera			1	--	--
Coleoptera			1	--	--
Zooplankton		Yes			
TOTAL	269	210	205	228	36
$-\sum p_i \ln p_i$	1.33	1.32	1.4	C.V. = 16%	
$\bar{H} = 1.35$				S.E. = 20.6	
H/H_{max}	.74	.68	.64		
$\bar{V} = .69$					

Natural Swamp - 10/19/78

TAXA (order)	A	B	C	$\bar{X}$	S.D.
		Individuals/0.0113 m^2			
Amphipoda	51	64	53	56	7
Oligochaeta	64	203	142	136	70
Diptera	5	7	9	7	2
Tricladida	8	7	16	10	5
Basommatophora	9	13	12	11	2
Odonata	1	3	4	3	2
Hemiptera		2	1	1	1
Coleoptera		1		--	--
Ephemeroptera			1	--	--
Zooplankton	Yes	Yes	Yes		
TOTAL	138	300	238	225	82
$-\sum p_i \ln p_i$	1.22	1.0	1.21	C.V. = 36%	
$\bar{H} = 1.14$				S.E. = 47.2	
H/H_{max}	.68	.48	.58		
$\bar{V} = .58$					

Guthion (1 ppb)

TAXA (order)	Sample No. 24	18	4	$\bar{X}$	S.D.
	Individuals/0.016 m^2				
Amphipoda	10	5	26	14	11
Oligochaeta	1		3	1.3	1.5
Diptera	9	5	3	6	3
Odonata	9	2	1	4	4
Basommatophora	2	5	5	4	2
Hemiptera		1		--	--
Cyprinodentiformes			1	--	--
Zooplankton	Yes				
TOTAL	31	18	39	29	11
$-\Sigma p_i \ln p_i$	1.37	1.47	1.12	C.V. = 36%	
$\bar{H}$ = 1.32				S.E. = 6.1	
H/H_{max}	.85	.91	.63		
$\bar{V}$ = .80					

Guthion (10 ppb)

TAXA (order)	Sample No. 30	16	12	8	$\bar{X}$	S.D.
	Individuals/0.016 m^2					
Odonata	11	4	8	3	7	4
Amphipoda	8	19	4		8	8
Diptera	4	2	2		2	2
Basommatophora	2	9	3	3	4	3
Oligochaeta		4		1	1	2
Homoptera			1	1	.5	.6
Hemiptera			1	1	.5	.6
Collembola				1	--	--
Zooplankton	Yes	Yes	--	Yes		
TOTAL	25	38	19	10	23	12
$-\Sigma p_i \ln p_i$	1.22	1.32	1.53	1.64	C.V. = 51%	
$\bar{H}$ = 1.43					S.E. = 5.9	
H/H_{max}	.88	.82	.85	.92		
$\bar{V}$ = .87						

Methyl Parathion (1 ppb)

TAXA (order)	Sample No. 13	5	1	$\bar{X}$	S.D.
	Individuals/0.016 m^2				
Diptera	3	3	3	3	0
Oligochaeta	3		1	1	1.5
Odonata	3	2	6	4	2
Amphipoda	3	27	19	16	12
Hemiptera	1			--	--
Cyprinodentiformes	1	2 (Dead)	1	1	.6
Basommatophora		2	5	2	2.5
Coleoptera			1	--	--
Zooplankton	Yes	Yes	Yes		
TOTAL	14	36	36	29	13
$-\sum p_i \ln p_i$	1.7	.9	1.42	C.V. = 44%	
$\bar{H}$ = 1.34				S.E. = 7.3	
H/H_{max}	.95	.56	.73		
$\bar{V}$ = .75					

Methyl Parathion (10 ppb)

TAXA (order)	Sample No. 32	28	25	17	$\bar{X}$	S.D.
	Individuals/0.016 m^2					
Odonata	6	6	9	1	6	3
Diptera	2	7	2		3	3
Amphipoda		12	5	5	6	5
Basommatophora		7	1	1	2	3
Hemiptera		2			--	--
Cyprinodentiformes			1	1	.5	.6
Zooplankton	Yes	Yes	Yes	Yes		
TOTAL	8	34	18	8	17	12
$-\sum p_i \ln p_i$	.56	1.49	1.27	1.07	C.V. = 72%	
$\bar{H}$ = 1.10					S.E. = 6.1	
H/H_{max}	.81	.93	.79	.77		
$\bar{V}$ = .83						

Furadan (1 ppb)

TAXA (order)	Sample No. 15	9	2	$\bar{X}$	S.D.
		Individuals/0.016 m^2			
Odonata	5	2	5	4	2
Amphipoda	16	7	24	16	9
Diptera	6	5	1	4	3
Lepidoptera	1			--	--
Oligochaeta	4	3	2	3	1
Basommatophora	1	7	11	6	5
Hemiptera			1	--	--
Zooplankton		Yes	Yes		
TOTAL	33	24	44	34	10
$-\sum p_i \ln p_i$	1.41	1.51	1.24	C.V. = 30%	
$\bar{H}$ = 1.39				S.E. = 5.8	
H/H_{max}	.79	.94	.69		
$\bar{V}$ = .81					

Furadan (10 ppb)

TAXA (order)	Sample No. 33	29	26	21	$\bar{X}$	S.D.
		Individuals/0.016 m^2				
Odonata	13	5	8	8	9	3
Hemiptera	2				--	--
Amphipoda	7	10	6	8	8	2
Diptera	4	14	7	4	7	5
Basommatophora	1		4	1	2	2
Oligochaeta			1		--	--
Zooplankton	Yes	Yes	Yes	--		
TOTAL	27	29	26	21	26	3.4
$-\sum p_i \ln p_i$	1.3	1.02	1.47	1.2	C.V. = 13%	
$\bar{H}$ = 1.25					S.E. = 1.8	
H/H_{max}	.81	.93	.91	.87		
$\bar{V}$ = .88						

Azodrin (1 ppb)

TAXA (order)	Sample No. 34	31	10	$\bar{X}$	S.D.
	Individuals/0.016 m^2				
Odonata	6	5	4	5	1
Diptera	7	6		4	4
Lepidoptera	2	1		1	1
Amphipoda	19	--	8	9	10
Basommatophora	4	1	6	4	3
Hemiptera		1	1	.5	.6
Homoptera			1	--	--
Zooplankton	Yes	Yes	Yes		
TOTAL	38	14	20	24	12
$-\sum p_i \ln p_i$	1.34	1.3	1.35	C.V. = 52%	
$\bar{H}$ = 1.33				S.E. = 7.2	
H/H_{max}	.83	.81	.84		
$\bar{V}$ = .83					

Azodrin (10 ppb)

TAXA (order)	Sample No. 35	23	22	11	$\bar{X}$	S.D.
	Individuals/0.016 m^2					
Odonata	9	5	8	7	7	2
Amphipoda	5	16	11	18	13	6
Diptera	1	6	6	2	4	2
Hemiptera	2	1	3		2	1
Lepidoptera	1				--	--
Basommatophora		1	6	9	4	4
Coleoptera			1		--	--
Cyprinodentiformes			1	1	.5	.6
Ephemeroptera				1	--	--
Zooplankton		Yes	Yes			
TOTAL	18	29	30	38	29	8
$-\sum p_i \ln p_i$	1.27	1.19	1.82	1.35	C.V. = 29%	
$\bar{H}$ = 1.41					S.E. = 4.1	
H/H_{max}	.79	.74	.94	.75		
$\bar{V}$ = .81						