EFFECTS OF WATER SOURCE, SUSPENDED SOLIDS, AND ACCLIMATION ON BIOTRANSFORMATION OF 2,4-DICHLOROPHENOXY ACETIC ACID IN AQUATIC SYSTEMS

THESIS

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CHAPTER I

INTRODUCTION

In recent years there has been a great deal of scientific interest in processes that affect the fate of organic chemicals in the environment. One main reason for this increased interest is due to greater environmental concern over accidental or purposeful release of these chemicals into the environment by man. A major environmental concern is the increased use of pesticides over the last few years. In the thirty years prior to 1978 the use of pesticides has increased by a factor of forty (Ridgeway et al., 1978). Recently the use of herbicides has been increasing, but that of insecticides has stabilized (Willis, 1983). Detectable amounts of organic pesticides can be found in many areas of the biosphere. For toxic organic chemicals to be used safely, researchers must have a clear understanding of the fate and persistence of these chemicals when they are released into the environment. This understanding will also allow the development of new products that, when properly used, will not produce adverse effects to man or the environment (Weber, 1972). According to the Toxic Substance Control Act (TSCA) any new or expanded-use chemical that might be released into the environment must be tested for environmental hazard.

Environmental hazard, according to Lee and Jones (1980), consists of two factors: the environmental toxicology of a chemical and the chemistry-fate of the chemical. The toxicity of a chemical is a function of its dose (concentration and duration of an exposure to an organism), whereas fate relates to the transport and disposition of the chemical in compartments of the environment (Staples et al., 1983). The fate processes therefore control the dose of the chemical acting on organisms in the environment. My research dealt principally with fate. Some of the fate processes acting on a chemical could be sorption, volatilization, hydrolysis, biodegradation, biotransformation, and photolysis.

My research focused on one aspect of the fate of one of the world's most widely used herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D) (Watson, 1977). The fate process investigated was biotransformation and how biotransformation of 2,4-D in aquatic systems is affected by suspended solids, source of water, and acclimation.

The reasons 2,4-D was chosen for this work are

1. 2,4-D is widely used in agriculture (Schwartz, 1967);
2. A great deal of literature exists regarding the fate of 2,4-D in soils (Altom and Stritzke, 1973; Watson et al., 1973; Norris and Greiner, 1967;

Audus, 1949, 1951, 1952, 1964);

3. There is relatively little information on the fate of the compound in aquatic systems (Nesbitt and Watson, 1980a,b; Steen et al., 1980; C.A.S.T., 1975);
4. 2,4-D belongs to a widely used class of pesticides, the chlorinated hydrocarbons;
5. 2,4-D has been found as a contaminant of water supplies (Schwartz, 1967);
6. Microbial degradation is the primary pathway for the degradation of 2,4-D in the environment (C.A.S.T., 1975);
7. Sorption to solids may affect the bioavailability of 2,4-D (Scott and Weber, 1967);
8. 2,4-D is a registered aquatic herbicide (Weed Science Society of America Herbicide Handbook, 1983);
9. 2,4-D is used in and around aquatic systems to control noxious weeds.

The U.S. Department of Agriculture has used 2,4-D to control weeds along river banks (Nesbitt and Watson, 1980a). Some uses of 2,4-D in aquatic systems have been to control water hyacinth, pond weed, and cattails (C.A.S.T., 1975). The main reasons for such widespread usage of 2,4-D are that it does not concentrate in the food chain, it does not persist from year to year, and it is much less toxic to animals than it is to plants (C.A.S.T., 1975) (Table I).

Even though 2,4-D has been widely studied, its mode of action is not wholly understood. It is known that this systemic herbicide causes plants to undergo abnormal growth response; 2,4-D also affects respiration, food reserves and cell division in the plant (Weed Science Society of America Herbicide Handbook, 1983).

If a compound, in an aquatic system, is associated with a solid, then it is no longer in solution. Staples et al. (1983) hypothesized that for a compound to exert toxicity (to be bioavailable) to water column organisms, it must be in the dissolved fraction of the system. Other researchers have presented data to support this hypothesis. Lee and Mariani (1977) showed that toxic chemicals in sediments are not available to act on aquatic organisms. It is suggested that these chemicals, while associated with the sediments, are not available because they are bound to the particulate matter of the sediments.

The potential of a chemical to sorb can be expressed by the adsorption coefficient (Kp) which is the ratio of chemical sorbed to chemical in solution. The Kp is generally a function of the properties of a chemical and the sorbing material (Lyman, 1982). Therefore, depending on the chemical structure, sorption may be one of the most important fate processes acting on a chemical (Baughman and Lassiter, 1978). The volatilization, photolysis, hydrolysis,

TABLE I

TOXICITY OF 2,4-D ACID FORMULATION TO ORGANISMS

Organism	LD_{50} (mg/kg)	LC_{50} (mg/l)
Rat	375	
Dog	100	
Guinea Pig	469	
Chicken	541	
Pigeon	668	
Mule Deer	400-800	
Bluegill		1000 (7 days)
Catfish		2000 (7 days)
Rainbow Trout		21.9 (48 hr)
Fathead Minnow		14-75 (48 hr)

Values taken from Way (1969).

biotransformation, and biodegradation of a chemical can be influenced by sorption of the chemical (Lyman, 1982). Bioavailability of a chemical may also be reduced by the interactions of the chemical with the abiotic and biotic solids in an aquatic system (Staples et al., 1983).

In aquatic systems, sources of sediments are diverse and include wastes from municipal, industrial, and agricultural sources, soil erosion, and decomposition of plants and animals within a water body (Weber, 1972). Suspended solids from municipal wastes are primarily organic substances and minerals. The input to aquatic systems from municipal waste is over 3.6 billion kilograms of suspended solids yearly (Weber, 1972). Manufacturing waste comes from four primary industries, paper, organic chemicals, petroleum and steel, and amount to over 8.1 billion kilograms of suspended solids added yearly to our waterways (Weber, 1972). However, the greatest volume of suspended solids comes from soil erosion. Soil erosion accounts for over 700 times the suspended solids introduced into aquatic systems as does sewage disposal (Weber, 1972). Suspended solids normally consist of sand, silt, and clays with thin films of organics and inorganics as well as metallic oxides attached to these particles. Microbial growth is often associated with these solids (Weber, 1972).

Suspended solids may affect the rate of biotransformation of a compound. As stated earlier, sorption of a compound to solids can affect the rate of biotransformation (Staples et al., 1983). Evans et al. (1973) showed that biodegradation of urea in river water increased under periods of high sediment loading. Nesbitt and Watson (1980a,b) correlated increased rates of degradation of 2,4-D with increased sediment loading in two Australian rivers. Simsiman and Chesters (1975) showed that the rate of biodegradation of endothall increased with suspended solids. Lee and Ryan (1979) investigated the effects of sediments on first-order biodegradation kinetics of p-chlorophenol, trichlorophenol, chlorobenzene, and trichlorophenoxy acetic acid. For these compounds the addition of 50 gm/l of sediments to estuary water enhanced the disappearance of the compounds. The first-order half-life of p-chlorophenol without sediments was reported as 20 days, with sediments the half-life was found to be 3 days. The half-lives of the other compounds were decreased, by addition of sediments, as follows: trichorophenol 90 days to 23 days; chlorobenzene 150 days to 75 days; and trichlorophenoxy acetic acid 1400 days to 95 days. Steen et al. (1980) showed that the degradation rate of chloropropham and di-n-butyl could be reduced by an increase in the amount of suspended solids. It was suggested that sorption to the solids rendered these compounds biologically unavailable (Steen et al., 1980). Adsorption of some herbicides, such as diquat and CIPC, by soil particles may also reduce their phytotoxicity because the herbicide is held near the surface of a soil particle rendering the herbicide less available to plants (Harris and Warren, 1963).

As the literature suggests, suspended solids can either increase, decrease, or not affect the rate of degradation or transformation of an organic compound in aquatic systems. Due to sorption, the bioavailability of a compound may be reduced. Adsorption is due to the interaction of the absorbent and the absorbate (Bailey and White, 1964).

The rate of degradation may increase for particular chemicals as a result of increased nutrients being released from the suspended solids to the water, increased microbial numbers contributed to the system from the suspended solids, or due to the suspended solids providing an interface for microbial-chemical interactions. The susceptibility of an organic compound to be biotransformed is controlled by the structure of the chemical and by environmental factors (Boethling and Alexander, 1979). For a herbicide to be biodegraded and/or biotransformed certain criteria must be met (Kearney et al., 1966).

1. The environment must be suitable for the microbes capable of transforming and/or degrading the compound.
2. The chemical must exist in the environment in a useable form for the microbes.

3. The compound must be available to the organisms.
4. The chemical must be capable of inducing the organisms to produce the necessary enzymes to breakdown the compound.
5. The environment must be suitable for the microbial population to grow and for the enzymes produced to function.

The inactivation/transformation of 2,4-D by soil microbes is well documented in the literature (Altom and Stritzke, 1973; Watson et al., 1973; Norris and Greiner, 1967; Schwartz, 1967; Aly and Faust, 1964; Audus, 1949, 1951, 1952, 1964; Klingman, 1964; Bollen, 1961; Bell, 1957; Rogoff and Reid, 1956; Walker and Newman, 1956; Evans and Smith, 1954; Jensen and Petersen, 1952; Newman and Walker, 1952; Akamine, 1951; Newman and Thomas, 1949; Brown and Mitchell, 1948; Derose and Newman, 1948). The inactivation/transformation of 2,4-D by microbes in aquatic systems has also been reported (Nesbitt and Watson, 1980a,b; Steen et al., 1980; Watson, 1977; C.A.S.T., 1975; Schultz, 1973; Hemmet and Faust, 1968; Demarco et al., 1967; Schwartz, 1967; Aly and Faust, 1964). The inactivation of 2,4-D has been attributed primarily to microbes (Nesbitt and Watson, 1980a,b; Watson et al., 1973; C.A.S.T., 1975; Jensen and Petersen 1952; Audus, 1949, 1951). Schultz (1973) found that there are at least eleven species of bacteria and two actinomycetes capable of degrading 2,4-D. Torstensson et al. (1975) also isolated species of bacteria and fungi capable of degrading 2,4-D as a sole carbon source.

There exist in the literature some controversy over the uptake of 2,4-D by bacteria. Wedemeyer (1966) suggests that there is a two step process in the uptake of the compound. The first step is sorption of 2,4-D to the cell wall of the bacteria followed by passive diffusion of the herbicide into the cytoplasm of the cell. Schwartz (1967), on the other hand, reported no sorption of 2,4-D to the cells of bacteria. If 2,4-D does sorb to the cell walls of microbes, then one would expect to find the compound sorbed to the microbes attached to suspended solids in an aquatic system. This could mean that the rate of transformation of 2,4-D might increase with the addition of suspended solids. The suspended solids may act as centers for microbial transformation of the compound.

The pathway of biodegradation of 2,4-D has also been studied extensively. Audus (1952) proposed the first step in this breakdown to be hydrolysis of the acetic acid side chain yielding a glycollic acid and a phenol. Evans and Smith (1954), Evans and Moss (1957), and Evans et al. (1961) proposed the first step in the breakdown of 2,4-D to be 6-hydroxy-2,4-dichlorophenoxyacetate followed by 3,5-dichlorocatechol and chloromuconic acid. Bell (1960) proposed 2,4-dichlorophenol as a metabolite of 2,4-D.

Tiedje et al. (1969) showed the degradation of 2,4-D, using Arthrobacter sp., to be characterized by cleavage of the ether linkage yielding 2,4-dichlorophenol and most likely glycollic acid. The glycollic acid is then converted to alpha-alanine. The 2,4-dichlorophenol is then oxidized forming 3,5-dichlorocatechol. The 3,5-dichlorocatechol is then further broken-down by oxidation.

One factor that may affect biotransformation rate of a compound is acclimation. In aquatic systems where the microbes have not recently been exposed to 2,4-D, the transformation rate of the compound may be less than in a system in which the microbes have recently been exposed to the compound. The literature contains references to lag phases in the degradation of 2,4-D (Nesbitt and Watson, 1980a; Norris and Greiner, 1967; Robson, 1966). During the lag phase the loss of compound is not significantly different from zero, the concentration of compound is relatively constant. This lag phase usually occurs when the organisms are initially exposed to a compound. The presence of lag phases may be an indicator of acclimation taking place prior to the compound actually being broken down. Other researchers have shown higher rates of microbial degradation of 2,4-D using acclimated cultures or in situations of redose than were shown in situations where the microbes have not previously been exposed to the compound (Nesbitt and Watson, 1980b; Watson, 1977; Newman and Walker, 1952; Audus, 1949, 1951; Newman and Thomas, 1949). Spain and Van Veld (1983) found that preexposure to 2,4-D enhanced the disappearance of the herbicide. In biodegradation experiments using water previously exposed to 2,4-D from the Escambia River, they reported less than 5% of the initial 2,4-D remaining after 40 hours. In non-preexposed river water over 80% of the herbicide remained after 100 hours. Spain and Van Veld (1983) also reported similar results for p-nitrophenol (PNP). In non-preexposed systems 70% of the compound remained after 120 hours. In preexposed systems about 10% of the PNP remained after 70 hours. The adaption of organisms to PNP reportly lasted seven weeks after initial exposure. Robson (1966) reported a lag phase when 2,4-D was introduced in low concentrations (0.5 mg/l) but not when the chemical was added in higher concentrations (5.0 mg/l) to water. Nesbitt and Watson (1980a) reported a lag phase of 6 to 12 days for the degradation of 2,4-D, in river water systems. The length of the lag phase was said to depend on environmental conditions.

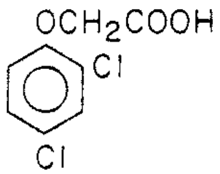
Chemical Parameters and Reported Half-Lives

The structure and some of the physical properties of 2,4-dichlorophenoxyacetic acid are shown in Table II. The values in this table came from literature sources including the Weed Science Society of America Herbicide Handbook

TABLE II

CHEMICAL STRUCTURE AND PHYSICAL PARAMETERS OF 2,4-D

Chemical Structure



Molecular Formula	. $C_8H_6Cl_2O_3$*
Molecular Weight.	.221.0
Melting Point	.135 to 138°C (Technical), 140 to 141°C (Pure)
Vapor Pressure.	.0.4 mm Hg at 160°C
Solubility Water.	.900 mg/l at 25°C 600 mg/l (Audus 1976)
pKa	.2.73 (Nelson and Faust 1969)
Koc	.330 (Neely and Mackay 1981)
Kow	.645 (Chiou et al. 1977) 11000 (Neely and Mackay 1981)

*Unless otherwise noted values are from the Weed Science Society of American Herbicide Handbook (1983).

(1983).

The reported half-life, or persistence, of 2,4-D in the literature varies greatly. Aly and Faust (1964) reported that 2,4-D remained in lake muds for up to 65 days, 35 days if the lake had previously been treated with the herbicide. They also reported 2,4-D persistance in the water column to be 120 days. C.A.S.T. (1975) reported the half-life of 2,4-D in soil to be 1-2 weeks. Schwartz (1967) reported that very little biodegradation of 2,4-D occurred in a non-sterile dilute salts media. He found that after 175 days only 11-23 % of the compound had been biodegraded. Klingman (1964) reported the persistance of 2,4-D in soils to be only 7 days, while Akamine (1951) reported the persistance of the compound in soils to be 98 days. Nesbitt and Watson (1980a) found the half-life of 2,4-D, in river waters to range from 10 to 50 days.

The goals of this research were as follows:

1. Determine some of the possible effects suspended solids have on the biotransformation rate of the herbicide 2,4-D;
2. Generate environmentally realistic biotransformation rate coefficients for 2,4-D. This involves using realistic concentrations of the chemical and also using realistic concentrations of suspended solids from the same source as the river water;

3. Determine if acclimation has an effect on the apparent biotransformation rate of 2,4-D;
4. Determine the effect that suspended solids have on the toxicity of the herbicide to Selenastrum capricornutum.

The following hypotheses were investigated in this work.

H1:Addition of suspended solids of 500 mg/l above background suspended solids concentration have no effect on the apparent biotransformation rate of 2,4-D in river waters.

H2:The source of water and suspended solids has no effect on the apparent biotransformation rate of 2,4-D.

H3:The apparent biotransformation rate coefficient of 2,4-D is best described by first-order kinetics.

H4:The organisms introduced from the suspended solids do not affect the apparent biotransformation rate of 2,4-D.

H5:The rate of biotransformation of 2,4-D is not affected by whether or not the system has previously been exposed to the herbicide.

H6: The toxicity of 2,4-D to Selenastrum capricornutum is not affected by the source or amount of suspended solids in the system.

CHAPTER II

MATERIALS AND METHODS

All biotransformation studies of 2,4-D that were conducted for this thesis used natural occurring waters and sediments. The waters and sediments are from three sources. The first source is the Trinity River in Dallas county, Texas. The second source of water and sediments is the Red River in Grayson county, Texas. The third source is the Mississippi River in Shelby county, Tennessee. The three rivers were chosen because of their proximity to industries and their importance as receiving systems of municipal, industrial, and agricultural wastes. The Mississippi River receives all of these wastes on a daily basis. The Red River, at the site chosen, does not have a great deal of industrial waste added to the upstream waters. The Trinity River, on the other hand, does have extensive agricultural runoff and a little industrial wastes added to its upstream waters.

From these three sites, water was collected in acid-washed 20-liter nalgene containers and transported to the laboratory. Sediments, from the three sites, were removed from the upper 2 cm of the river beds and placed in 1-liter nalgene containers. The sediments, prior to use, were sieved through a 277-um sieve to promote uniformity. Sediments and water that were not to be used immediatly were stored at 4^{o}C.

To help account for variations in the biotransformation studies between the river systems, water quality and sediment chemistry parameters were quantified. Analytical methods for water quality can be found in Table III and methods for the sediment properties are in Table IV. Some of the water quality data for the Mississippi River were obtained from the STORET (USEPA, 1984) data base.

The biotransformation rates for 2,4-D were found using a shake-flask design with an initial concentration of approximately 2 mg/l of 2,4-D. This concentration is an environmentally realistic concentration since it is well within the concentration recommended on the labels of the aquatic licensed formulation. The disappearance of 2,4-D was followed for at least two half-lives. The vessels used were 250-ml screw-top Erlenmeyer flasks. Screw-top flasks were used to aid in maintaining sterility of the controls. Each flask initially contained 200 ml of one of the river waters with the appropriate amount of solids added, either 0 mg/l or 500 mg/l. The biotransformation tests were performed in the dark to prevent any photodegradation of the herbicide. Significant photodegradation of 2,4-D ester has been reported in the literature under laboratory conditions (Hansen and Buchholt, 1952; Crosby and Tutass, 1966; Bell,

TABLE III

ANALYTICAL METHODS FOR WATER QUALITY PARAMETERS

Parameter	Method	Reference*
Ammonia	Specific Ion Probe	417E
Calcium	Flame Atomia Absorption	303A
Dissolved Oxygen	YSI Model 54A Meter	208A.2.C
Iron	Flame Atomic Absorption	303A
Nitrate	Specific Ion Probe	418B
Orthophosphate	Ascorbic Acid	424F
pH	Markson pH Meter	423.2
Sodium	Flame Atomic Absorption	303A
Temperature	YSI Model 54A Meter	212
Total Phosphate	Persulfate Digestion/ Ascorbic Acid	424C&F

*All references from Standard Methods (1980).

TABLE IV

ANALYTICAL METHODS FOR SEDIMENT PARAMETERS

Parameter	Method	Reference
Ammonia	Specific Ion Probe	Standard Methods 417E, 1980
Loss on Ignition	Heating to 550°C	Standard Methods 209G, 1980
Nitrate	Specific Ion Probe	Standard Methods 418B, 1980
Particle Size	Hydrometric Analysis	Black et al., 1965

1956; Aly and Faust, 1964). Aly and Faust (1964) reported 2,4-D acid as the breakdown product of the ester and that the acid did not undergo any further breakdown. Performing these experiments in the dark is probably not necessary since the acid and not an ester is being used. The shake flasks were maintained at room temperature and shaken on a rotary shaker at 100 revolutions per minute (RPM). One hundred RPM was sufficient agitation to keep most of the solids in suspension and to maintain dissolved oxygen concentrations in the flasks above 4 mg/l. There were four replicates of each treatment.

To account for any losses of the compound by other than biological means, autoclaved controls, also in replicates of four, were maintained with the test flasks. The complete experimental matrix is shown in Figure 1. For each river system, the matrix consisted of the following:

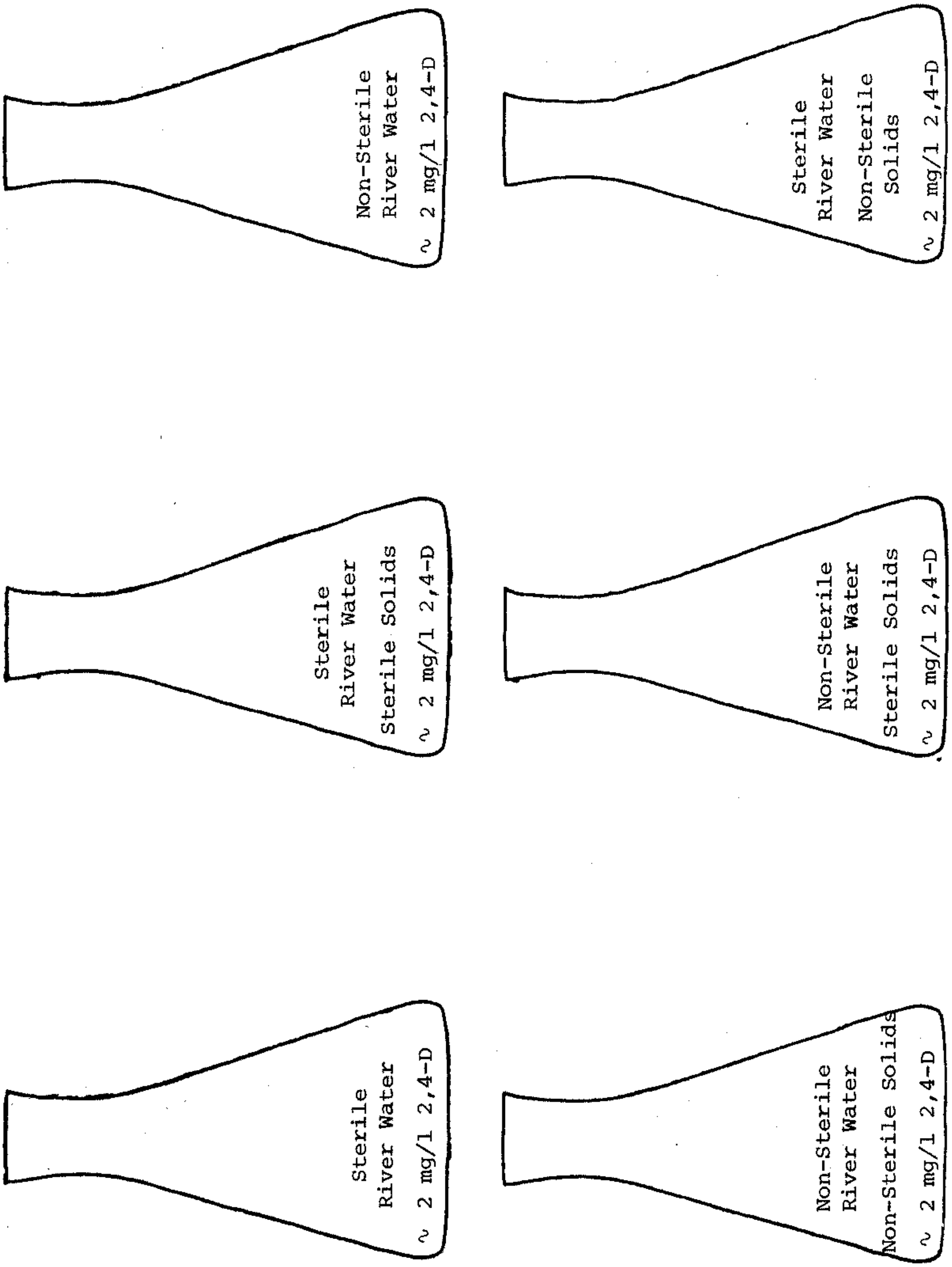
Four flasks containing river water with no additional solids(T1-T4).

Four flasks containing river water and 500 mg/l additional solids (T5001-T5004).

Four flasks containing sterile river water and 500 mg/l non-sterile solids (NSS1-NSS4).

Four flasks containing non-sterile river water and 500 mg/l sterile solids (SS1-SS4).

Fig. 1--Experimental matrix of the biotransformation studies.


Sterile
River Water
~ 2 mg/l 2,4-D
Sterile
River Water
Sterile Solids
~ 2 mg/l 2,4-D
Non-Sterile
River Water
~ 2 mg/l 2,4-D
Non-Sterile
River Water
Non-Sterile Solids
~ 2 mg/l 2,4-D
Non-Sterile
River Water
Sterile Solids
~ 2 mg/l 2,4-D
Sterile
River Water
Non-Sterile
Solids
~ 2 mg/l 2,4-D

Four flasks containing sterile river water only (C1-C4).

Four flasks containing sterile river water and 500 mg/l sterile solids (C5001-C5004).

On day zero and then periodically throughout each experiment, samples were removed from each of the flasks for 2,4-D analysis. Samples were also removed from each flask on day zero and periodically throughout the experiments for estimates of bacteria in the systems.

The effects of the suspended solids concentration on the rate of biotransformation of 2,4-D were determined by comparing the biotransformation rates of the herbicide in the presence of 0 mg/l (T) and 500 mg/l (T500) additional solids for each of the three sources of sediments and water. A concentration of 500 mg of solids per liter of river water was chosen because the suspended solids in natural waters typically range from 10 mg/l to 10,000 mg/l (Wetzel, 1975). The suspended solids concentration was chosen closer to the lower end of the typical suspended solids concentration range to represent more closely the majority of river systems. Also, if significant differences are shown with 500 mg/l of additional suspended solids, then that would indicate that small changes in the suspended solids loading of a river will alter the biotransformation rates of hazardous chemical significantly.

The effect of the sediment microbes on biotransformation rates of 2,4-D was analyzed by comparing rates of biotransformation in shake-flasks containing non-sterile water and non-sterile solids (T500) with rates found in flasks containing non-sterile water and sterile solids (SS). A comparison of biotransformation rates in the flasks that contain non-sterile water and sterile solids (SS) with rates of flasks containing sterile water and non-sterile solids (NSS) may indicate the fraction of transformation of the compound that the water or sediment microbes contribute to the total biotransformation of the herbicide.

Nesbitt and Watson (1980a,b) reported a correlation between the nutrients of suspended solids and the rate of degradation of 2,4-D in river water. They also showed a correlation between organic matter in the system and biotransformation of the compound. Keeping this in mind, correlations between organic matter, nutrients, and the rate of biotransformation of 2,4-D were analyzed using the Statistical Analysis System (SAS) release 82.4.

To investigate the possibility of higher biotransformation rates in situations of acclimation redosing experiments were performed using Red and Trinity river waters and solids. Redosing was not performed on the

Mississippi River samples because the rate of biotransformation in the Mississippi was initially fast, very little lag was seen and according to STORET (1984), the Mississippi, at the sampling site, has a background level of the herbicide present. Due to lack of sensitivity, of the analytical method used to quantify the herbicide, these background concentrations were not seen. The redosing procedure consisted of decanting the liquid from a test flask into four sterile 50-ml centrifuge tubes. The liquid was then centrifuged for 15 minutes in a International model HN (International Equipment Company) centrifuge on high (1600 RPM). The pellet was then resuspended in 200 ml of sterile river water that had been dosed with approximately 2 mg/l of 2,4-D. The resuspension was accomplished by adding approximatly 20 ml of the water to each of the centrifuge tubes and vortexing each tube for 1 minute on a Thermolyne Maxi-Mix. The liquid was then decanted back into the original Erlenmeyer flask and 20 ml of fresh, dosed sterile water was again added to each centrifuge tube and vortexed. After this liquid was added to the original flask, additional sterile dosed water was added to make a total volume of 200 ml.

The number of bacteria in the flasks of the before mentioned experiments were estimated by standard pour plates using 0.1% plate count agar (Difco) and 1% agar (Difco). The pour plates were incubated for 120 hours at

$20^{\circ}C$ and then counted using a Quebec Colony counter. Plate counts were performed on all flasks including the autoclaved controls. The presence of microbes in the controls indicated contamination and voided any data collected from that control since the last plate count where no contamination was observed.

Analytical Protocol for 2,4-D

Analytical procedures for 2,4-D were modified from methods described by Nesbitt and Watson (1980a,b) and Hammarstrand (1979). On the day of analysis, 3 ml of water were removed from each flask. To this aliquot, 4 ml of reagent-grade methanol and 1 ml of concentrated hydrochloric acid were added. The mixtures were then incubated at $60^{\circ}C$ ($\pm 2^{\circ}C$) in a water bath for 18 hours. This procedure resulted in the formation of the methyl ester of the 2,4-D acid (Hammarstrand, 1979). The methyl ester was then extracted from the aliquot into 3 ml of pesticide-grade n-hexane, by vortexing the sample vial containing the derived 2,4-D for 5 minutes on a Thermolyne Maxi-Mix. The concentration of the methyl ester in the hexane was then analyzed via gas liquid chromatography (GLC) using a 50-cm, 2-mm i.d. column containing GP 5% DEGS-PS on 100/120 Supelcorport. The carrier gas was a mixture of argon with 10% methane. The gas chromatograph used was a Tracor 560 equipped with an electron capture detector (ECD). A

Hewlett-Packard integrater was used to quantify the methyl ester of the 2,4-D. External standards were used during analysis to insure accuracy of results.

The reagents used in this analysis were obtained from several sources. 2,4-D acid (99.68%) was obtained from the Quality Assurance section of USEPA, Research Triangle Park, North Carolina. From Union Carbide, 99% or purer 2,4-D methyl ester was obtained. Reagent-grade methanol, pesticide-grade n-hexane and reagent-grade concentrated hydrochloric acid were purchased from the Fisher Scientific Company.

The following quality control procedures were followed during analyses of the herbicide concentration: (a) extraction efficiencies were determined at the same concentration level as the samples; (b) at five or six sample intervals, standards of known concentration were injected; and (c) procedure blanks were injected for each analysis.

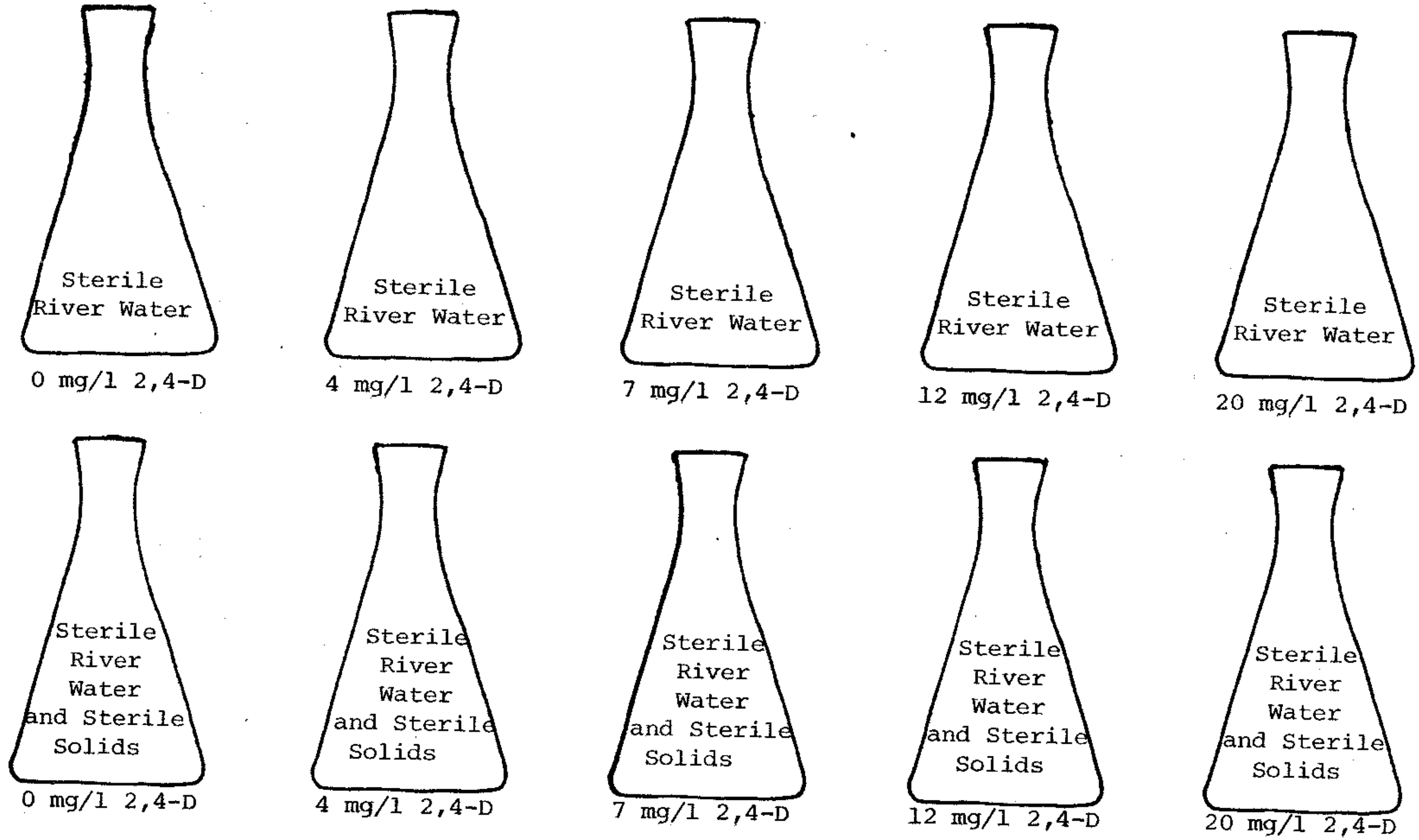
Preliminary Algal Bioassay Test

To test the effect of suspended solids on toxicity and bioavailability of 2,4-D to Selenastrum capricornutum, a modified algal assay bottle test was performed. The test used was a modification of the test as described by Miller et al. (1978). This experiment consisted of inoculating sterile 1-liter Erlenmeyer flasks containing 200 ml of sterile Trinity River and varying concentrations of 2,4-D with Selenastrum capricornutum yielding an initial algal concentration of approximatly 1000 cells per ml of river water. The concentrations of 2,4-D used were 0 mg/l, 4 mg/l, 7 mg/l, 12 mg/l, and 20 mg/l. Six flasks contained each of the above concentrations. Sterile Trinity River solids were added to three of each of the before mentioned six flasks. Enough sediment was added to yield a final concentration of 500 mg/l. These are the same sediments and the same concentration as used in the biotransformation test. The complete experimental design can be seen in Figure 2. The flasks were incubated at room temperature and illuminated at 300 foot-candles. The flasks were shaken at least once a day for 8 days. On the eighth day the number of algal cells per milliliter were determined by microscopic examination using a hemacytometer. Concentrations of algal cells in the flasks with sediment were compared with concentrations of cells in the flask containing sediments using an analysis of covariance procedure (SAS 82.4).

Analysis of Data

The reaction order (zero, first or second) was determined by plotting the percent of the 2,4-D remaining, the natural log of the percent remaining, and the reciprocal of the percent remaining versus time. If the reaction order is zero-order, then the plot of the percent of 2,4-D

Fig. 2--Experimental matrix of the preliminary algal bioassay.



To All Flasks *Selenastrum capricornutum* was Added.

remaining versus time will result in a linear plot. If the reaction order is first-order, then the plot of the natural log of the percent remaining versus time will be linear, and if the reaction rate is second-order, then the plot of the reciprocal of the percent remaining versus time will be linear (Williams et al., 1978). The regression coefficients for each of these plots was determined using the regression procedure of SAS (82.4).

If the reaction order is determined to be zero-order then the rate coefficient (k_0) is equal to the slope of the line of best fit of the percent of the compound remaining versus time multiplied by negative one. If the reaction order is determined to be first-order, then the rate coefficient (k_1) is equal to the slope of the line of best fit of the log of the percent of compound remaining versus time multiplied by negative one. If the rate is determined to be second-order, then the rate coefficient (k_2) is determined by the equation $k_2 = \frac{k_1}{[B]}$ where k_1 is the first-order rate coefficient and [B] is the biomass of microbes as estimated by plate counts (CFU/ml) (Paris et al., 1981).

If the rate of loss of the compound in the sterile controls is significantly different than zero, it is necessary to subtract the rate of loss in the sterile control flasks (C or C500) from the test flasks (T or T500) to account for losses of the chemical as a result of photolysis, volatilization, sorption, and/or other physical and chemical processes that may be occurring. A comparison of slopes was used to determine if any of the various experimental systems significantly differ from each other in their apparent biotransformation rate coefficients (Zar, 1974). These comparisons were accomplished using the analysis of covariance procedure of SAS (82.4).

CHAPTER III

RESULTS AND DISCUSSION

The results found during this research are presented under seven major topic areas:

1. Results of the analytical method used to quantify the herbicide 2,4-dichlorophenoxyacetic acid;
2. Characterization of the waters and sediments used in this research;
3. Results of the biotransformation tests for the Red River, the Trinity River, and the Mississippi River waters and waters plus solids;
4. Results of the biotransformation tests designed to determine the effect of acclimation on the rate of biotransformation of the herbicide in the three river systems;
5. Comparison of experimentally determined biotransformation rates with biotransformation rates found in the literature;
6. Development of predictive models to predict the biotransformation rate of 2,4-D in aquatic systems;
7. Results of the preliminary algal bioassay test used to determine both the toxicity of the 2,4-D

to *Selenastrum capricornutum* and to determine if the addition of solids reduces the toxicity of the herbicide to the alga.

Analytical Method to Determine the Concentration of 2,4-D

The method used to determine the concentration of the 2,4-D for this research is not the most sensitive available. This method is, however, more than adequate for the concentrations used in this study. Percent recovery experiments yielded an overall recovery efficiency of 76% for the three river systems. The actual percent recoveries for the various waters can be seen in Table V. As can be easily recognized from this table, the method yielded consistent percent recoveries for the three river waters.

This method yielded minimum detectable levels for 2,4-D of 0.1 mg/l. Below this concentration, the resulting 2,4-D peak could not be reliably resolved from the base line of the GLC. Since the initial dose of the herbicide into the test systems was approximatly 2 mg/l, this minimum detectable limit was more than acceptable since this sensitivity allowed for the biotransformation of the compound to be followed for over two half-lives.

This method, even though it requires 18-20 hours between sampling and analysis for derivatization to be

TABLE V

PERCENT RECOVERIES OF 2,4-D FROM THE MISSISSIPPI RED, AND TRINITY RIVER WATERS

River	Percent Recovered			Mean	Standard Deviation
Mississippi	71	78	77	75.3	3.8
Red	80	75	73	76	3.7
Trinity	84	72	77	77.7	6.0

accomplished, takes less than 10 minutes of personnel time for complete analysis. This time estimate includes sampling, derivatizing, extraction, and quantification. The low labor intensiveness of this method allows for a great number of samples to be analyzed by a single technician.

Water and Sediment Chemistry

The water chemistry for the Red River, the Trinity River, and the Mississippi River can be found in Table VI. As can be seen from this water chemistry data, the three rivers are by no means identical. The pH of the three waters is similar with that of the Red being slightly more basic than the pH of the other two waters. The alkalinity of the Red and the Mississippi river waters is almost identical and the alkalinity of the Trinity River water is somewhat lower. The hardness of the Red River water is very low, yet the hardness in the other two river waters is moderate to hard. In examining the data collected concerning phosphates, nitrates, and ammonia for the three waters, a ranking of nutrient concentrations in the waters is suggested. This ranking would place the Red River with lowest nutrient concentration and the Trinity with the highest nutrients. The nutrient concentration of the Mississippi falls between the other two waters and could be considered as moderate. Suspended solids content in the

TABLE VI

RESULTS OF WATER CHEMISTRY ANALYSIS FOR MISSISSIPPI, RED, AND TRINITY RIVER WATERS

Parameter	Mississippi River	Red River	Trinity River
pH	7.3	7.8 ± 0	7.2 ± 0.03
Alkalinity (mg $CaCO_3$/l)	102.1 ± 16.5*	126.7 ± 5.8	76.7 ± 2.9
Hardness (mg $CaCO_3$/l)	150 ± 20.9*	8.5 ± 0	159.7 ± 2
Orthophosphates (mg PO_4^- P/l)	0.23 ± 0.07*	$6.9 \times 10^{-4} \pm 9 \times 10^{-5}$	NA**
Total phosphates	0.7 ± 0.3*	$2.7 \times 10^{-3} \pm 4 \times 10^{-4}$	NA
Ammonia (mg NH_3 N/l)	0.075 ± 0.07*	BDL***	2.6 ± 0.2
Nitrate (mg NH_3^- N/l)	0.025 ± 0.02*	0.03 ± 0.04	8.4 ± 0.06
Total Suspended Solids (mg/l)	170.8 ± 131.1	33.3 ± 16	50 ± 23
Chlorides (mg/l)	16.9 ± 4.1	312 ± 56	72.6 ± 0.3
Total Carbon (mg/l)	29.6	32 ± 1.7	33 ± 1.1
Inorganic Carbon (mg/l)	21.0	31 ± 0.49	30.4 ± 0.6
Organic Carbon (mg/l)	8.6 ± 4.2	1 ± 1.9	2.6 ± 0.6
Calcium (mg/l)	33.0 ± 0.1	101 ± 0.6	40.5 ± 0.1
Sodium (mg/l)	11.2 ± 1.3	126 ± 1.8	101 ± 0.6
Iron (mg/l)	0.49 ± 0.02	0.03 ± 0.02	1.2 ± 0.02

*Values from STORET (1971).

**NA = Not available.

***BDL = Below Detectable Limit for Ammonia BDL = .03 mg NH_3 N/l.

Red and the Trinity river waters is shown to be similar, with the background suspended solids in the Mississippi River to be three to five times greater. Carbon analysis of the three waters shows similarities in the concentration of total carbon and a higher concentration of organic carbon for the Mississippi River water. These water quality data may suggest a possibility of higher rates of biotransformation in the Trinity River waters than in the other two systems because of increased nutrients of the Trinity. This possibility is in agreement with the correlations shown by Nesbitt and Watson (1980a,b).

In viewing the sediment characteristics (Table VII) of the three systems, the particle size data, nutrient data, and the percent volatile matter should be noted. The particle size data show that the Red River has the most sand, over 86%, followed by the Mississippi, with over 62%, and then the Trinity with only 42% sand. Sand is inert and should not affect the bioavailability of the compound. The clay and silt fractions of the sediments may affect the bioavailability of a compound as a result of sorption. The Trinity River sediments contain almost 40% clay and over 20% silt. The Mississippi sediments contain less then 18% clay and 20% silt. The silt and clay content of the Red River sediments are 0 and 14%, respectively. This particle

TABLE VII

SEDIMENT CHARACTERISTICS OF THE MISSISSIPPI, RED, AND TRINITY RIVERS

Parameter	Mississippi River	Red River	Trinity River
pH	7.0	7.3	6.7
% Sand	62.4	86.4	41.8
% Silt	19.8	0	21.2
% Clay	17.8	13.6	37.0
CFU/gr	5.2×10^6	1.9×10^7	1.6×10^7
Cation Exchange (meq/100 gr)	NA	41	427.2
Nitrogen (mg NH_3^- N/gr wet wt)	0.0632	0.0529	0.198
Total Phosphate (PO_4^- P/gr wet wt)	3.7	0.88	6.01
Volatile Matter (mg/kg)	60533 $\pm$ 932	5456 $\pm$ 196	5429 $\pm$ 191

NA = Not available.

size data indicates that if any of the sediments are going to affect the bioavailability of the 2,4-D, it should be the sediments of the Trinity River.

In considering the nutrient content of the sediments, as indicated by nitrate and total phosphate concentrations, the Trinity River has greater than an order of magnitude higher concentration than the Red River sediments. The sediments of the Mississippi River fall between the sediments of the Red and the Trinity in nutrient concentration. This ranking of the nutrient concentrations of the sediments is of the same order as the ranking of the nutrient concentration of the waters. This similarity in the rankings of the nutrients in the water and sediment compartments of the three river systems is expected since the nutrient content of the sediments is in most cases dictated by the nutrients in the overlying water. Sediments act as a sink for nutrients.

The volatile matter data of the three sediments indicate that the Trinity and the Red river sediments have almost the same volatile matter (5,400 mg/kg), and the Mississippi River has over an order of magnitude greater volatile matter (60,000 mg/kg). The volatile matter can be used as an indicator of the carbon content of the sediments. These data would suggest that the Mississippi River sediments contain more carbon than the other two sediments. This increased carbon content may increase the amount of 2,4-D that is sorbed to the sediments, therefore affecting the biotransformation rate of the compound.

Biotransformation Tests

Results of selected biotransformation tests are discussed below. The following results will indicate the effects that the source of water (Red River, Trinity River, or Mississippi River) has on the biotransformation first-order rate coefficient. Also included in this section are effects that the presence or absence of additional solids has on biotransformation of 2,4-D. The zero-order and the second-order rate coefficient are also included in this section and are discused below. The regression coefficients for the various rate coefficients and the calculated first-order half-lives are also presented. For the twenty-six non-sterile studies conducted, the first-order rate coefficients showed a better regression coefficient than the second-order rate coefficient fourteen times. A rate coefficient is termed "better" if its regression coefficient is 0.01 units greater than the regression coefficient of the the other rate coefficients. The second-order rate coefficients are better than the first-order coefficients only one time. Surprising is the fact that in twelve cases the zero-order rate coefficients have a better regression coefficient than the first-order coefficients. The first-order coefficients are better than the zero-order coefficients seven times. To aid in deciding whether zero, first, or second order kinetics should be used to describe the disappearance of the herbicide a comparisons of the coefficients of variation of the experimental rate coefficients was performed. The rate coefficients (zero, first, and second) for each of the non-sterile experiment were used to determine a coefficient of variation for the biotransformation rate of 2,4-D for each of the three kinetic orders. These 24 experiments included all three river systems, experiments with and without additional solids, and experiments using both acclimated an non-acclimated organisms. The order which yields the lowest coefficient of variation should best describe the disappearance of the compound since its rate coefficients had the least variation over all of the experiments conducted. This analysis resulted in similar results between zero and first order kinetics with second-order kinetics a distant third. The coefficients of variation for the various reaction orders are 80.1, 82, and 97 for zero-order, first-order and second-order respectively. From these comparison of regression coefficients, it can be seen that the biotransformation of 2,4-D in the conditions described is either zero or first order. In all of the biotransformation studies, the first-order rate coefficient adequately described the disappearance of the compound. In none of the non-sterile test was the zero-order regression coefficient 0.1 units greater then the first-order regression coefficients. Therefore, the discussions that follow will deal mainly with the first-order rate coefficients.

The results from the biotransformation test incorporating sterile river waters and additional non-sterile solids (NSS), and the biotransformation test containing non-sterile river waters and additional sterile solids (SS) are also presented and discussed below. These latter two experimental matrix are designed to indicate the contribution that sediment or water column associated microbes have on the biotransformation of the compound.

Biotransformation of 2,4-D in the Red River System

The results of the biotransformation studies on the disappearance of 2,4-D in the Red River can be found in Table VIII. These results are a culmination of four independent biotransformation experiments. Selected graphical depictions of the disappearance of the compound

TABLE VIII

RESULTS OF THE BIOTRANSFORMATION STUDIES OF 2,4-D IN THE RED RIVER

Exper-iment	Water	Solids	Biomass (cfu/ml)	Rate Coefficients: Zero-Order*	r	First-Order**	r	Second-Order***	r	First-Order Half-Life (days)
1	Sterile	None Added	0	0.35	0.407	0.004	0.422	0	0.437	173.3
1	Sterile	Sterile	0	0.095	0.173	0.001	0.173	0	0.173	693
1	Non-Sterile	None Added	19500	0.22	0.492	0.002	0.506	$1x10^{-7}$	0.519	301.3
1	Non-Sterile	Non-Sterile	44750	3.38	0.734	0.11	0.738	$2x10^{-6}$	0.739	6.3
2	Non-Sterile	None Added	46250	1.59	0.76	0.06	0.745	$1x10^{-6}$	0.729	11.94
2	Non-Sterile	Non-Sterile	78000	2.01	0.899	0.07	0.847	$9x10^{-6}$	0.802	9.62
3	Sterile	None Added	0	0.19	0.416	0.002	0.415	0	0.414	364.7
3	Sterile	Sterile	0	0.07	0.092	0.0007	0.07	0	0.036	949.3
3	Non-Sterile	None Added	26750	1.70	0.601	0.05	0.567	$2x10^{-6}$	0.522	14.15
3	Non-Sterile	Non-Sterile	42250	3.12	0.795	0.09	0.719	$2x10^{-6}$	0.617	7.87
4	Sterile	None Added	0	0.008	0.315	0.002	0.316	0	0.293	364.7
4	Sterile	Sterile	0	-0.20	0.414	-0.002	0.409	0	0.404	-330
4	Non-Sterile	None Added	42750	4.464	0.789	0.15	0.788	$3x10^{-6}$	0.792	4.78
4	Non-Sterile	Non-Sterile	57750	3.005	0.731	0.10	0.734	$2x10^{-6}$	0.727	7.02

*Unit expressed as % day^{-1}.

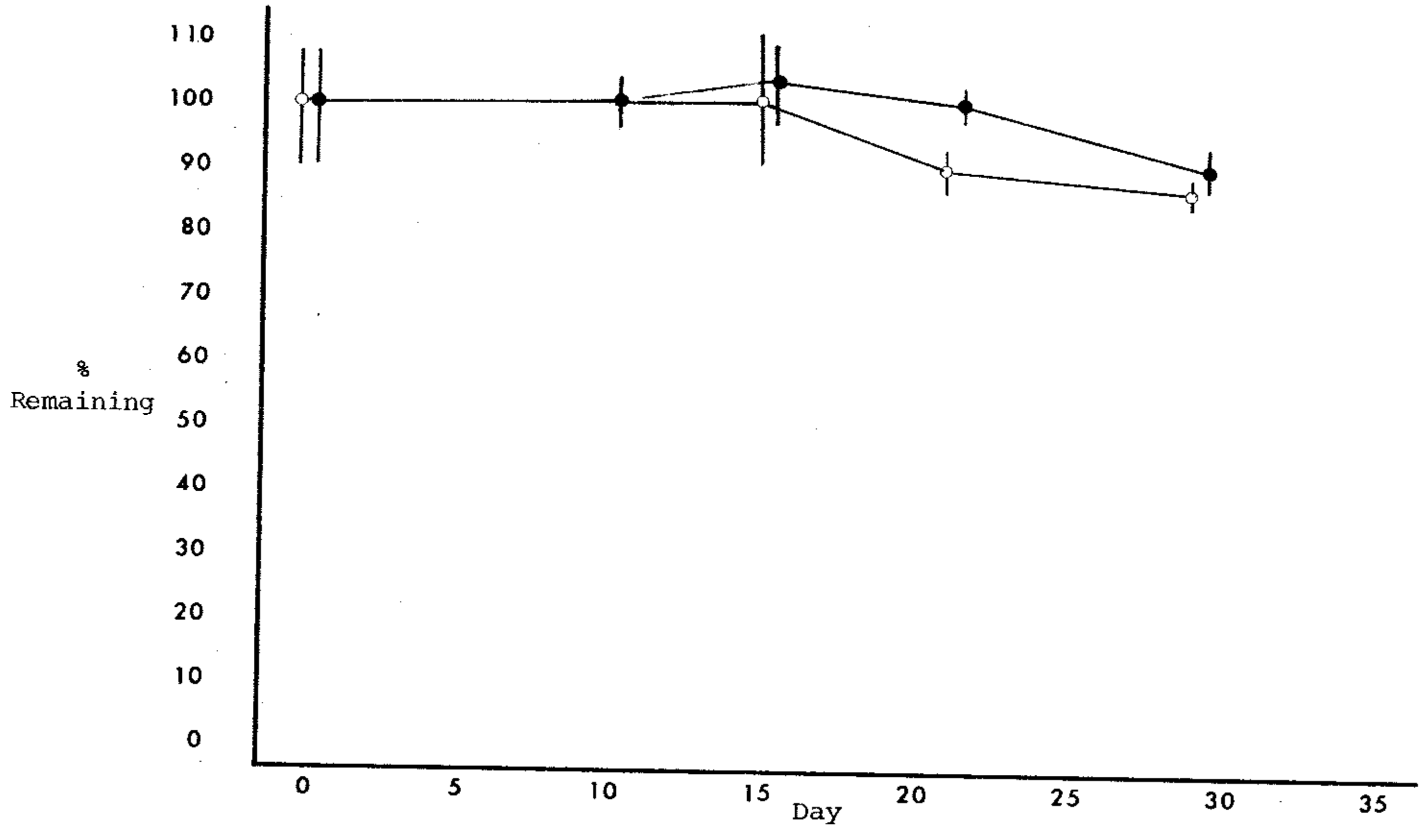
**Unit expressed as day^{-1}.

***Unit expressed as %$(cfu/ml)^{-1} day^{-1}$.

can be found in Figures 3-8. These figures indicate the disappearance of the compound in tests flask (T or T500) as compared to the disappearance of the herbicide in the pertinent sterile control flasks (C or C500). The points depict the mean percent remaining in four replicate flask versus time. Also included in these figures are points depicting a range of one standard deviation on both sides of the reported means. It should be noted that five out of the six slopes of the line of best fit for the disappearance of the compound in the sterile controls (C or C500) are not significantly different from zero (P=0.05). All of the lines of best fit for the disappearance of the herbicide in the non-sterile flasks (T or T500) have slopes that are significantly different (P=0.05) or highly significantly different (P=0.01) from zero (Table IX).

With the exception of one set of replicates (RRAT), the derived first-order rate coefficients for the biotransformation of 2,4-D in the Red River water ranged from 0.05 day^{-1} to 0.14 day^{-1}. These coefficients relate to half-lives from just over 14 days to just under 5 days. The first-order rate coefficient for test flasks RRAT is 0.002 day^{-1}, which would yield a half-life of over 300 days. The abnormality of this test (RRAT) might be explained by the low numbers of microbes present in these systems. The

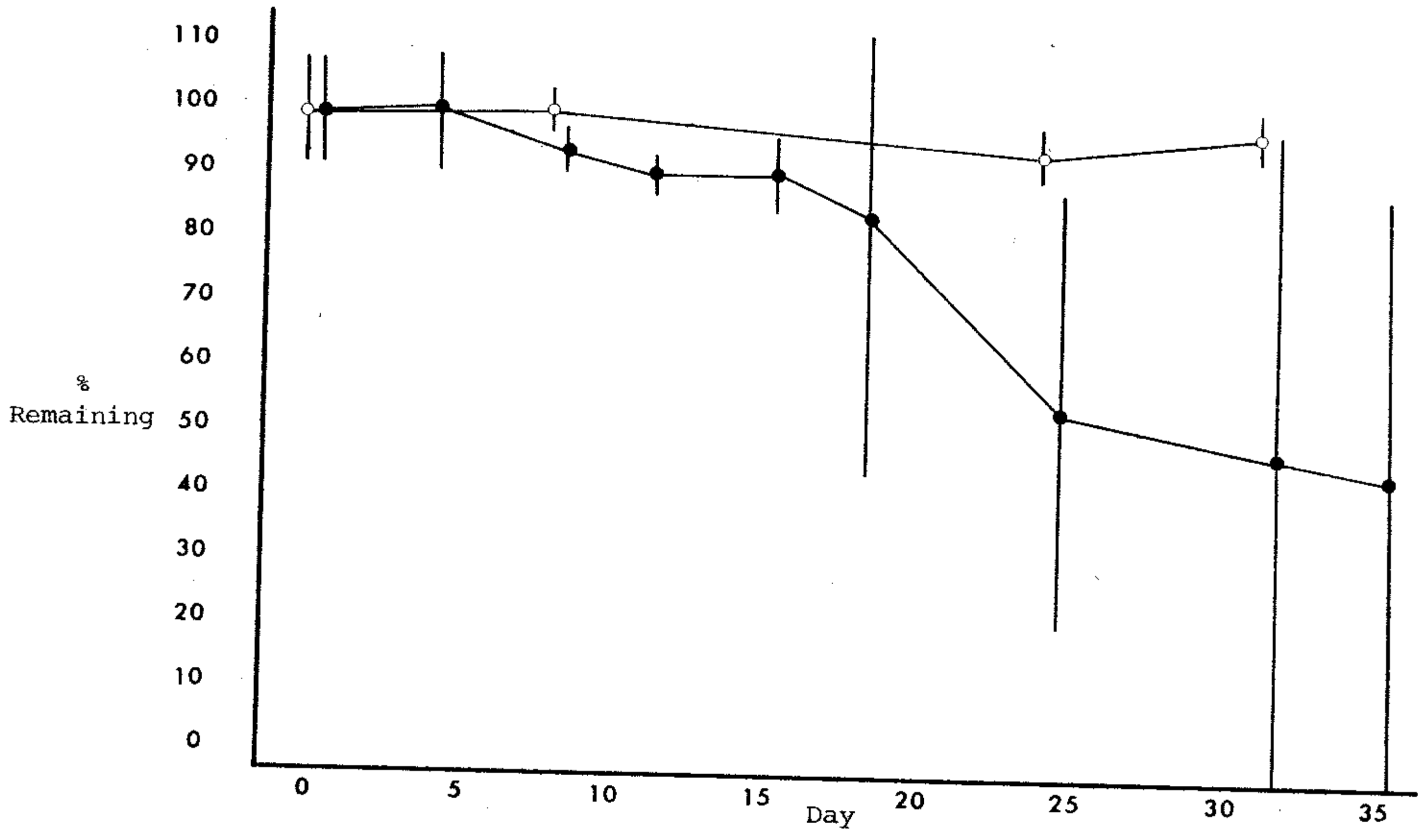
Fig. 3--Loss of 2,4-D through time in the systems containing non-sterile Red River water only in the first Red River experiment.



ϕ = 2,4-D Concentration in Sterile Controls.
♦ = 2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

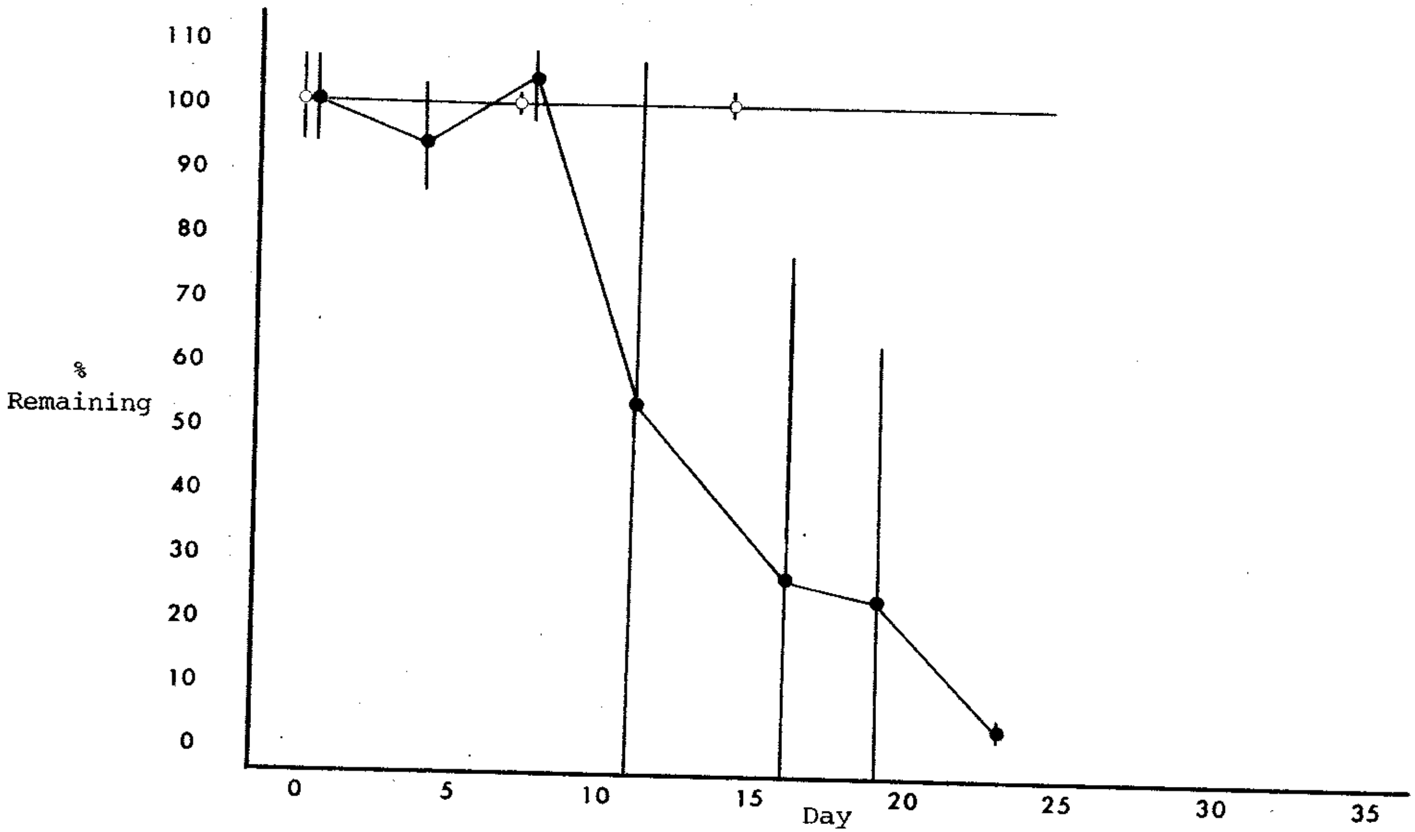
Fig. 4--Loss of 2,4-D through time in the systems containing non-sterile Red River water only in the third Red River experiment.



ϕ=2,4-D Concentration in Sterile Controls.
●=2,4-D Concentration in Non-Sterile Tests.

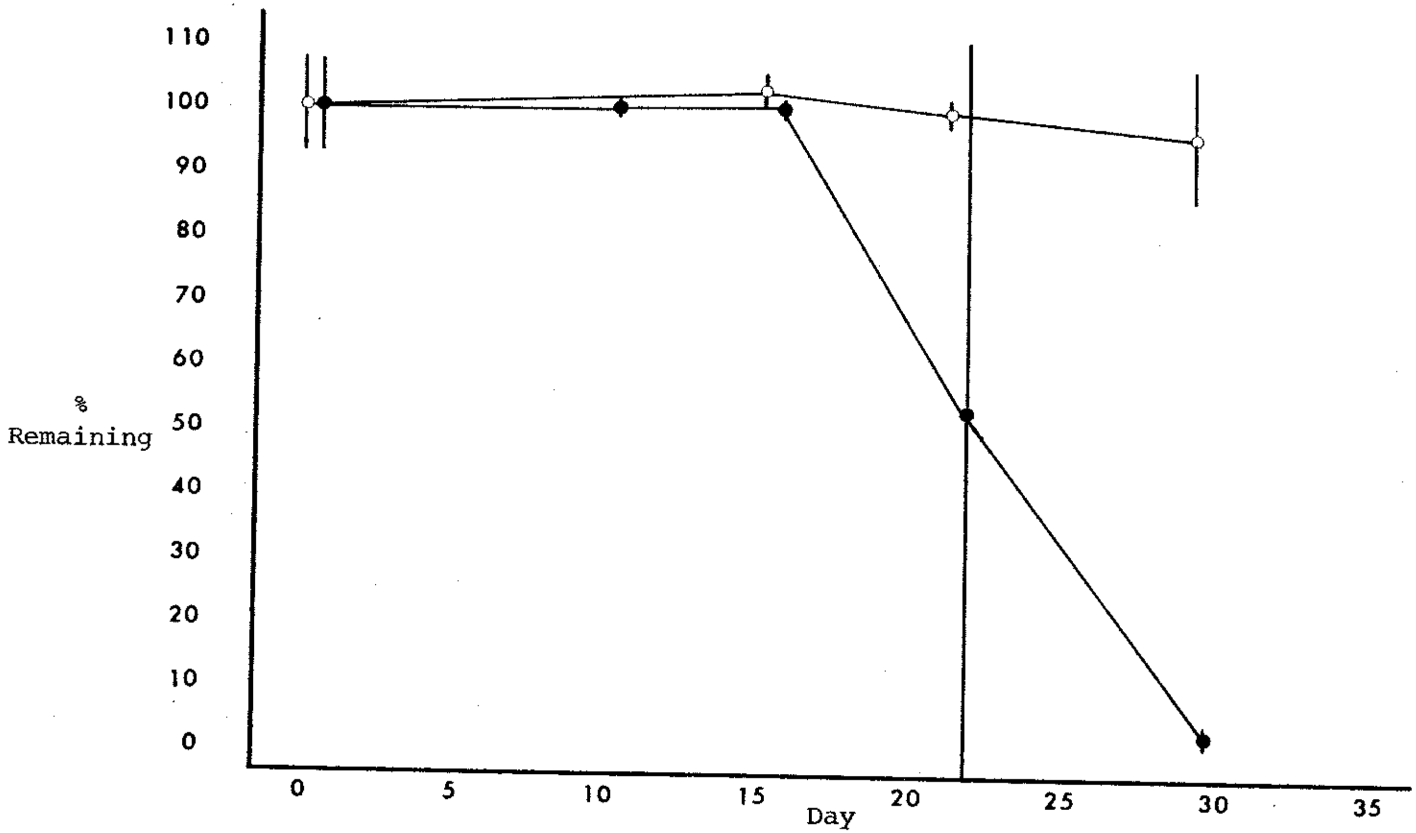
Points depict Mean ± One Standard Deviation.

Fig. 5--Loss of 2,4-D through time in the systems containing non-sterile Red River water only in the fourth Red River experiment.



ϕ = 2,4-D Concentration in Sterile Controls.
● = 2,4-D Concentration in Non-Sterile Tests.
Points depict Mean ± One Standard Deviation.

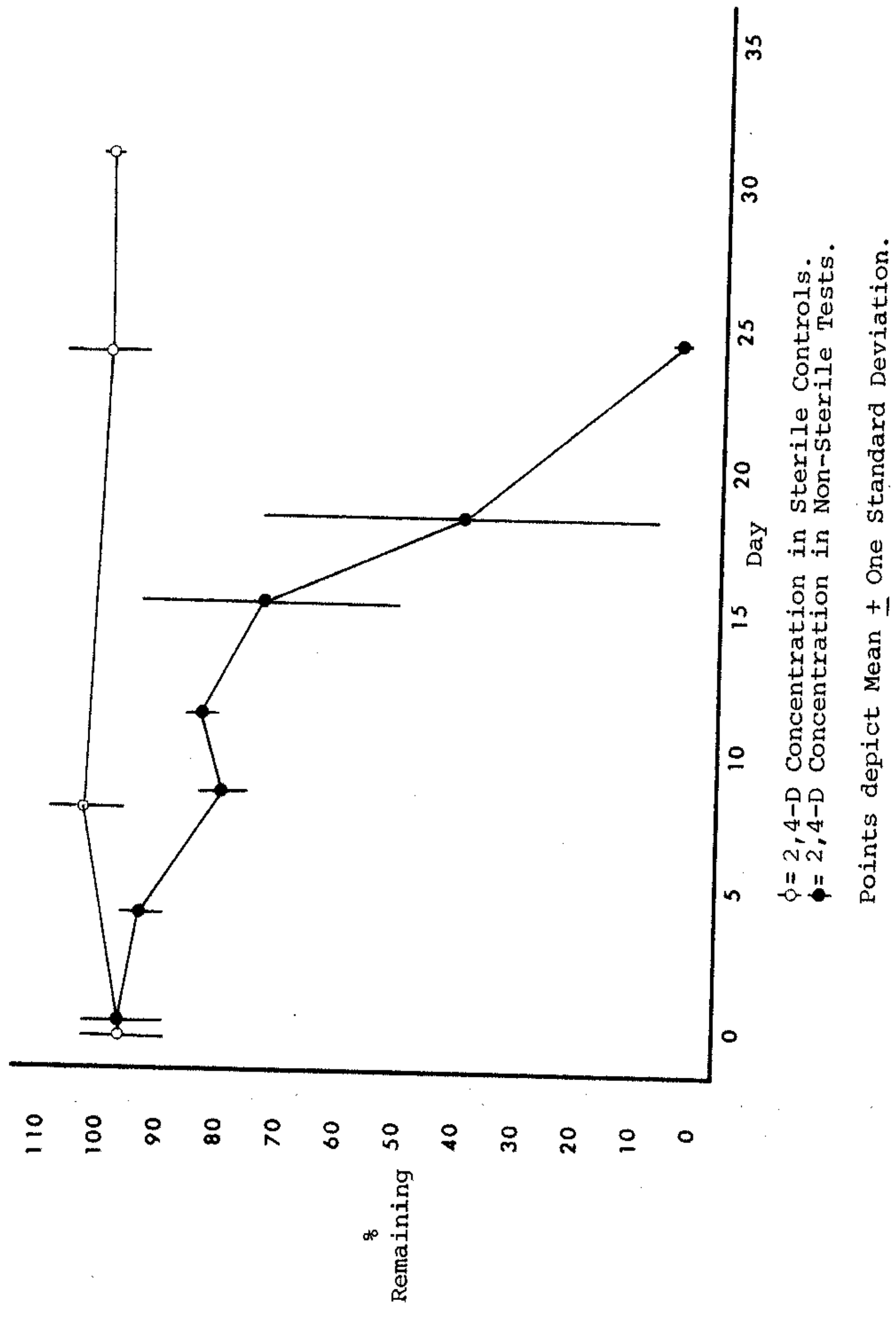
Fig. 6--Loss of 2,4-D through time in the systems containing non-sterile Red River water and non-sterile solids in the first Red River experiment.



ϕ=2,4-D Concentration in Sterile Controls.
⧫=2,4-D Concentration in Non-Sterile Tests.

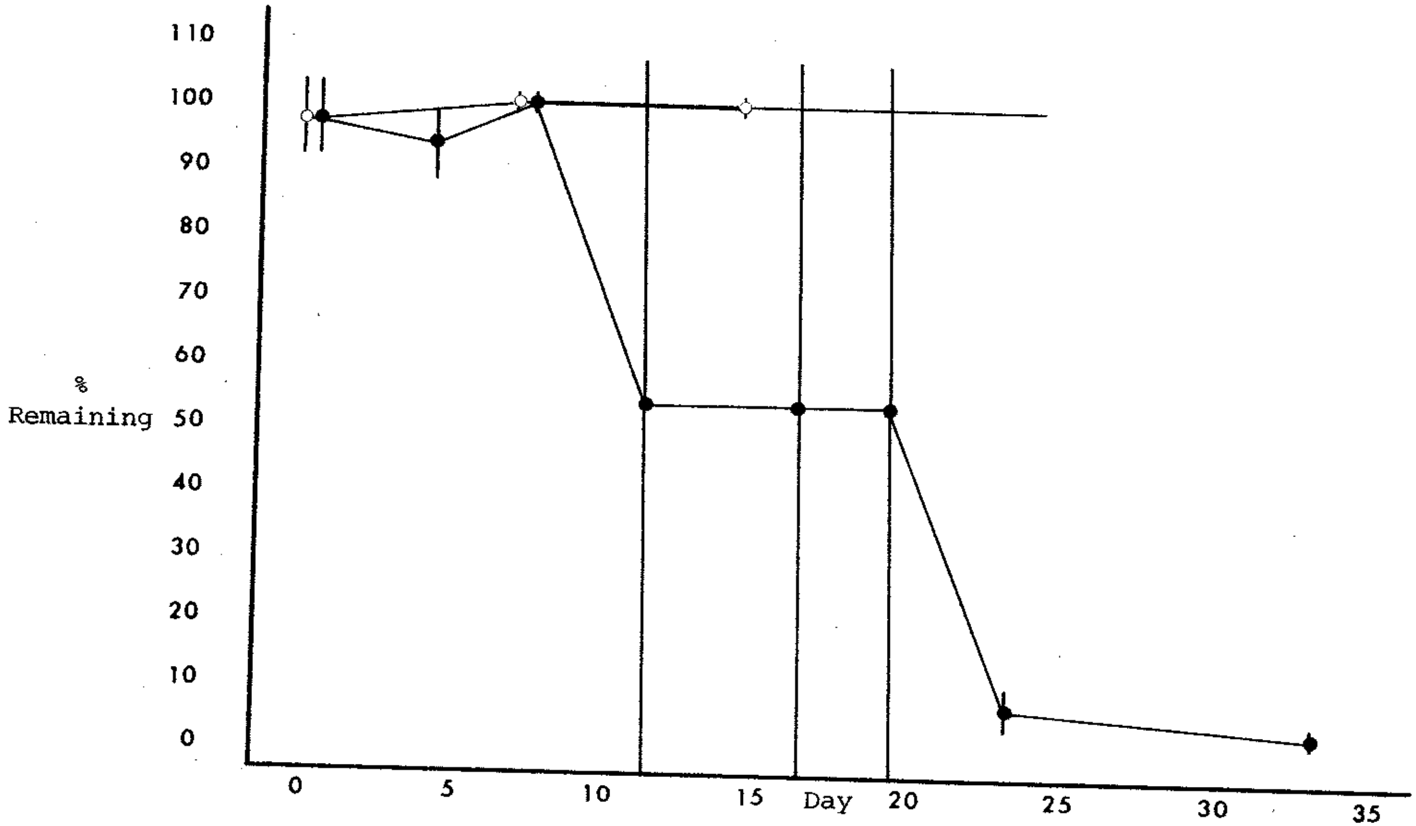
Points depict Mean ± One Standard Deviation.

Fig. 7--Loss of 2,4-D through time in the systems containing non-sterile Red River water and non-sterile solids in the third Red River River experiment.



% Remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Day
= 2,4-D Concentration in Sterile Controls.
= 2,4-D Concentration in Non-Sterile Tests.
Points depict Mean ± One Standard Deviation.

Fig. 8--Loss of 2,4-D through time in the systems containing non-sterile Red River water and non-sterile solids in the fourth Red River experiment.



ϕ=2,4-D Concentration in Sterile Controls.
●=2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

TABLE IX

SIGNIFICANCE OF THE LINES OF BEST FIT FOR BIOTRANSFORMATION STUDIES OF THE RED RIVER

Experiment	Water	Sediment	Slope Significantly Different From Zero	P
1	Sterile	None Added	No	0.0513
1	Sterile	Sterile	No	0.5031
1	Non-Sterile	None Added	Highly	0.0068
1	Non-Sterile	Non-Sterile	Highly	0.0001
2	Non-Sterile	None Added	Highly	0.001
2	Non-Sterile	Non-Sterile	Highly	0.001
3	Sterile	None	Yes	0.016
3	Sterile	Sterile	No	0.3607
3	Non-Sterile	None Added	Highly	0.0001
3	Non-Sterile	Non-Sterile	Highly	0.0001
4	Sterile	None Added	No	0.9836
4	Sterile	Sterile	No	0.1029
4	Non-Sterile	None	Highly	0.0001
4	Non-Sterile	Non-Sterile	Highly	0.0001

microbes in these four replicate flask averaged 20,000 CFU/ml, which is the lowest estimate of microbes in any of the non-acclimated test systems. However, no correlation between the microbial population and the rate of biotransformation of the herbicide was observed. A R-square of 0.12 was found when the microbial plate counts were correlated with the first-order biotransformation half-lives of the compound in the Red River test flask that contained non-sterile sediments and/or non-sterile water (T or T500).

Analysis of covariance showed that in all cases but one (RRAT), the biotransformation rate of 2,4-D in the flasks that contained non-sterile solids and/or non-sterile Red River water (T or T500) are either significantly different (P=0.05) or highly significantly different (P=0.01) from the rate of disappearance of the compound in the sterile control flasks (C or C500) (Table X). Statistical analysis also showed that there are no significant differences in the disappearance of the compound in the flasks containing sterile Red River water only (C) and the disappearance in the flask containing both sterile Red River water and sterile Red River solids (C500). These findings indicate that the disappearance of the herbicide in the non-sterile flasks (T and T500) is due to biological rather physical or chemical means such as volatilization, photolysis, and

TABLE X

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF STERILE CONTROL SYSTEMS AND NON-STERILE TEST SYSTEMS

Experiment	First Conditions		Second Conditions		Direction of Significance*	P
	Water	Solids	Water	Solids		
1	Sterile	None Added	Non-Sterile	None Added	=	0.9246
1	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0001
3	Sterile	None Added	Non-Sterile	None Added	<	0.0005
3	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0001
4	Sterile	None Added	Non-Sterile	None Added	<	0.003
4	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0297

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

hydrolysis.

The addition of solids (T500) to the Red River water caused varied effects on the first-order biotransformation rate of 2,4-D. In three out of four Red River, experiments the addition of solids increased the biotransformation rate of the herbicide (Table XI). Two of these three increased rates are significantly (P=0.05) different from the biotransformation rate in the experiments containing Red River water only (T). Out of these two significantly greater biotransformation rates, one is an abnormality. This abnormality results from comparing the biotrans-formation rate in the flasks containing water and solids (RRAT500) with the flasks containing water only (RRAT). 2,4-D in the flasks labeled RRAT was found to have a half-life of over 300 days. Therefore, there is only one set of flasks those containing water and solids (T500) that has a truly significantly greater biotransformation rate than flasks that contain only Red River water (T). In one experiment, the biotransformation rate in the flasks containing Red River water only (T) is significantly greater (P=0.05) than the biotransformation rate in the flasks containing water and solids (T500) from the Red River (Table XI). From these four experiments, it does not appear that the addition of solids consistently affects the

TABLE XI

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN RED RIVER SYSTEMS CONTAINING NON-STERILE WATER WITH AND WITHOUT SOLIDS

Exper-iment	First Conditions		Second Conditions		Direction of Significance*	P
	Water	Solids	Water	Solids		
1	Non-Sterile	None Added	Non-Sterile	Non-Sterile	<	0.0001
2	Non-Sterile	None Added	Non-Sterile	Non-Sterile	=	0.2777
3	Non-Sterile	None Added	Non-Sterile	Non-Sterile	<	0.0034
4	Non-Sterile	None Added	Non-Sterile	Non-Sterile	>	0.0456

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

biotransformation of 2,4-D in the Red River experiments. If the data found using the test RRAT are omitted from consideration because of the low biotransformation rate, then the mean plus and minus one standard deviation first-order half-life for the flasks containing Red River water only (T) is 10.29 days $\pm$4.9 days. The mean plus and minus one standard deviation for the systems containing Red River water plus 500 mg/l additional solids (T500) is 7.7 days $\pm$1.4 days. A non-parametric Man-Whittney U test showed that at the P=0.05 level there is no significant difference in these biotransformation rates.

The results of the biotransformation test using Red River water and sterile Red River solids (SS) yielded a mean first-order half-life of 6.3 days. In all of the Red River studies, the test systems with additional sterile solids (SS) has as high or higher biotransformation rates as the test systems containing non-sterile water only (T) or the system containing non-sterile water and non-sterile solids (T500) (Table XII). Analysis of covariance showed that the test systems with sterile solids (SS) produced significantly higher rates of biotransformation than the other test systems (Table XIII). In one of the four comparisons, the biotransformation rate of the systems containing sterile solids and non-sterile Red River water

TABLE XII

BIOTRANSFORMATION RATES OF 2,4-D IN SYSTEMS CONTAINING NON-STERILE WATER AND STERILE SOLIDS, AND IN SYSTEMS CONTAINING STERILE WATER AND NON-STERILE SOLIDS IN THE RED RIVER EXPERIMENTS

				Rate Coefficients						
Experiment	Water	Solids	Biomass (cfu/ml)	Zero-Order*	r	First-Order**	r	Second-Order***	r	First-Order Half-Life (days)
1	Sterile	Non-Sterile	160000	0.38	0.655	0.004	0.663	0	0.67	173.3
3	Non-Sterile	Sterile	49000	2.61	0.699	0.08	0.712	$2x10^{-6}$	0.703	8.28
4	Non-Sterile	Sterile	64750	4.60	0.752	0.16	0.775	$2x10^{-6}$	0.783	4.35

*Unit expressed as % day^{-1}.

**Unit expressed as day^{-1}.

***Unit expressed as %$(cfu/ml)^{-1}day^{-1}$.

TABLE XIII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN RED RIVER SYSTEMS CONTAINING NON-STERILE SOLIDS AND/OR NON-STERILE WATER WITH THE SYSTEMS CONTAINING EITHER STERILE WATER OR STERILE SOLIDS

Experiment	First Conditions		Second Conditions		Direction of Significance*	P
	Water	Solids	Water	Solids		
1	Sterile	Non-Sterile	Non-Sterile	Non-Sterile	<	0.0001
3	Non-Sterile	Sterile	Non-Sterile	None Added	>	0.0024
3	Non-Sterile	Sterile	Non-Sterile	Non-Sterile	=	0.7502
4	Non-Sterile	Sterile	Non-Sterile	None Added	>	0.0267
4	Non-Sterile	Sterile	Non-Sterile	Non-Sterile	>	0.0456

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

(SS) is highly significantly different (P=0.01), then the rate of biotransformation in the flask containing Red River water only (T). In one experiment, the rate of biotransformation in the systems containing non-sterile Red River water and sterile solids (SS) is significantly (P=0.05) different from the systems containing non-sterile Red River water only (T). The biotransformation rate of 2,4-D in the systems containing non-sterile Red River water and sterile solids (SS) is in one experiment significantly different (P=0.05) and in another experiment not significantly different from the rate of biotransformation in systems containing non-sterile Red River water and non-sterile Red River solids (T500) (Table XIII).

As can be seen from Table XII the biotransformation rate of 2,4-D in the system containing sterile Red River water and non-sterile solids (NSS) is quite low. The first-order half-life for this experiment is over 170 days. The biotransformation of 2,4-D in these systems (NSS) is highly significantly different (P=0.01) from the biotransformation of the herbicide in the systems containing non-sterile Red River water and solids (T500 or SS). The biotransformation rate in these systems with only microbes from the solids most closly resembles the disappearance of the compound in the sterile controls (C and C500). Whereas the biotransformation rates of most of the experimental systems that contain non-sterile water with or without solids (even sterile solids) are one to three orders of magnitude greater than the rate of the sterile controls, the biotransformation rate of the test system with sterile water and non-sterile solids (NSS) only vary from the sterile controls by a factor of 1 to 3. The data just presented indicate that the water column-associated microbes are principally responsible for biotransformation of 2,4-D in the Red River experiments.

It is very interesting to note the high count of microbes in the systems that initially contained sterile water and non-sterile solids (NSS). The estimated microbial number in these systems is an order of magnitude higher then the counts estimated in most of the other test systems. Even with these unusually high microbial counts, a very low rate of biotransformation is seen. Since these flasks start with only the microbes associated with the solids, the initial counts are lower then the systems containing non-sterile water. Steady-state microbial estimates were seen in the flask that contained non-sterile solids and sterile water (NSS) on the same day as steady-state in the systems with non-sterile water. This would indicate that more microbial growth and activity is taking place in the systems containing non-sterile solids and sterile water.

The types of microbes present in the various systems were not identified during this study, but there did not appear to be differences in the types of colonies growing on the plate count agar in any of the systems. Additional research with a focus on the identification of the microbes actively transforming the compound is needed. This additional research may help explain the role of water column and sediment associated microbes in the transformation of 2,4-D. The quantification of active transformers may also help to explain the variation in the observed biotransformation rates of the herbicide.

Biotransformation of 2,4-D in the Trinity River System

The results of the biotransformation studies of the disappearance of 2,4-D in the Trinity River systems can be found in Table XIV. Graphical depictions of the disappearance of the herbicide in the various Trinity River experiments can be found in Figures 9-12. These figures plot the mean percent remaining of the compound versus time. To indicate the precision of this data, one standard deviation on either side of each mean is shown. Also included on these figures is the percent remaining of 2,4-D

TABLE XIV

RESULTS OF BIOTRANSFORMATION STUDIES OF 2,4-D IN THE TRINITY RIVER

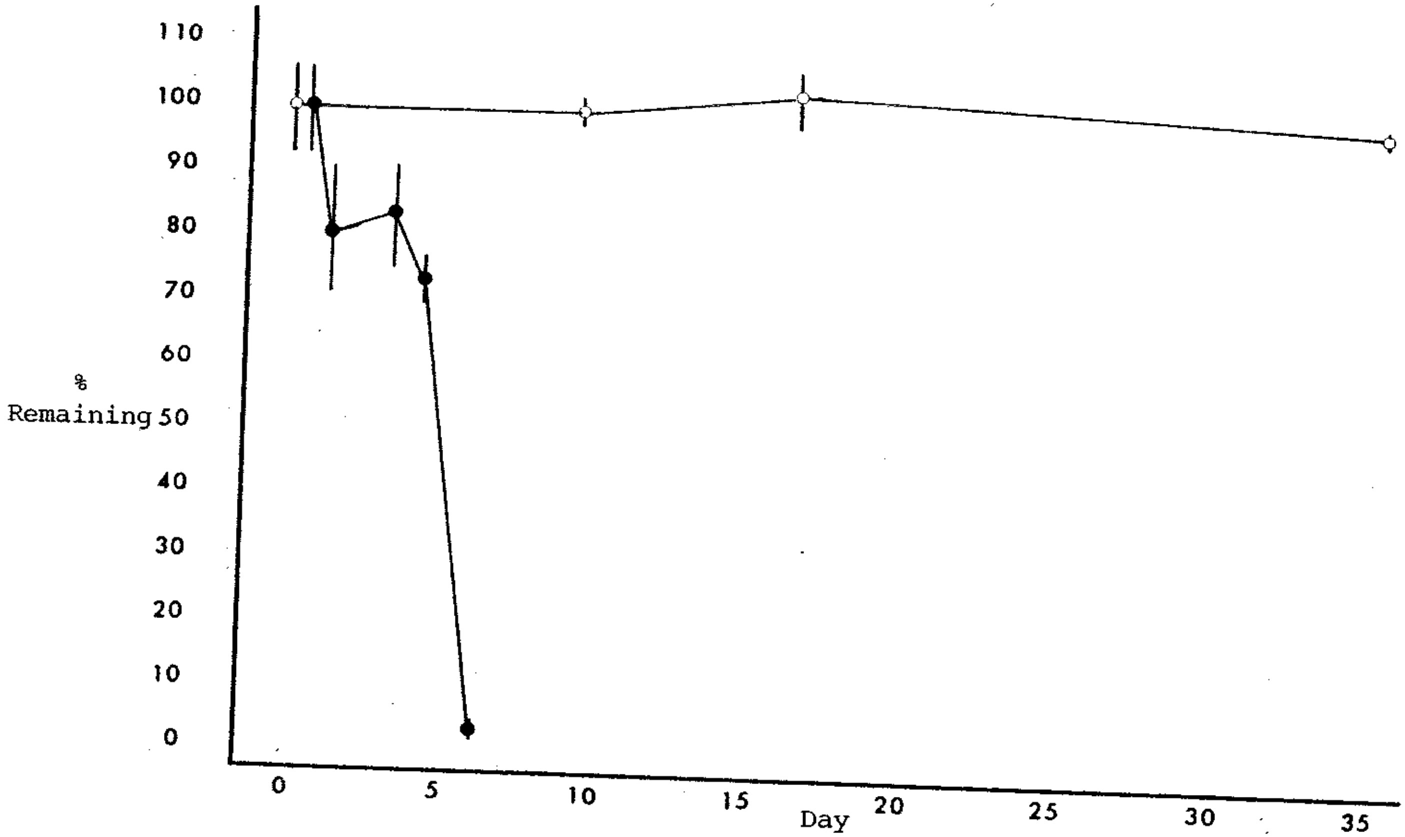
Exper-iment	Water	Solids	Biomass (cfu/ml)	Rate Coefficients: Zero-Order*	r	First-Oder**	r	Second-Order***	r	First-Order Half-Life (days)
1	Sterile	None Added	0	0.14	0.26	0.0014	0.273	0	0.221	495
1	Sterile	Sterile	0	0.10	0.185	0.0011	0.033	0	0.221	613.3
1	Non-Sterile	None Added	245000	14.06	0.905	0.49	0.93	$2x10^{-6}$	0.937	1.42
1	Non-Sterile	Non-Sterile	98000	14.64	0.931	0.48	0.938	$5x10^{-6}$	0.939	1.44
2	Non-Sterile	None Added	100500	13.31	0.872	0.45	0.802	$4x10^{-6}$	0.763	1.55
2	Non-Sterile	Non-Sterile	91250	13.99	0.914	0.47	0.815	$5x10^{-6}$	0.74	1.48

*Unit expressed as % day^{-1}.

**Unit expressed as day^{-1}.

***Unit expressed as $\%(cfu/ml)^{-1}day^{-1}$.

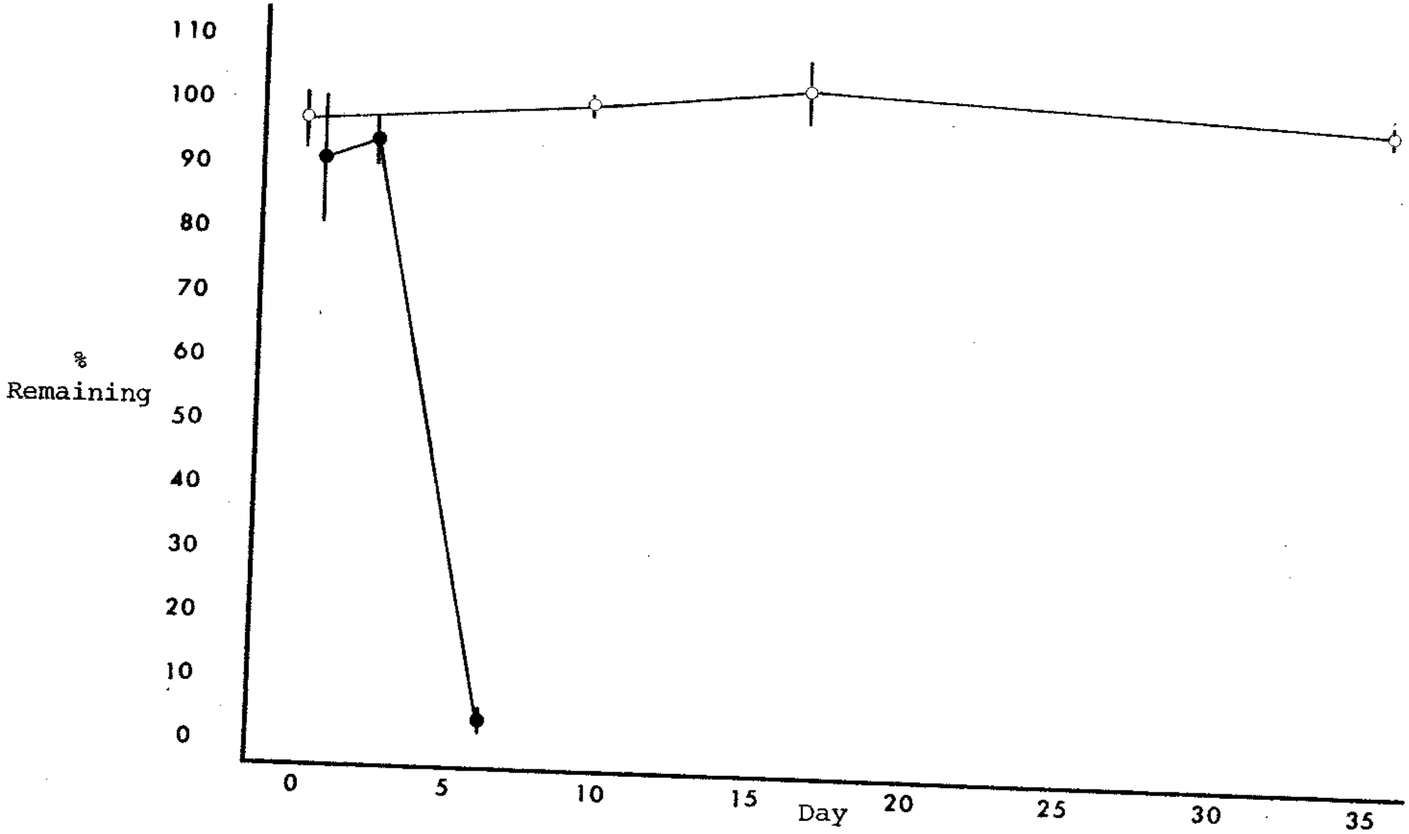
Fig. 9--Loss of 2,4-D through time in the systems containing non-sterile Trinity River water only in the first Trinity River experiment.



ϕ = 2,4-D Concentration in Sterile Controls.
♦ = 2,4-D Concentration in Non-Sterile Tests.

Points depict Mean $\pm$ One Standard Deviation.

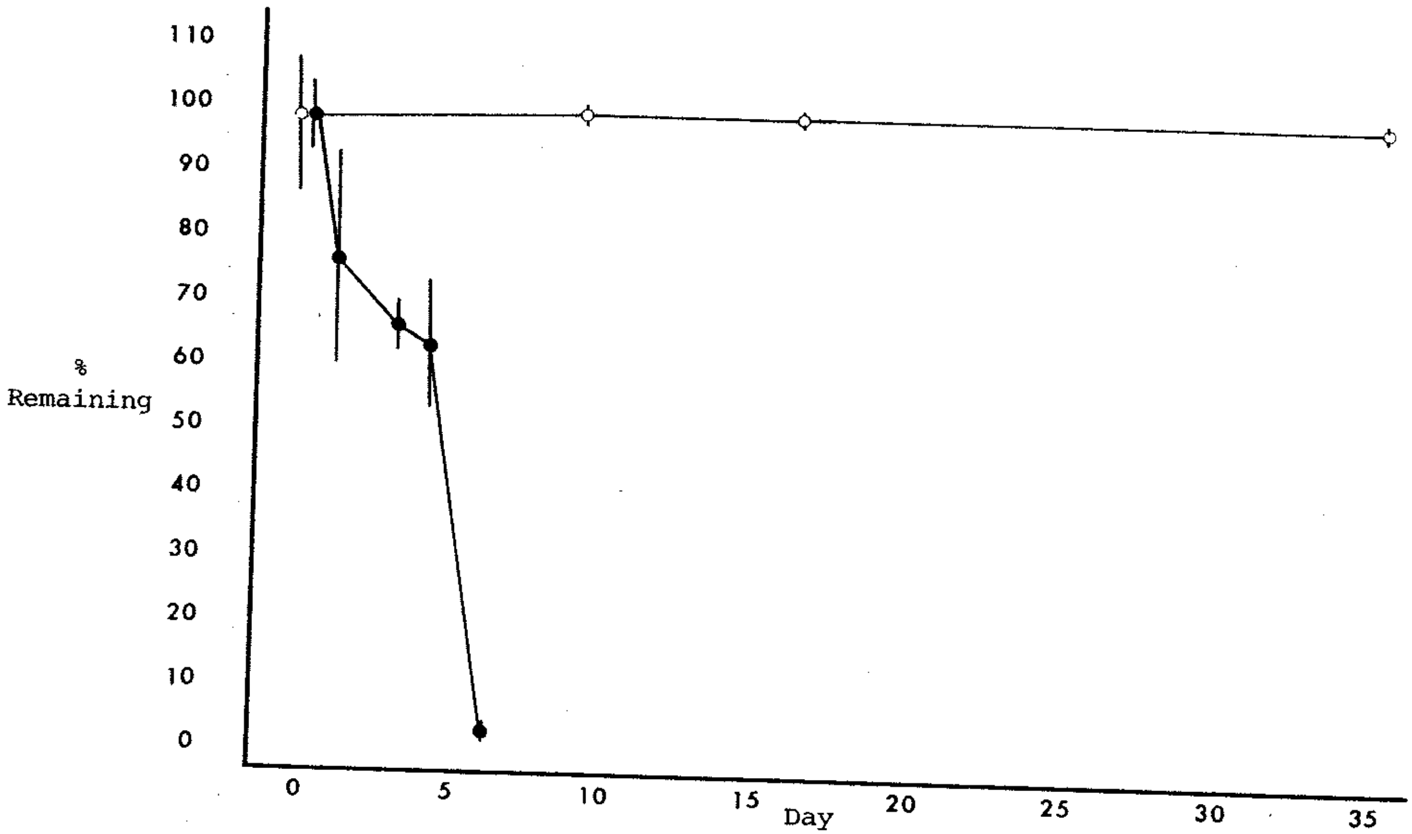
Fig. 10--Loss of 2,4-D through time in the systems containing non-sterile Trinity River water only in the second Trinity River experiment.



ϕ= 2,4-D Concentration in Sterile Controls.
●= 2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

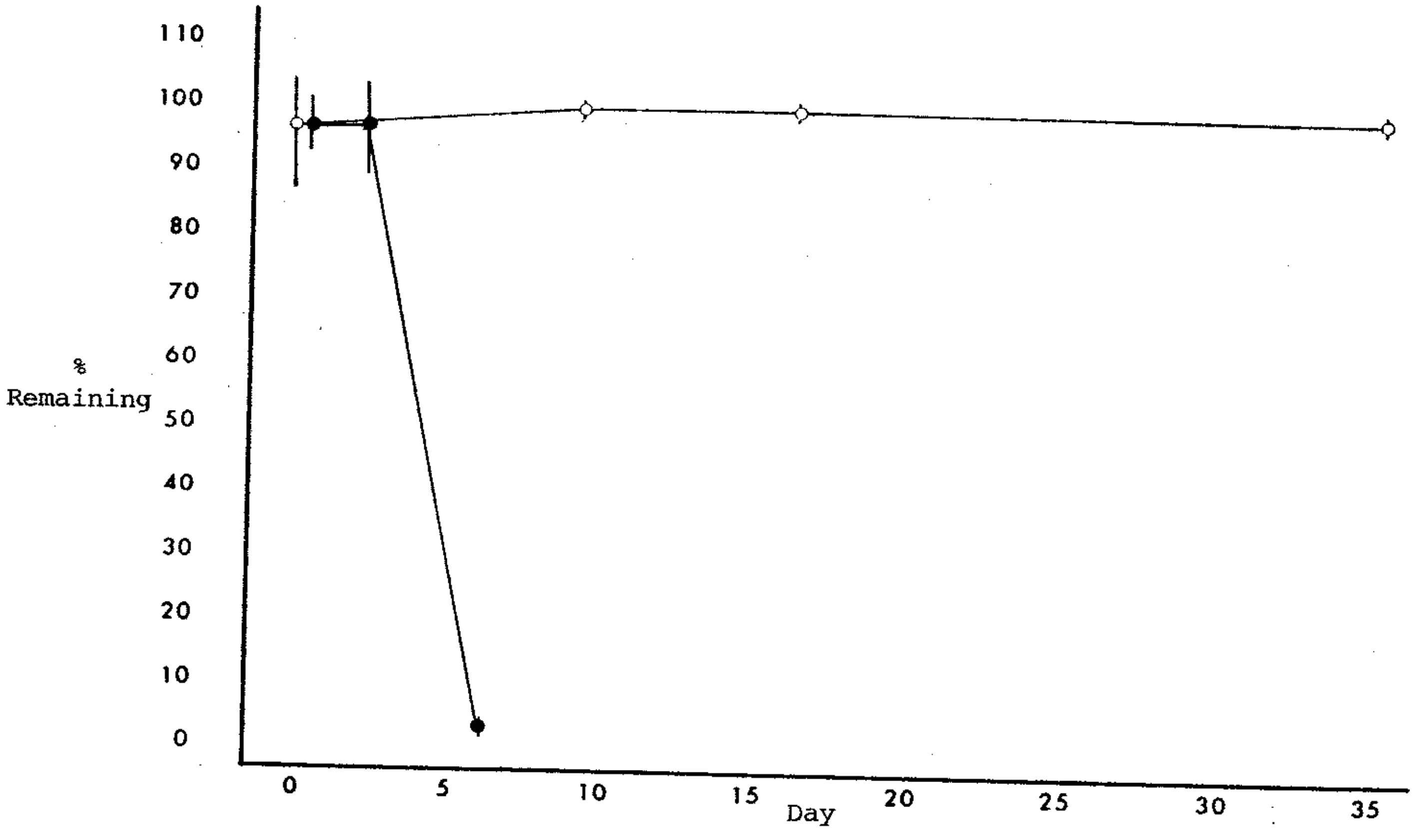
Fig. 11--Loss of 2,4-D through time in the systems containing non-sterile Trinity River water and non-sterile solids in the first Trinity River experiment.



ϕ= 2,4-D Concentration in Sterile Controls.
♦= 2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

Fig. 12--Loss of 2,4-D through time in the systems containing non-sterile Trinity River water and non-sterile solids in the second Trinity River experiment.



◊=2,4-D Concentration in Sterile Controls.
♦=2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

in the sterile controls (C or C500). The percent remaining in the controls is shown for visual comparisons of the amount of the compound lost by other than biological means to the amount lost by biotransformation.

The slope of the lines of best fit for the percent of the compound remaining in the sterile controls (C or C500) is not significantly different from zero. The lines of best fit of the percent of the herbicide remaining in the systems containing non-sterile solid and/or non-sterile water (T or T500) are all shown to be highly significantly different from zero (Table XV).

The first-order biotransformation rate coefficient for disappearance of 2,4-D in the Trinity River systems containing non-sterile water only (T) or non-sterile water and non-sterile solids (T500) ranged from 0.45 day^{-1} to 0.49 day^{-1}. These rate coefficients yield a calculated half-life of 1.55 to 1.42 days, respectively. The experimental rate coefficients for the systems with and without non-sterile solids are very similar.

Analysis of covariance indicated in all cases the biotransformation rate coefficients of the control (sterile) systems are highly significantly different (P=0.01) from the experimental flask with non-sterile solids and/or non-sterile water (T or T500) (Table XVI). Analysis of

TABLE XV

SIGNIFICANCE OF LINES OF BEST FIT FOR BIOTRANSFORMATION STUDIES OF THE TRINITY RIVER

Exper-iment	Water	Sediment	Slope Significantly Different From Zero	P
1	Sterile	None Added	No	0.1879
1	Sterile	Sterile	No	0.2191
1	Non-Sterile	None Added	Highly	0.0001
1	Non-Sterile	Non-Sterile	Highly	0.0001
2	Non-Sterile	None Added	Highly	0.0001
2	Non-Sterile	Non-Sterile	Highly	0.0001

TABLE XVI

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF STERILE CONTROL SYSTEMS AND NON-STERILE TEST SYSTEMS IN THE TRINITY RIVER

Exper-iment	First Conditions		Second Conditions		Direction of Significance*	P
	Water	Solids	Water	Solids		
1	Sterile	None Added	Non-Sterile	None Added	<	0.0001
1	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0001
2	Sterile	None Added	Non-Sterile	None Added	<	0.0001
2	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0001

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

covariance also showed that there is no difference between the biotransformation rate coefficient of the flasks that contain non-sterile Trinity River water only (T) and the flasks that contain non-sterile Trinity River water and additional non-sterile solids (T500) (Table XVII). The mean first-order biotransformation rate coefficient for the systems that contained non-sterile Trinity River water (T) is 0.48 day^{-1}, with a half-life of 1.45 days. The mean first-order biotransformation rate coefficient for the systems that contain non-sterile Trinity River water and non-sterile additional solids (T500) is 0.47 day^{-1} with a corresponding half-life of 1.47 days. These data indicate that the addition of 500 mg/l of non-sterile Trinity River solids does not effect the biotransformation rate of 2,4-D in Trinity River waters.

The results of the Trinity River biotransformation test involving non-sterile river water with sterile solids added (SS) and sterile water with non-sterile solids added (NSS) can be found in Table XVIII. Figures 13 and 14 depict the percent of 2,4-D remaining versus time for these systems. These figures also include the lines of best fit for the percent of 2,4-D remaining in the pertinent sterile control (C500) and for the flasks with non-sterile Trinity River water and non-sterile solids (T500). As can be seen from

TABLE XVII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN TRINITY RIVER SYSTEMS CONTAINING NON-STERILE WATER WITH AND WITHOUT SOLIDS

Exper-iment	First Conditions Water	First Conditions Solids	Second Conditions Water	Second Conditions Solids	Direction of Significance*	P
1	Non-Sterile	None Added	Non-Sterile	Non-Sterile	=	0.8154
2	Non-Sterile	None Added	Non-Sterile	Non-Sterile	=	0.8635

*A = sign indicates that there is no difference in the two biotransformation rates.

TABLE XVIII

BIOTRANSFORMATION RATES OF 2,4-D IN SYSTEMS CONTAINING NON-STERILE WATER AND STERILE SOLIDS, AND IN SYSTEMS CONTAINING STERILE WATER AND NON-STERILE SOLIDS OF THE TRINITY RIVER EXPERIMENTS

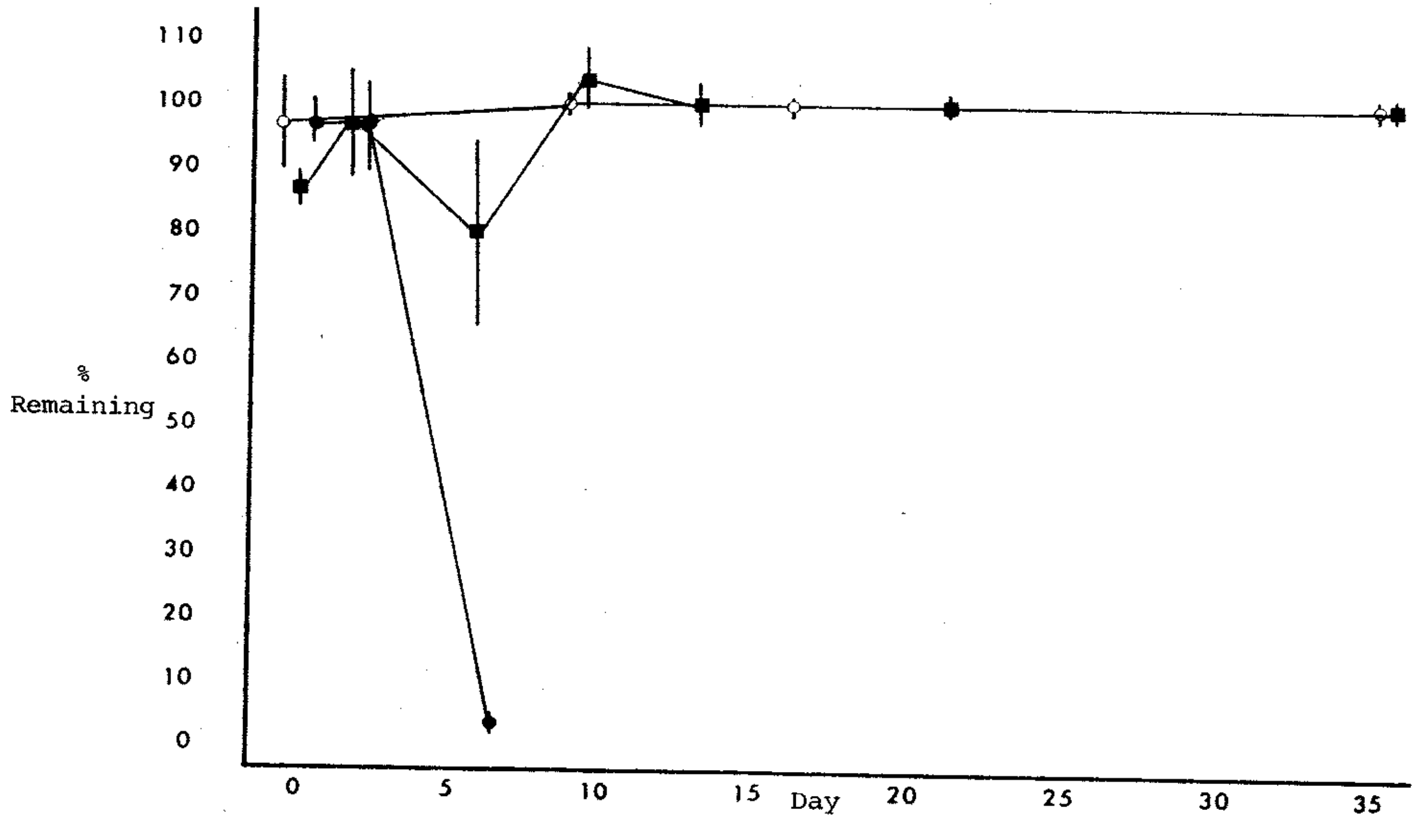
				Rate Coefficients						
Experiment	Water	Solids	Biomass (cfu/ml)	Zero-Order*	r	First-Order**	r	Second-Order***	r	First-Order Half-Life (days)
1	Sterile	Non-Sterile	502500	-0.38	0.449	-0.004	0.414	$-1x10^{-7}$	0.37	-169
1	Non-Sterile	Sterile	84750	16.0	0.977	0.53	0.963	$6x10^{-6}$	0.952	1.3

*Unit expressed as % day^{-1}.

**Unit expressed as day^{-1}.

***Unit expressed as %$(cfu/ml)^{-1}day^{-1}$.

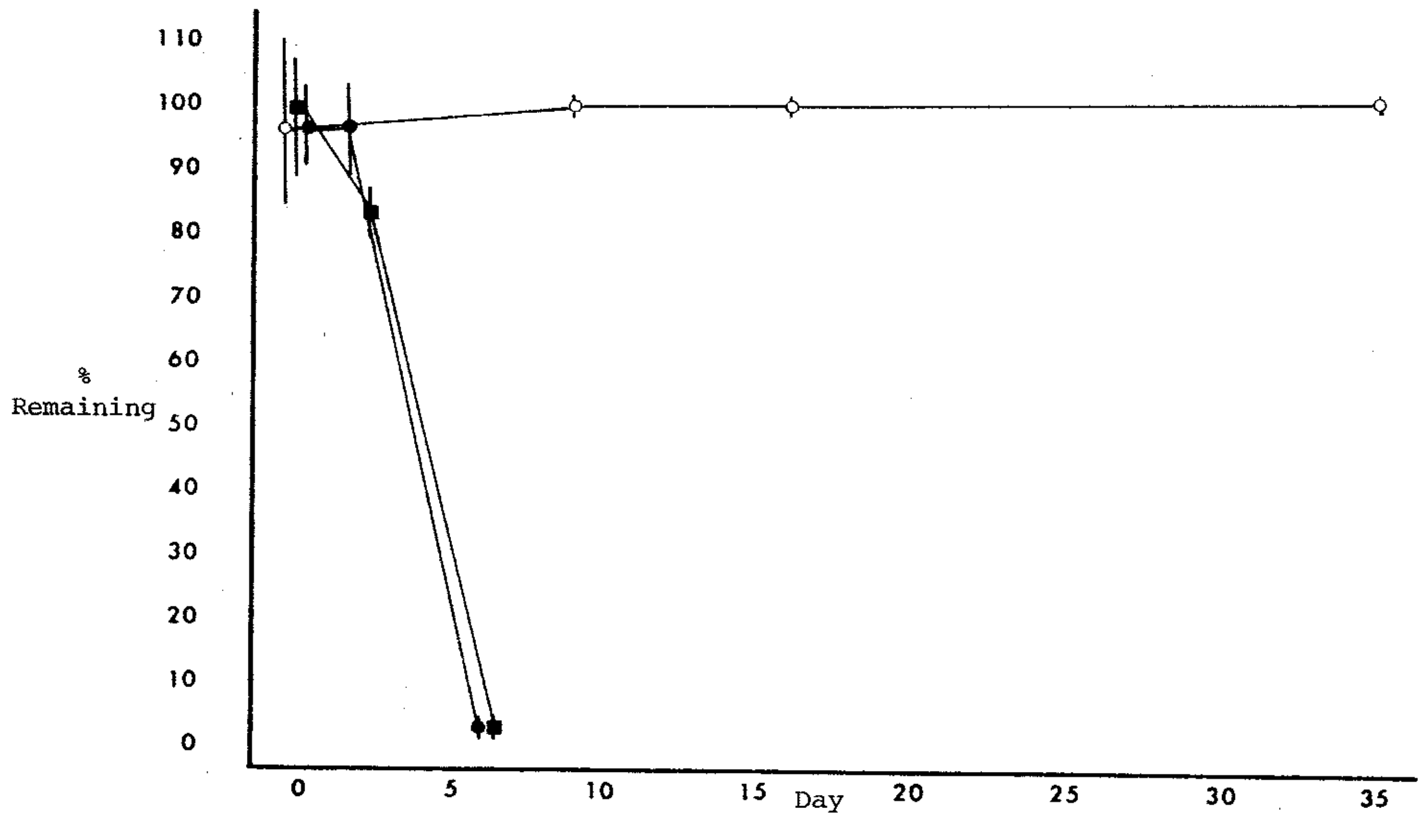
Fig. 13--Loss of 2,4-D through time in the Trinity River systems which initially contained non-sterile water and sterile solids.



ϕ=2,4-D Concentration in Sterile Controls.
⧫=2,4-D Concentration in Non-Sterile Tests.
■=2,4-D Concentration in Flasks Initially Containing Sterile Water and Non-Sterile Solids.

Points depict Mean ± One Standard Deviation.

Fig. 14--Loss of 2,4-D through time in the Trinity River systems which initially contained sterile water and non-sterile solids.



○=2,4-D Concentration in Sterile Controls.
●=2,4-D Concentration in Non-Sterile Tests.
■=2,4-D Concentration in Flasks Initially Containing Non-Sterile Water and Sterile Solids.

Points depict Mean ± One Standard Deviation.

Table XVIII and Figure 13, the biotransformation rate of the 2,4-D in the flasks containing non-sterile Trinity River water and sterile solids (SS) is greater than the biotransformation rate in the systems that contain Trinity River water only (T). The biotransformation is also faster in the systems containing non-sterile water and sterile solids (SS) than in the systems containing non-sterile water and non-sterile solids (T500). Analysis of covariance demonstrates that there is no difference in the biotransformation rate of the system containing non-sterile water and sterile solids (SS) and the systems that contain non-sterile solids and/or non-sterile Trinity River water (T and T500) (Table XIX). Tables XVIII and XIX, and Figure 14 indicate the recalcitrant nature of 2,4-D in the system that initially contained sterile Trinity River water and non-sterile solids (NSS). Biotransformation of 2,4-D in the systems containing sterile Trinity River water and non-sterile solids (NSS) is not significantly different from the disappearance of the compound in the sterile controls (C and C500). In these systems with solids as the only source of microbes, very little, if any, biotransformation is taking place. It is interesting to note, that similar to the Red River study, these systems that initially contained sterile water and non-sterile solids

TABLE XIX

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN TRINITY RIVER SYSTEMS CONTAINING NON-STERILE SOLIDS AND/OR NON-STERILE WATER WITH SYSTEMS CONTAINING EITHER STERILE WATER OR STERILE SOLIDS

Experiment	First Conditions		Second Conditions		Direction of Significance*	P
	Water	Solids	Water	Solids		
2	Non-Sterile	Sterile	Non-Sterile	None Added	=	0.2945
2	Non-Sterile	Sterile	Non-Sterile	Non-Sterile	=	0.2111
2	Non-Sterile	Sterile	Sterile	Sterile	$>$	0.0001
2	Sterile	Non-Sterile	Sterile	Sterile	=	0.6887
2	Sterile	Non-Sterile	Non-Sterile	None Added	$<$	0.0001
2	Sterile	Non-Sterile	Non-Sterile	Non-Sterile	$<$	0.0001

*A $<$ sign indicates that the biotransformation in the first condition is slower than in the second condition.

A $>$ sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

(NSS) have the highest microbial counts of any of the systems. This again indicates that the biotransformation of the compound is principally due to the microbes initially found in the water column.

Biotransformation of 2,4-D in the Mississippi River System

The results of the biotransformation studies of 2,4-D in the Mississippi River can be found in Table XX. Graphical representation of the biotransformation in these systems can be seen in Figures 15 and 16.

The systems containing sterile Mississippi water only (C) indicated some disappearance of the compound. These control systems had a first-order half-life of just under 35 days. Analysis of covariance indicated that the line of best fit for the disappearance of the compound in these systems versus time had a slope significantly different from zero (Table XXI). However the systems containing sterile Mississippi River water and sterile solids (C500) have a similar first-order half-life, just under 35 days, and the slope of the line of best fit is not significantly different from zero. The disappearance of the compound in the systems containing non-sterile water only (T) and the systems containing non-sterile water and solids (T500) is over an order of magnitude greater then the disappearance

TABLE XX

RESULTS OF BIOTRANSFORMATION STUDIES OF 2,4-D IN THE MISSISSIPPI RIVER

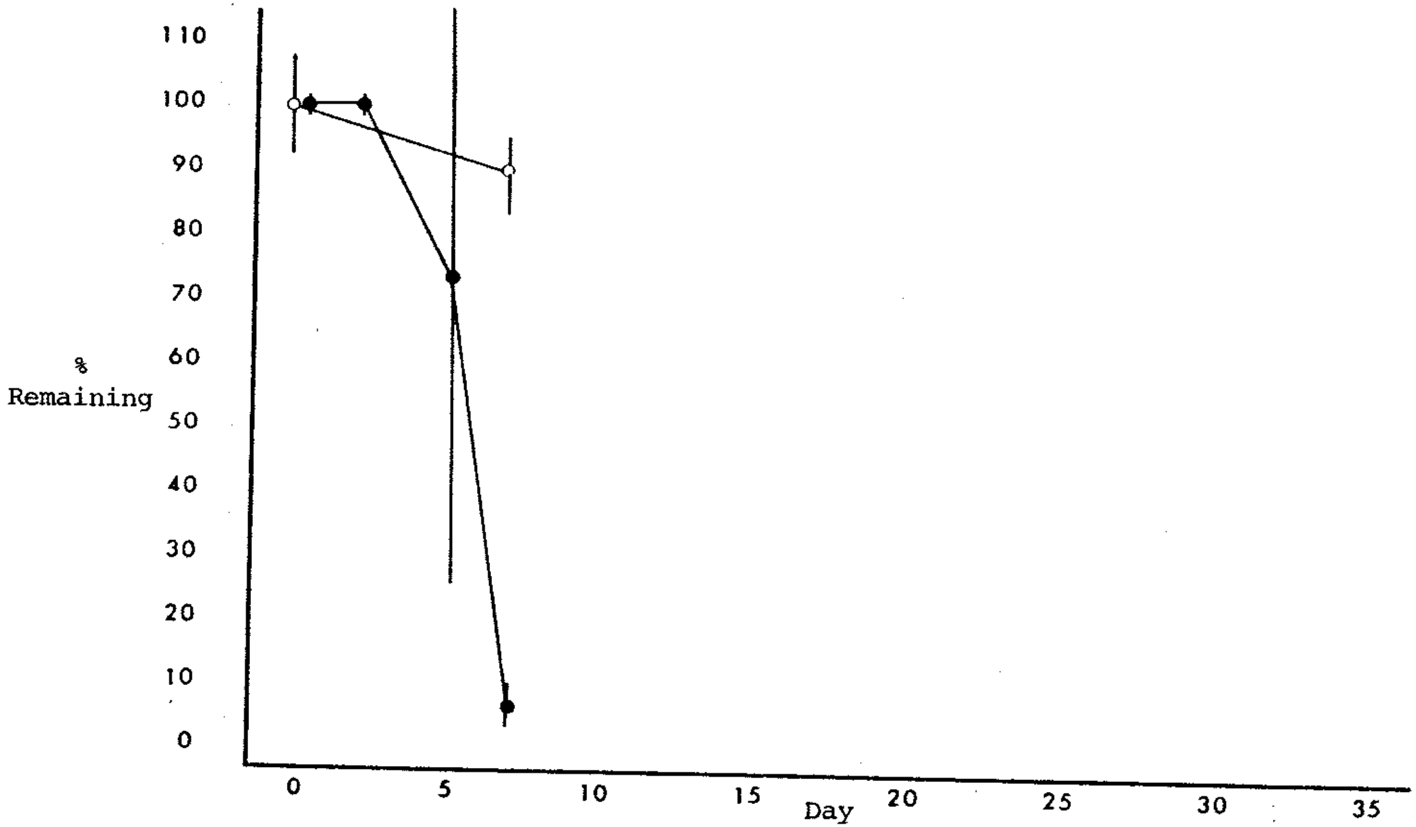
				Rate Coefficients						
Experiment	Water	Solids	Biomass (cfu/ml)	Zero-Order*	r	First-Order**	r	Second-Order***	r	First-Order Half-Life (days)
1	Sterile	None Added	0	1.5	0.748	0.02	0.748	0	0.747	34.65
1	Sterile	Sterile	0	1.86	0.586	0.02	0.595	0	0.602	34.65
1	Non-Sterile	None Added	82500	12.5	0.77	0.35	0.757	$4x10^{-6}$	0.727	1.98
1	Non-Sterile	Non-Sterile	48250	17.4	0.906	0.57	0.921	$1x10^{-6}$	0.926	1.21
1	Sterile	Non-Sterile	86750	19.2	0.909	0.51	0.909	$6x10^{-6}$	0.909	1.35
1	Non-Sterile	Sterile	35000	13.2	0.883	0.40	0.856	$1x10^{-5}$	0.829	1.73

*Unit expressed as % day^{-1}.

**Unit expressed as day^{-1}.

***Unit expressed as %$(cfu/ml)^{-1}day^{-1}$.

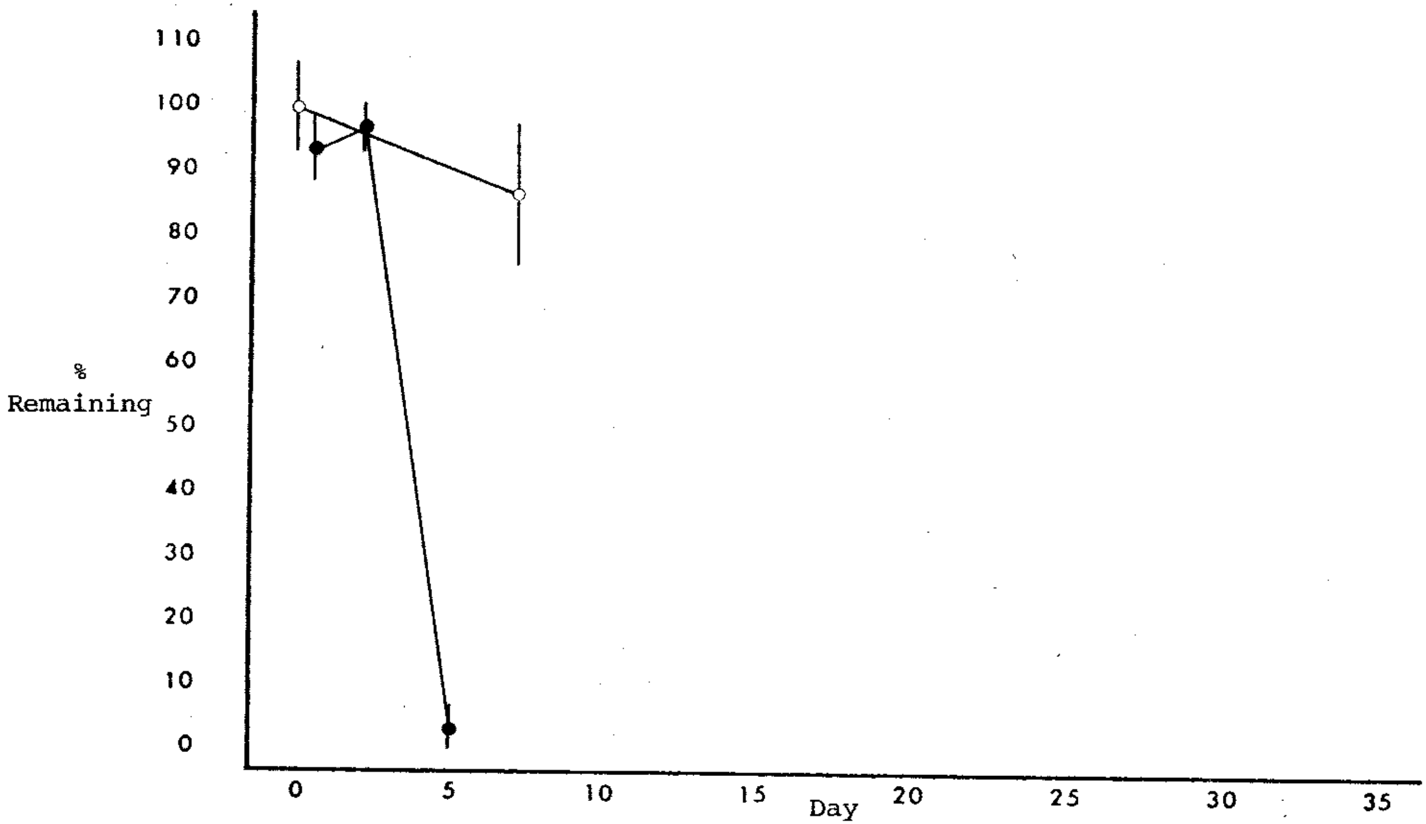
Fig. 15--Loss of 2,4-D through time in the Mississippi River systems containing non-sterile water only.



ϕ=2,4-D Concentration in Sterile Controls.
⧫=2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

Fig. 16--Loss of 2,4-D through time in the Mississippi River systems containing non-sterile water and solids.



ϕ=2,4-D Concentration in Sterile Controls.
♦=2,4-D Concentration in Non-Sterile Tests.

Points depict Mean ± One Standard Deviation.

TABLE XXI

SIGNIFICANCE OF LINES OF BEST FIT FOR BIOTRANSFORMATION STUDIES OF THE MISSISSIPPI RIVER

Experiment	Water	Sediment	Slope Significantly Different From Zero	P
1	Sterile	None Added	Yes	0.0197
1	Sterile	Sterile	No	0.0739
1	Non-Sterile	None Added	Highly	0.0003
1	Non-Sterile	Non-Sterile	Highly	0.0001

of the compound in the sterile controls (C and 500). Analysis of covariance indicated that these non-sterile systems (T and T500) have a first-order biotransformation coefficient significantly diffeient from zero. Statistical analysis also showed these biotransformation rate coefficients are significantly different from the rate coefficients of the disappearance of the compound in the sterile controls (C and C500) (Table XXII). Statistical analysis indicates that the systems containing non-sterile Mississippi River water and non-sterile solids (T500) have a slightly higher biotransformation rate coefficient then the systems containing non-sterile water only (T). The first-order biotransformation rate coefficient for systems containing Mississippi River water and solids is 0.57 day^{-1} with a half-life of 1.21 days. The first-order biotransformation rate coefficient for the test systems containing non-sterile Mississippi River water only (T) is 0.35 day^{-1}, which calculates to a half-life of 1.98 days. The increase in rate coefficients in systems containing non-sterile water and solids (T500) may indicate that the solids in the Mississippi River contribute to the biotransformation of 2,4-D.

Another indicator that the solids contribute to the biotransformation of the compound in the Mississippi system

TABLE XXII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF STERILE CONTROL SYSTEMS AND NON-STERILE TEST SYSTEMS IN THE MISSISSIPPI RIVER

	First Conditions		Second Conditions			
Exper-iment	Water	Solids	Water	Solids	Direction of Significance*	P
1	Sterile	None Added	Non-Sterile	None Added	<	0.0001
1	Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0001
1	Non-Sterile	None Added	Non-Sterile	Non-Sterile	<	0.0185

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

is the high rate coefficient (0.51 day^{-1}) found in the systems that initially contained sterile Mississippi River water and non-sterile solids (NSS). In both the Red River systems and Trinity River systems, similarly designed experiments yielded biotransformation rate coefficients approaching that of the sterile controls. In the Mississippi River experiments, the systems that containing sterile water and non-sterile solids (NSS) have biotransformation rate coefficients that are not significantly different from the other non-sterile test systems (Table XXIII). Table XXIII also shows that the biotransformation of the compound in the systems that initially contained non-sterile water and sterile solids (SS) is not significantly different from the biotransformation of the herbicide in the systems containing non-sterile water only (T), but is significantly different from the biotransformation in the systems containing non-sterile water and non-sterile solids (T500). These experiments suggest that in the Mississippi River systems the solids play an important role in the biotransformation of the herbicide 2,4-D.

The results of these biotransformation tests in the Mississippi River systems can be found in Table XX. Graphical representation of the actual disappearance of the herbicide can be found in Figures 17 and 18. These figures

TABLE XXIII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN MISSISSIPPI RIVER SYSTEMS CONTAINING NON-STERILE SOLIDS AND/OR NON-STERILE WATER WITH SYSTEMS CONTAINING EITHER STERILE WATER OR STERILE SOLIDS

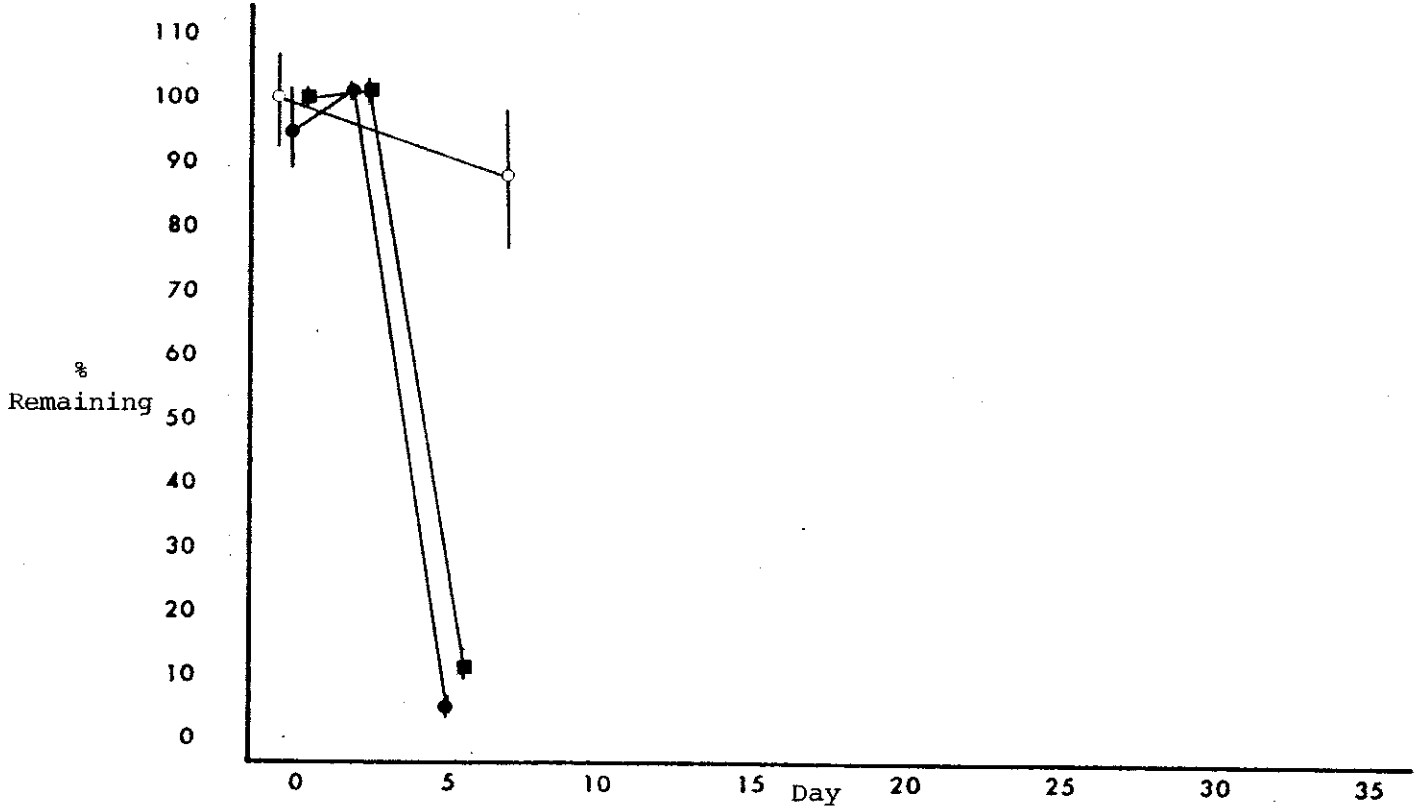
	First Conditions		Second Conditions			
Experiment	Water	Solids	Water	Solids	Direction of Significance*	P
1	Sterile	Non-Sterile	Non-Sterile	Sterile	=	0.2455
1	Sterile	Non-Sterile	Non-Sterile	None Added	=	0.1238
1	Sterile	Non-Sterile	Non-Sterile	Non-Sterile	=	0.5636
1	Non-Sterile	Sterile	Non-Sterile	None Added	=	0.5547
1	Non-Sterile	Sterile	Non-Sterile	Non-Sterile	<	0.0458

*A < sign indicates that the biotransformation in the first condition is slower than in the second condition.

A > sign indicates that the biotransformation in the first condition is faster than in the second condition.

A = sign indicates that there is no difference in the two biotransformation rates.

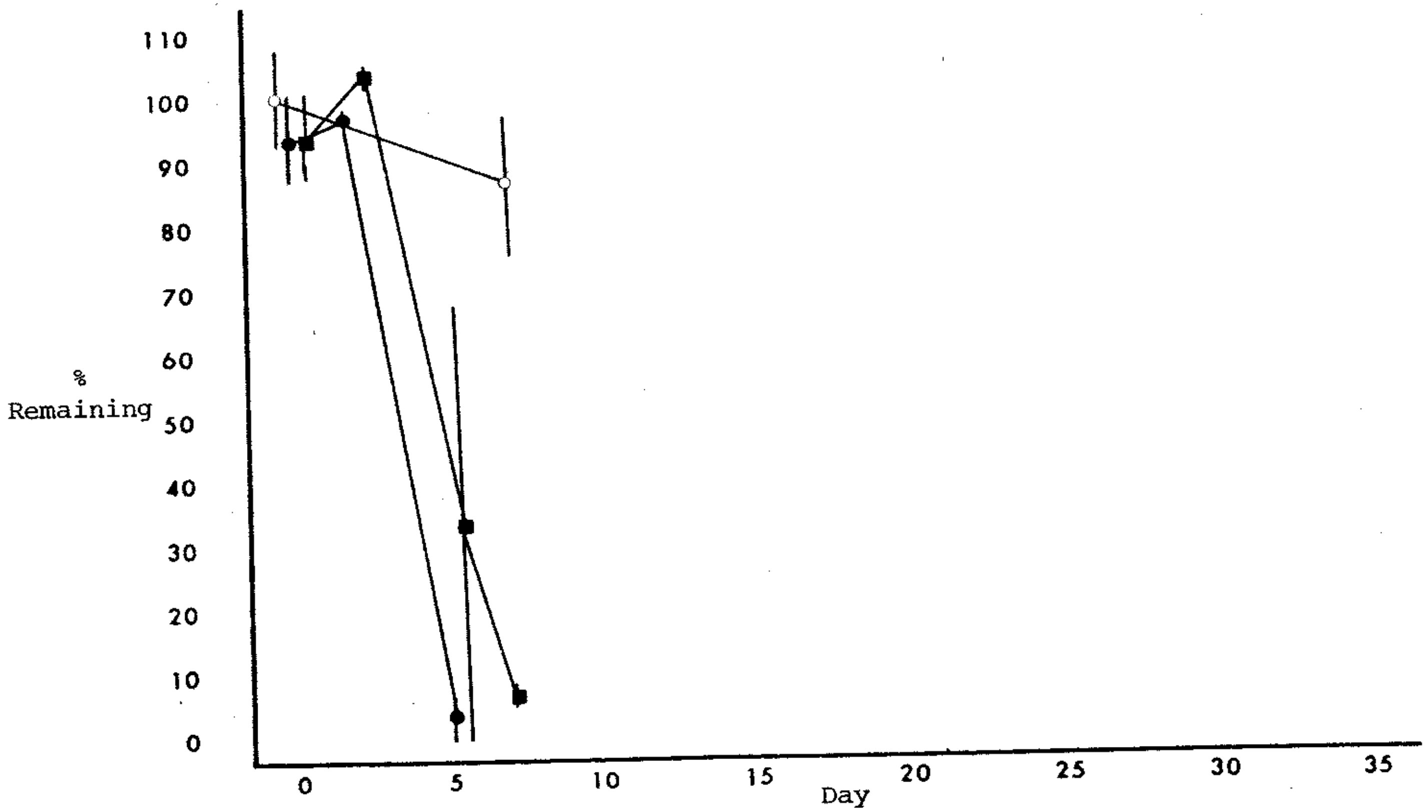
Fig. 17--Loss of 2,4-D through time in the Mississippi River systems initially containing sterile water and non-sterile solids.



◊=2,4-D Concentration in Sterile Controls.
●=2,4-D Concentration in Non-Sterile Tests.
■=2,4-D Concentration in Flasks Initially Containing Sterile Water and Non-Sterile Solids.

Points depict Mean ± One Standard Deviation.

Fig. 18--Loss of 2,4-D through time in the Mississippi River systems initially containing non-sterile water and sterile solids.



◊=2,4-D Concentration in Sterile Controls.
♦=2,4-D Concentration in Non-Sterile Tests.
■=2,4-D Concentration in Flasks Initially Containing Non-Sterile Water and Sterile Solids.

Points depict Mean ± One Standard Deviation.

depict the percent of the compound remaining versus time.

Comparisons of the Biotransformation of 2,4-D in the Three River Systems

The data from the biotransformation studies previously presented demonstrate that in all three river systems biotransformation is a major fate process for 2,4-D. Biotransformation rate coefficients for the herbicide in the Mississippi and the Trinity river systems are similar. Biotransformation in the Red River systems is less than biotransformation in the other two river systems. Analysis of covariance comparisons of the disappearance of the herbicide in the systems containing non-sterile water only (T) in the three river systems can be found in Table XXIV. Table XXV includes the statistical comparisons for the systems that contained both non-sterile water and solids (T500). These tables show that in every comparison of biotransformation of 2,4-D between the Red River systems and either of the other two rivers systems, highly significant differences exist. In comparisons between the Trinity and the Mississippi river systems, no significant differences are seen. The reason for increased rates of biotransformation in the Mississippi and the Trinity river systems is not totally understood but one possibility that will be discused later is the higher nutrient levels in

TABLE XXIV

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN SYSTEMS CONTAINING NON-STERILE WATER ONLY, BETWEEN MISSISSIPPI, RED, AND TRINITY RIVERS

Comparison*	Significantly Different	Direction of Significance	P
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0043
MR vs. TR	No	MR = TR	0.3421
MR vs. TR	No	MR = TR	0.2088
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001

*MR = Mississippi River.
RR = Red River.
TR = Trinity River.

TABLE XXV

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN SYSTEMS CONTAINING NON-STERILE WATER AND NON-STERILE SOLIDS, BETWEEN MISSISSIPPI, RED, AND TRINITY RIVERS

Comparison*	Significantly Different	Direction of Significance	P
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. RR	Highly	MR > RR	0.0001
MR vs. TR	No	MR = TR	0.3448
MR vs. TR	No	MR = TR	0.4053
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001

*MR = Mississippi River.
RR = Red River.
TR = Trinity River.

these two rivers. Another possibility is that in both of these river systems, Trinity and Mississippi, the microbes have previously been exposed to the herbicide. The data from STORET (1984) indicate low levels of the herbicide in the Mississippi River water. Since the minimum detectable limit of the analytical method used to analyze 2,4-D is 0.1 mg/l concentrations, less than this may be present in Trinity River water, or sometime in the last month water containing the herbicide may have flowed through the sample area.

It is interesting that the solids in the Mississippi River systems seem to be important in biotransformation of the herbicide but did not apparently contribute to the biotransformation of the herbicide in the other two rivers.

Acclimation Studies

Results of the biotransformation studies in which the microbes had previously been exposed to 2,4-D can be found in Table XXVI. Disappearance of the herbicide in these experiments is graphically depicted in Figures 19-22. These figures include disappearance of the compound in flasks that have previously been exposed to the herbicide, the flasks that contain non-sterile water that has received only one dose of the herbicide, and also the sterile controls. Since the data from STORET (1984) indicated a

TABLE XXVI

RESULTS OF BIOTRANSFORMATION STUDIES DEALING WITH ACCLIMATION

System[a]	Water	Solids	Biomass (cfu/ml)	Rate Coefficients: Zero-Order[b]	r	First-Order[c]	r	Second-Order[d]	r	First-Order Half-Life (days)
RR	Non-Sterile	None Added	19500	0.22	0.492	0.002	0.506	$1x10^{-7}$	0.519	301.3
RR	Acclimated	None Added	11500	6.84	0.702	0.23	0.722	$2x10^{-5}$	0.729	3.01
RR	Non-Sterile	Non-Sterile	44750	3.38	0.734	0.11	0.738	$2x10^{-6}$	0.739	6.3
RR	Acclimated	Acclimated	11525	9.56	0.999	0.30	1	$3x10^{-5}$	1	2.27
RR	Non-Sterile	None Added	46250	1.59	0.76	0.06	0.745	$1x10^{-6}$	0.729	11.94
RR	Acclimated	None Added	42500	6.62	0.709	0.25	0.696	$6x10^{-6}$	0.644	2.78
RR	Acclimated	Acclimated	38250	18.13	0.863	0.68	0.856	$2x10^{-5}$	0.794	1.01
RR	Non-Sterile	Non-Sterile	78000	2.01	0.899	0.07	0.847	$9x10^{-7}$	0.802	9.62
TR	Non-Sterile	None Added	100500	13.31	0.872	0.45	0.802	$4x10^{-6}$	0.763	1.55
TR	Acclimated	None Added	126750	33.02	0.975	1.12	0.955	$9x10^{-6}$	0.942	0.61
TR	Non-Sterile	Non-Sterile	91250	13.99	0.914	0.47	0.815	$5x10^{-6}$	0.74	1.48
TR	Acclimated	Acclimated	132250	32.67	0.985	1.125	0.961	$9x10^{-6}$	0.942	0.61
MR	Non-Sterile	None Added	82500	12.5	0.77	0.35	0.757	$4x10^{-6}$	0.727	1.98
MR	Non-Sterile	Non-Sterile	48250	17.4	0.906	0.57	0.921	$1x10^{-5}$	0.926	1.21

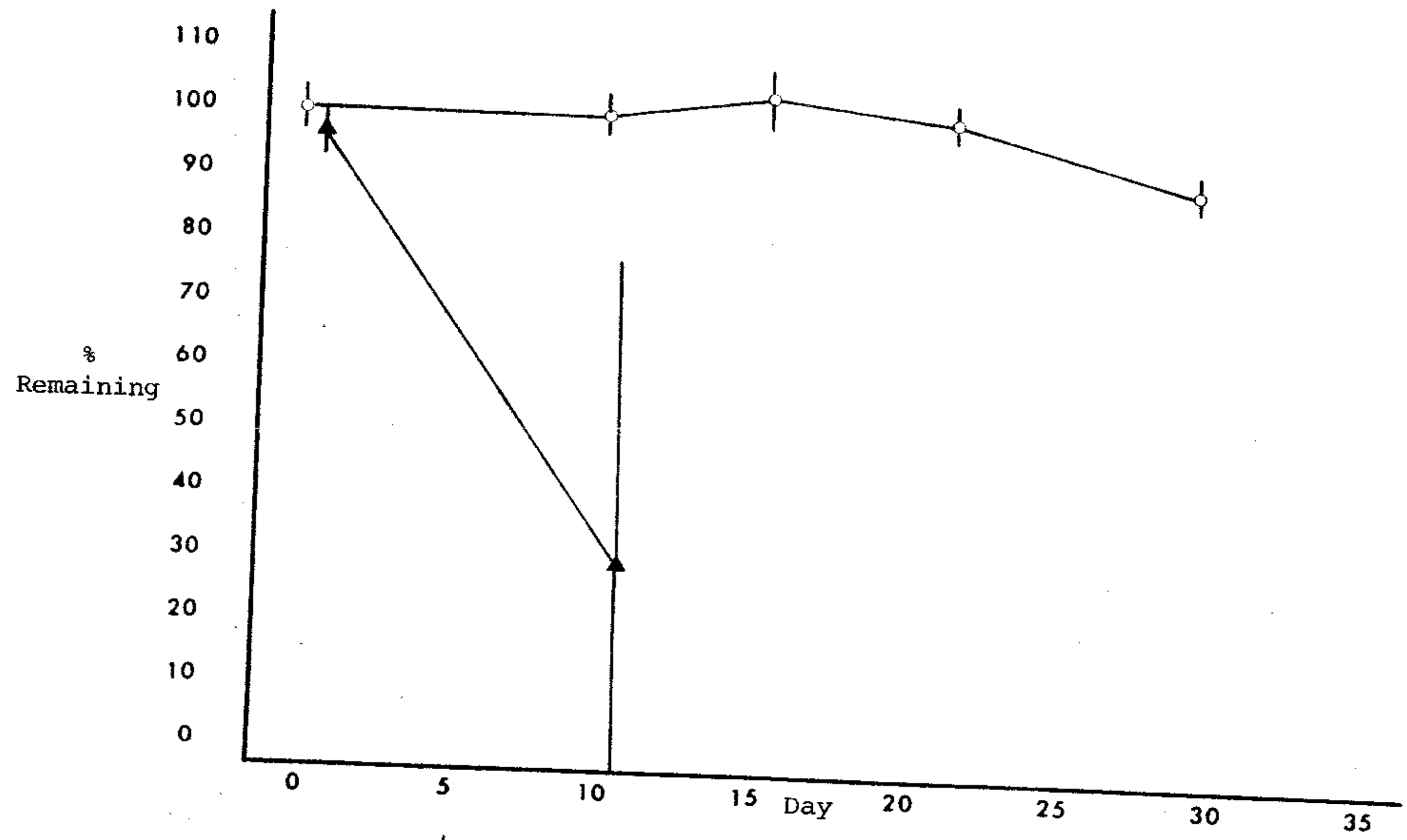
[a] RR = Red River; TR = Trinity River; MR = Mississippi River.

[b] Unit expressed as % day^{-1}.

[c] Unit expressed as day^{-1}.

[d] Unit expressed as $\%(cfu/ml)^{-1}day^{-1}$.

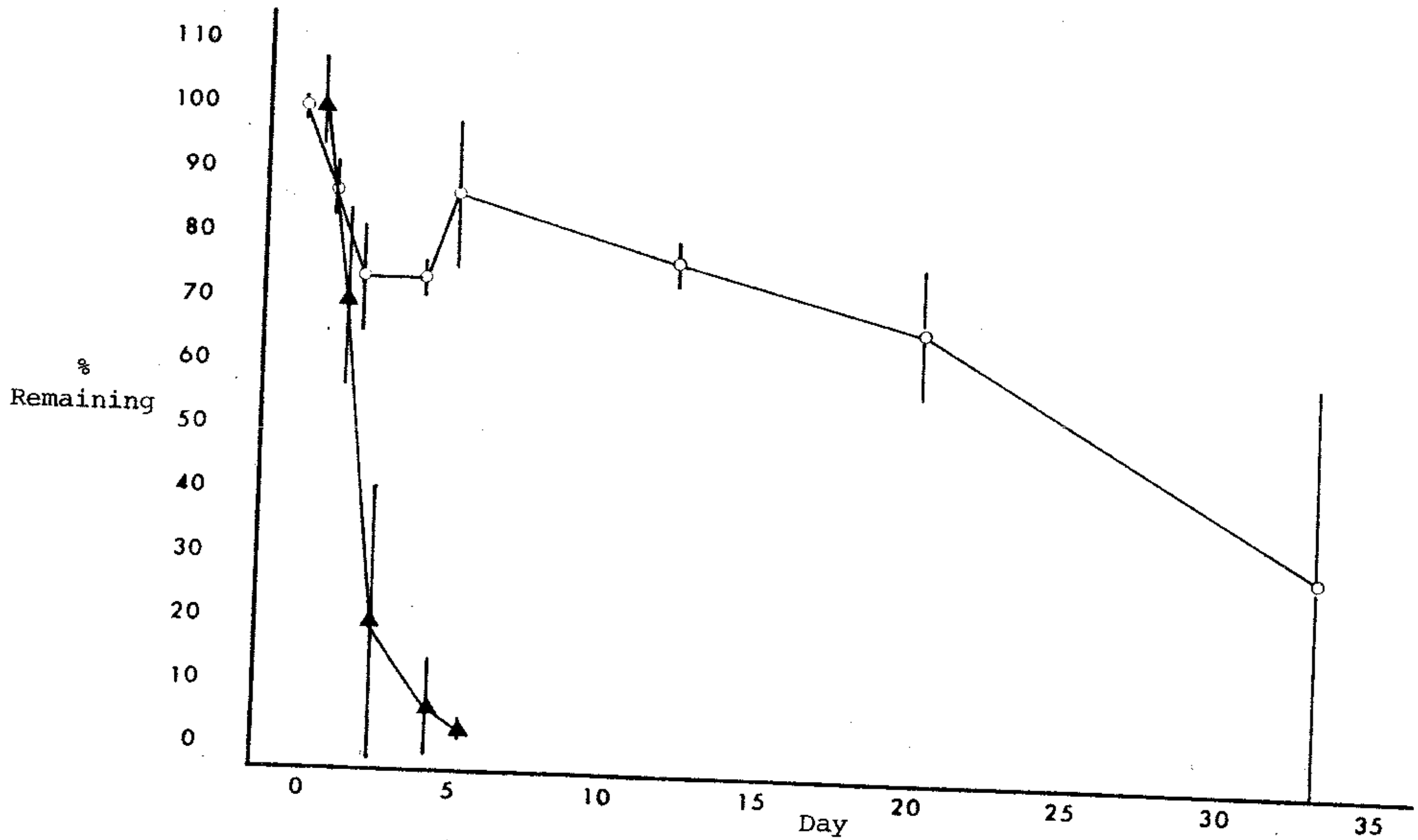
Fig. 19--Loss of 2,4-D through time in the acclimated Red River systems containing water only.



ϕ=2,4-D Concentration in Non-Acclimated Test Flasks.
▲=2,4-D Concentration in Acclimated Test Flasks.

Points depict Mean ± One Standard Deviation.

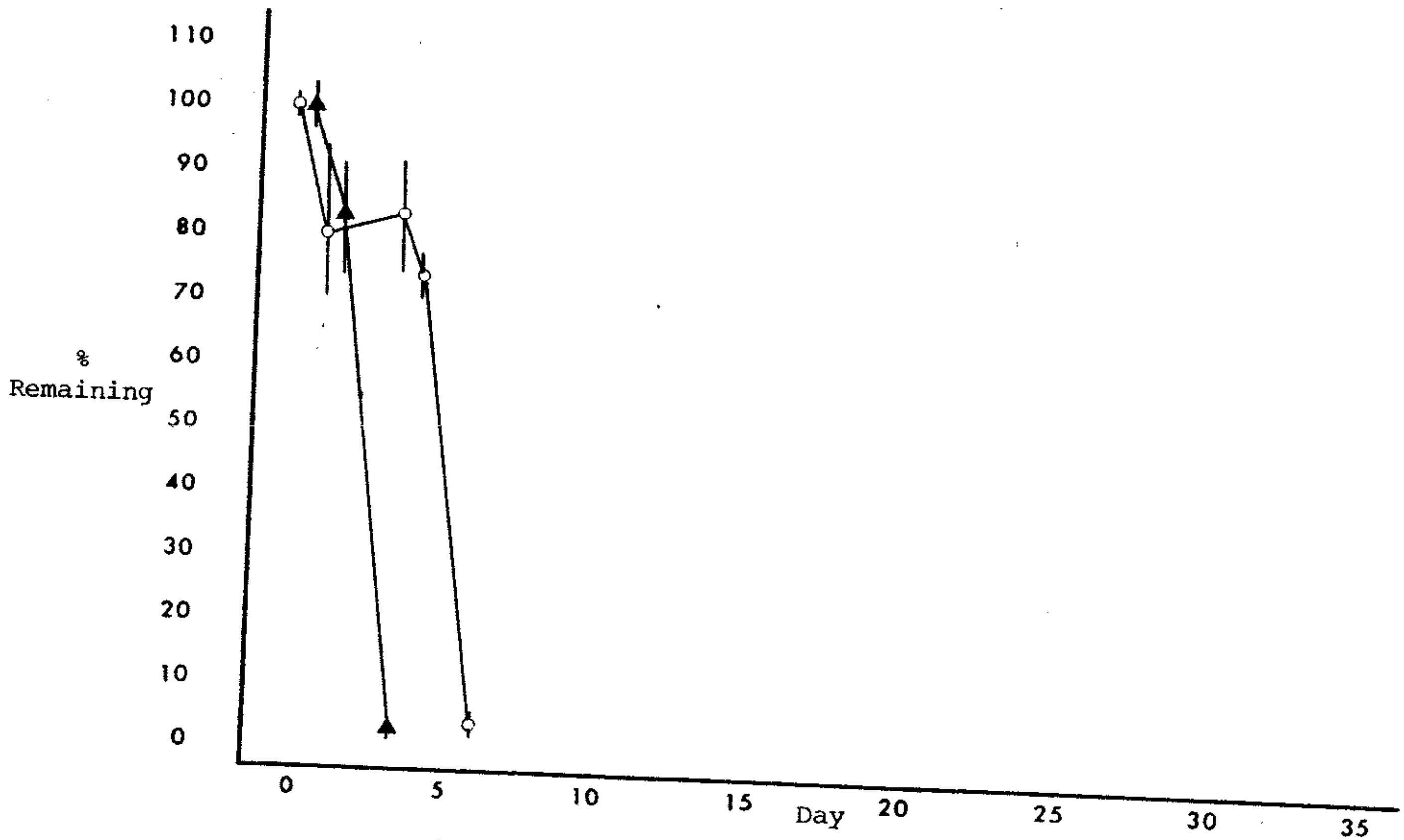
Fig. 20--Loss of 2,4-D through time in the acclimated Red River systems containing water and solids.



◊= 2,4-D Concentration in Non-Acclimated Test Flasks.
▲= 2,4-D Concentration in Acclimated Test Flasks.

Points depict Mean ± One Standard Deviation.

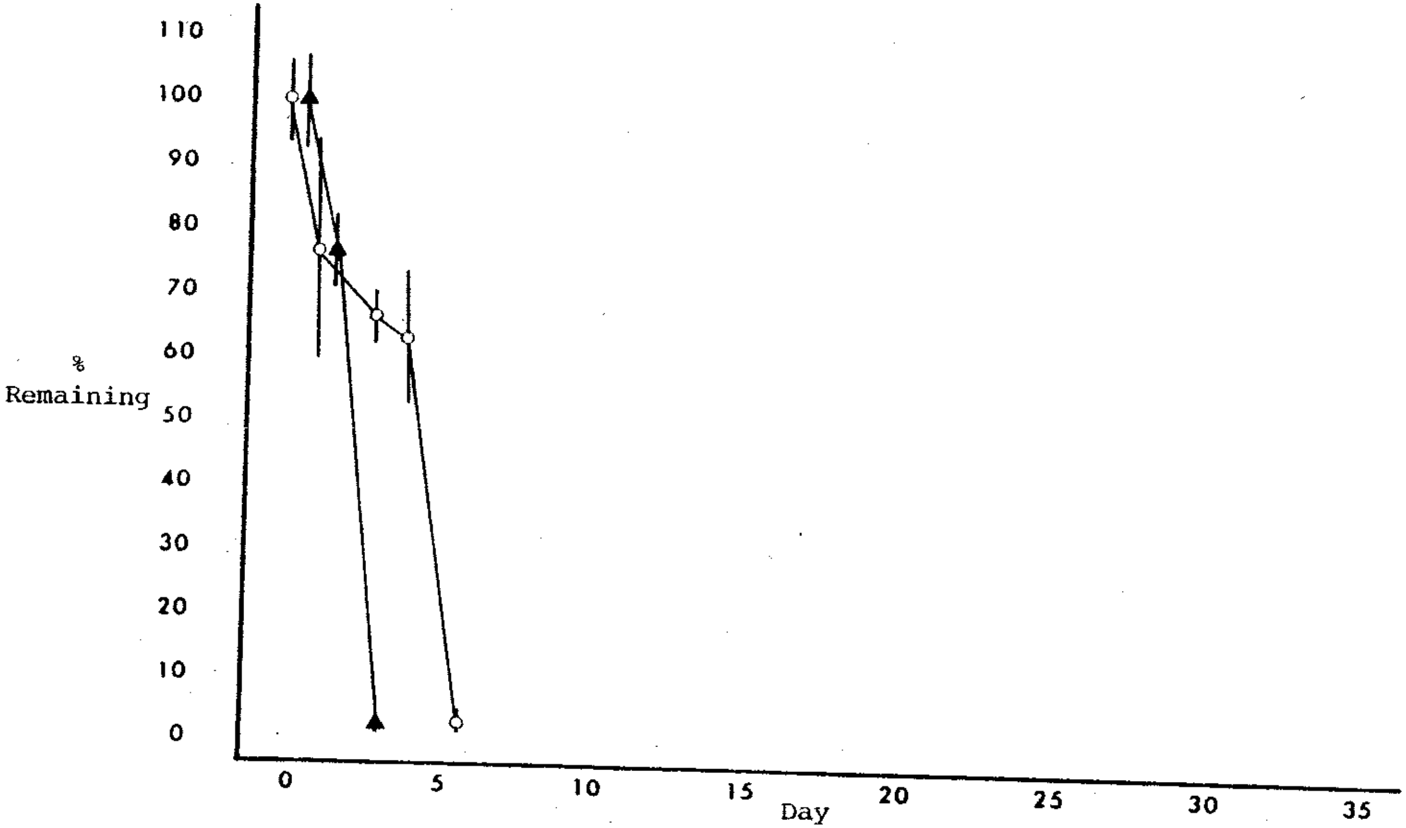
Fig. 21--Loss of 2,4-D through time in the acclimated Trinity River systems containing water only.



◊=2,4-D Concentration in Non-Acclimated Test Flasks.
▲=2,4-D Concentration in Acclimated Test Flasks.

Points depict Mean ± One Standard Deviation.

Fig. 22--Loss of 2,4-D through time in the acclimated Trinity River systems containing water and solids.



○=2,4-D Concentration in Non-Acclimated Test Flasks.
▲=2,4-D Concentration in Acclimated Test Flasks.

Points depict Mean ± One Standard Deviation.

background level of 2,4-D in the Mississippi River water and little lag phase was seen in Mississippi River experiments, no redosing was performed on the Mississippi River experiments. The biotransformation study previously presented incorporating Mississippi water and solids will be used for comparisons with the redosed systems of the other two rivers.

As can be seen from these figures (19-22) as well as the figures previously presented the disappearance of the herbicide in the Red and Trinity river systems are characterized by an initial lag phase. The lag phase in the Red River systems ranged from 6 to 15 days. The lag phase in the Trinity River system was shorter than the lag phase of the Red. In the Trinity the longest lag phase seen was 4 days. The significance of the long lag phase seen in the Red River system can be best realized if it is remembered that in 15 days over 12 half-lives of biotransformation of 2,4-D can take place in the Mississippi River systems.

In the Red River system, use of acclimated organisms increased the biotransformation rate coefficient by factors ranging from 2 to 10. In the Trinity River systems, the use of acclimated organisms increased the biotransformation rate coefficient by a factor slightly greater than 2. These increased rates of biotransformation occured even though the initial biomass of the acclimated flask were less then the biomass of the initial test systems. On day 0 the number of microbes in the acclimated systems is anywhere from 10% to 50% of the number of microbes in the test systems. After 3 or 4 days there does not appear to be a difference in the biomass of the test and acclimated systems. Table XXVII includes statistical comparisons of biotransformation rate coefficients in the non-acclimated systems with the acclimated systems' rate coefficients. The biotransformation rate coefficients in all cases of redosing are highly significantly different from the biotransformation rate coefficients in the non-redosed system.

Table XXVIII shows comparisons of the acclimated rate coefficients between the three river systems. In three out of four experiments, acclimation in the Red River systems increased the rate of biotransformation to a degree that no significant difference could be shown between biotransformation coefficients of 2,4-D in the Mississippi River and the Red River.

This increase in the biotransformation rates after acclimation may have far reaching implications in both herbicidal treatments of aquatic vegetation and in hazard assessment. If redosing of a body of water to eliminate

TABLE XXVII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES BEFORE AND AFTER ACCLIMATION

System*	Solids	Significantly Different	P
RR	None	Highly	0.0001
RR	None	Highly	0.0001
RR	Yes	Highly	0.0001
RR	Yes	Highly	0.0001
TR	None	Highly	0.0001
TR	Yes	Highly	0.0001

*RR = Red River, TR = Trinity River.

TABLE XXVIII

STATISTICAL COMPARISONS OF BIOTRANSFORMATION RATES OF 2,4-D IN MISSISSIPPI, RED, AND TRINITY RIVER ACCLIMATED SYSTEMS

Comparison*	Significantly Different	Direction of Significance	P
MR vs. RR	No	MR = RR	0.1672
MR vs. RR	No	MR = RR	0.1949
MR vs. TR	Highly	MR < TR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
TR vs. RR	Highly	TR > RR	0.0001
MRS vs. RRS	Highly	MRS > RRS	0.0096
MRS vs. RRS	No	MRS = RRS	0.4048
MRS vs. TRS	Highly	MRS < TRS	0.0041
TRS vs. RRS	Yes	TRS > RRS	0.0215
TRS vs. RRS	Highly	TRS > RRS	0.0001

*MR = Mississippi River water only.
TR = Trinity River water only.
RR = Red River water only.
MRS = Mississippi River water and solids.
RRS = Red River water and solids.
TRS = Trinity River water and solids.

nuisance vegetation is performed, then this dose may need to be increased to yield the same degree of "kill" as the initial dose. In deciding upon the dose the effects on non-target species must be taken into account. This consideration of non-target species needs to be considered for both initial doses and for any redosing that occurs. In deciding on the concentration of the herbicide to use the laws and regulations (FIFRA) also need to be considered. In the case of time-released formulation of herbicides, this increased biotransformation should be accounted for to assure the desired results. In hazard assessment, the potential hazard of a spill of a compound may be decreased if the microbes have previously been exposed to the compound. This may be particularly important for industries whose effluents contain 2,4-D or similar compounds. The affects of the effluents, to the environment, may be reduced, due to increased rates of biotransformation, if the wastes contains continuous levels of the compound rather than intermittent releases of the toxicant.

Comparison of Experimentally Found Rates with Literature Values

The literature values for the biotransformation and/or degradation of 2,4-D vary greatly. The persistance of 2,4-D in nature or laboratory studies were reported to range from a few days to months. The half-lives for 2,4-D in the Red River studies were similar to the half-lives reported by Nesbitt and Watson (1980a) and C.A.S.T. (1975). The half-lives of the herbicide in the Red River experiments ranged from 5 days to 14 days. The half-lives reported by Nesbitt and Watson (1980a) for 2,4-D in river waters ranged from 10 days to 50 days. C.A.S.T (1975) reported the half-life of 2,4-D in soils to range from 1 to 2 weeks. The biotransformation rates observed in the Trinity and Mississippi rivers are greater than those reported by Nesbitt and Watson (1980a) or C.A.S.T. (1975). The half-life of 2,4-D in the Trinity and Mississippi river experiments ranged from one to two days. Biotransformation rates this great have also been reported by Klingman (1964). Klingman reported the persistance of 2,4-D in soils to be 7 days. Results of the acclimation experiments conducted for this thesis agree with the results of Spain and Van Veld (1983). Spain and Van Veld reported less than 5% of the initial herbicide remaining after 40 hours in pre-exposed systems. After 40 hours in acclimated Trinity River water almost 3 half-lives have occured leaving only 15% of the herbicide not transformed. In Red River acclimated systems, after 40 hours only 25% to 50% of 2,4-D remains untransformed.

Use of Rate Constants in Predicting Fate of 2,4-D in Aquatic Systems

The first-order rate constants found in this research for the disappearance of 2,4-D indicate that biotransformation is the major fate process of the herbicide. Other processes such as hydrolysis, photolysis, and volatilization if occurring do so at a much lower rate than biotransformation. If biotransformation is the major fate process of a compound and the half-life of that compound can be measured in days or weeks then to predict the fate of that compound, accurate biotransformation rate constants must be known. If the rate constants are not accurate and are used to predict the overall fate of a compound large errors in these predictions would occur. If biotransformation is not a major fate process, or the half-life is measured in years, then the errors introduced by inaccurate biotransformation rate constants would be insignificant. Since biotransformation is the major fate process for 2,4-D, accurate rate constants are needed. First-order rate constants found in this study, for the non-acclimated systems range from 0.05 day^{-1} to 0.57 day^{-1}. This range of biotransformation rate constants includes all three river systems with and without additional solids added. The range of rate constants for each individual river system is smaller. The range of rate constants for the Trinity River is 0.45 day^{-1} to 0.49 day^{-1}. The first-order biotransformation rate constants for the Mississippi River systems range from 0.35 day^{-1} to 0.57 day^{-1} and the range for the Red River systems is 0.049 day^{-1} to 0.14 day^{-1}. From these results it is apparent that the rate of biotransformation of a compound in one aquatic system may be different than in another aquatic system. The range of first-order rate coefficients for the acclimated experiments is 0.23 day^{-1} to 1.12 day^{-1}. As can be seen from these data acclimation reduces some of the variation in the observed rate coefficients.

The use of models to predict the first-order biotransformation rate constant may help account for the variation in the rate constants between various aquatic systems. These models should incorporate the water chemistry and sediment properties of the aquatic system. These models should also include if the system has previously been exposed to the compound since acclimation as already been shown to increase the biotransformation in an system. The following section will introduce and discuss two possible models.

Predictive Models of the Biotransformation Rate of 2,4-D

The results of the biotransformation experiments were incorporated into statistical models. These models are designed to yield first-order biotransformation rate coefficients for 2,4-D. The usefulness of these models is facilitated because they only require the user to know simple water quality parameters and a little history about the water body.

Two statistical models were developed, with the aid of SAS (Stepwise procedure), to predict these rate coefficients. The first model is designed to predict the first-order rate coefficient in waters with low suspended solid (less then 100 mg/l). The second model is designed to predict the rate coefficient in waters with a wider range of suspended solids (less then 600 mg/l).

In the development of the first model, the following parameters were evaluated: the origin or source of the water, the concentration of ammonia, the concentration of nitrate, the amount of organic carbon present, and whether the system had previously been exposed to the compound. Phosphates were not considered for these models since data for phosphates in the Trinity River were lacking. The resulting model did not incorporate the origin of the water or nitrate concentration. Addition of the either of these parameters to the model did not improve the R-square value sufficiently (0.15) to warrant its inclusion. The model for low suspended concentrations is as follows:

Kl=(0.189*ammonia)+(0.043*organic carbon)+(0.333*acclimation)-0.363

Where Kl is the first-order rate coefficient for 2,4-D

ammonia is measured in mg/l as NH_3 N

organic carbon is measured in mg/l

and acclimation is equal to one if the system has not previously been exposed to the compound and two if previously exposed.

In developing the second model, the following parameters were evaluated: the origin of the water, ammonia concentration, nitrate concentration, the concentration of organic carbon, acclimation, volatile matter of the suspended solids and the percent of sand, silt, and clay of the suspended solids. The resulting model is as follows:

Kl=(0.375*acclimation)+(5.56*ammonia)-(1.66*nitrate)-0.290

Where Kl is the first-order biotransformation rate coefficient acclimation is equal to one if the system has not been previously exposed to the herbicide and two if previously exposed

ammonia is in mg/l as NH_3 N

nitrate is in mg/l as NH_3 N

The other variables evaluated did not meet the 0.15 significance level for entry into the model.

Both of these generated models are significant. The first model has a probability of a greater F value of 0.012 and an R-Square of 0.869. The second model has an R-square value of 0.809 with a probability of a greater F value of 0.0001.

The usefulness of these models is facilitated by not requiring elaborate characterization of the water or suspended solids of the system being studied. In a matter of hours, a researcher can have an approximate first-order rate coefficient specific for a water body. This first-order rate coefficient can be found without first conducting a month-long biotransformation study. These models also demonstrate relationships between biotransformation rates and acclimation, and nutrients. In both models the affect of acclimation is an increase in the first-order rate coefficient of over 0.3 day^{-1}. This increase is significant when you consider that the non-acclimated rates in the Red River experiments range from 0.05 day^{-1} to 0.16 day^{-1}. These models also indicate that as nutrients are increased so is biotransformation.

Preliminary Algal Bioassay

The results of the preliminary algal bioassay to determine the effects of suspended solids on the bioavailability of 2,4-D to Selenastrum capricornutum can be found in Table XXIX. Analysis of variance and Duncans multiple range test were performed on these data to determine if the herbicide was toxic to the algae. These statistical tests indicated that there is no difference in the concentration of algal cells in the various concentrations of the herbicide used. The concentration of the herbicide ranged from 0 mg/l to 20 mg/l. The experimental matrix for this experiment was Trinity River water without additional suspended solids. Statistical analysis of the data from the bioassay experiment conducted using Trinity River water with the addition of 500 mg/l of suspended solids indicated that difference in the concentration of algal cells existed over the concentrations used (Table XXX). The concentration of 2,4-D in this experiment also ranged from 0 to 20 mg/l.

TABLE XXIX

RESULTS FROM PRELIMINARY ALGAL BIOASSAY WITHOUT SOLIDS

Concentration of 2,4-D (mg/l)	Cell Count (10^7 cell/l)*	Significantly Different From Controls
0	2.65 ± 1.8	No
4	3.88 ± 3.8	No
7	2.44 ± 1.04	No
12	1.68 ± 0.71	No
20	0.68 ± 0.50	No

*$\bar{X}$ ± Standard Deviation.

TABLE XXX

RESULTS FROM PRELIMINARY ALGAL BIOASSAY WITH SOLIDS

Concentration of 2,4-D (mg/l)	Cell Count (10^7 cell/l)*	Significantly Different From Controls
0	1.64 $\pm$ 0.63	No
4	1.90 $\pm$ 0.09	No
7	1.5 $\pm$ 0.4	No
12	1.11 $\pm$ 0.3	Yes
20	0.72 $\pm$ 0.06	Yes

*$\bar{X}$ $\pm$ Standard Deviation.

To test the hypothesis of no difference in the bioavailability of 2,4-D to Selenastrum capricornutum to the presence and absence of 500 mg/l additional suspended solids, analysis of covariance was performed on the results from the preliminary algal bioassay. This statistical test failed to reject the null hypothesis of no difference at the P=0.05 level.

Since differences in the bioavailability of the herbicide were not detected and the dose required to show toxicity is far greater then environmentally realistic concentrations, further algal bioassay experiments were not performed.

CHAPTER FOUR

CONCLUSIONS

The objectives and hypotheses of this research address the role of acclimation, suspended solids, and the source of water in biotransformation of the chlorinated hydrocarbon, 2,4-dichlorophenoxy acetic acid. Goals of this work also included generating realistic biotransformation rate coefficients, evaluating the reaction order of biotransformation and to determine if suspended solids altered the bioavailability of the herbicide. The objectives of this research were accomplished and all hypotheses were evaluated with varying degrees of success. The conclusions which can be drawn from this work are as follows:

1. Addition of 500 mg/l of suspended solids may affect the biotransformation rate of 2,4-D. The biotransformation rate in the Mississippi River systems was increased by the addition of solids ($T_{1/2}$=2.0 days versus $T_{1/2}$=1.2 days). The biotransformation rate in the Trinity River systems was not altered significantly by the addition of the solids. In the Red River the addition of suspended solids (500 mg/l) caused mixed results. In two experiments the addition of solids increased

biotransformation ($T_{1/2}$=12 days versus $T_{1/2}$=9.6 days and $T_{1/2}$=14.2 days versus $T_{1/2}$=7.9 days). In one experiment the addition of solids decreased biotransformation of 2,4-D ($T_{1/2}$=4.8 days versus $T_{1/2}$=7.02 days).

2. The source of water and suspended solids affects the biotransformation of the herbicide 2,4-D. The first-order biotransformation rate of the herbicide was shown to be less in the Red River systems than in the other two systems. In the Red River systems the highest first-order biotransformation rate was 0.14 day^{-1} in the Trinity and Mississippi the smallest rate coefficients were 0.45 day^{-1} and 0.35 day^{-1} respectively.

3. The apparent biotransformation rate coefficient was adequately described by first-order kinetics although zero-order kinetics may also describe the disappearance of the compound. Zero and first order kinetics better described the disappearance of the herbicide than second-order kinetics. Second-order rate coefficients were more variable than zero or first order rate coefficients.

4. The results of experiments using non-sterile solids and sterile water indicate that the microbes associated with the suspended solids of the Trinity and Red rivers did not contribute to

biotransformation of the compound. The microbes associated with the suspended solids of the Mississippi River did contribute to the biotransformation of 2,4-D.

5. Acclimation or redose increased the rate of biotransformation of the herbicide as much as an order of magnitude. Acclimation increased the rate of biotransformation in one of the Red River experiments from 0.07 day^{-1} to 0.68 day^{-1}.
6. Based on three sources of water and solids, and an initial concentration of 2,4-D of approximately 2 mg/l environmentally realistic first-order biotransformation rate coefficients range from 1.12 day^{-1} to 0.05 day^{-1} depending on environmental conditions.
7. The toxicity of the herbicide to *Selenastrum capricornutum* was not reduced by addition of suspended solids to Trinity River water. Little toxicity of 2,4-D to the algae was demonstrated even at 2,4-D concentrations of 20 mg/l.

APPENDIX I

RAW DATA OF BIOTRANSFORMATION EXPERIMENTS

--------------------- VAR=MRC T=0 ---------------------

OBS	VAR	T	CONC	INIT	BIOMASS
1	MRC	0	1.88	1.84	.
2	MRC	0	1.85	1.84	.
3	MRC	0	1.70	1.84	.
4	MRC	0	1.92	1.84	.

--------------------- VAR=MRC T=7 ---------------------

OBS	VAR	T	CONC	INIT	BIOMASS
5	MRC	7	1.75	1.84	.
6	MRC	7	1.61	1.84	.
7	MRC	7	1.56	1.84	.
8	MRC	7	1.64	1.84	.

------------------- VAR=MRC500 T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
9	MRC500	0	2.01	1.88	.
10	MRC500	0	1.73	1.88	.
11	MRC500	0	1.77	1.88	.
12	MRC500	0	1.99	1.88	.

------------------- VAR=MRC500 T=7 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
13	MRC500	7	1.61	1.88	.
14	MRC500	7	1.49	1.88	.
15	MRC500	7	1.54	1.88	.
16	MRC500	7	2.14	1.88	.

------------------- VAR=MRNSS T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
17	MRNSS	0	1.12	1.12	48000
18	MRNSS	0	1.12	1.12	81000
19	MRNSS	0	1.12	1.12	180000
20	MRNSS	0	1.12	1.12	38000

------------------- VAR=MRNSS T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
21	MRNSS	2	1.48	1.12	48000
22	MRNSS	2	1.53	1.12	81000
23	MRNSS	2	1.62	1.12	180000
24	MRNSS	2	1.84	1.12	38000

-------------------- VAR=MRNSS T=5 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
25	MRNSS	5	0.1	1.12	48000
26	MRNSS	5	0.1	1.12	81000
27	MRNSS	5	0.1	1.12	180000
28	MRNSS	5	0.1	1.12	38000

-------------------- VAR=MRSS T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
29	MRSS	0	1.55	1.74	77000
30	MRSS	0	1.92	1.74	77000
31	MRSS	0	1.55	1.74	35000
32	MRSS	0	1.92	1.74	35000
33	MRSS	0	1.55	1.74	17000
34	MRSS	0	1.92	1.74	17000
35	MRSS	0	1.55	1.74	11000
36	MRSS	0	1.92	1.74	11000

-------------------- VAR=MRSS T=2 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
37	MRSS	2	1.91	1.74	77000
38	MRSS	2	1.99	1.74	35000
39	MRSS	2	2.34	1.74	17000
40	MRSS	2	2.08	1.74	11000

-------------------- VAR=MRSS T=5 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
41	MRSS	5	0.94	1.74	77000
42	MRSS	5	0.10	1.74	35000
43	MRSS	5	1.25	1.74	17000
44	MRSS	5	0.10	1.74	11000

-------------------- VAR=MRSS T=7 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
45	MRSS	7	0.1	1.74	77000
46	MRSS	7	0.1	1.74	35000
47	MRSS	7	0.1	1.74	17000
48	MRSS	7	0.1	1.74	11000

-------------------- VAR=MRT T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS

49	MRT	0	1.55	1.55	200000
50	MRT	0	1.55	1.55	35000
51	MRT	0	1.55	1.55	78000
52	MRT	0	1.55	1.55	17000

-------------------- VAR=MRT T=2 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
53	MRT	2	2.13	1.55	200000
54	MRT	2	2.03	1.55	35000
55	MRT	2	1.87	1.55	78000
56	MRT	2	1.73	1.55	17000

-------------------- VAR=MRT T=5 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
57	MRT	5	1.58	1.55	200000
58	MRT	5	0.10	1.55	35000
59	MRT	5	1.68	1.55	78000
60	MRT	5	1.19	1.55	17000

-------------------- VAR=MRT T=7 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
61	MRT	7	0.10	1.55	200000
62	MRT	7	0.10	1.55	35000
63	MRT	7	0.17	1.55	78000
64	MRT	7	0.10	1.55	17000

------------------ VAR=MRT500 T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
65	MRT500	0	2.22	2	61000
66	MRT500	0	1.78	2	61000
67	MRT500	0	2.22	2	68000
68	MRT500	0	1.78	2	68000
69	MRT500	0	2.22	2	31000
70	MRT500	0	1.78	2	31000
71	MRT500	0	2.22	2	33000
72	MRT500	0	1.78	2	33000

------------------ VAR=MRT500 T=2 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
73	MRT500	2	2.60	2	61000
74	MRT500	2	1.99	2	68000
75	MRT500	2	1.91	2	33000

------------------ VAR=MRT500 T=5 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
76	MRT500	5	0.1	2	61000
77	MRT500	5	0.1	2	68000
78	MRT500	5	0.1	2	31000
79	MRT500	5	0.1	2	33000

------------------- VAR=RRAC T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
80	RRAC	0	2.22	2.29	.
81	RRAC	0	2.01	2.29	.
82	RRAC	0	2.63	2.29	.
83	RRAC	0	2.29	2.29	.

------------------- VAR=RRAC T=15 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
84	RRAC	15	1.96	2.29	.
85	RRAC	15	2.15	2.29	.
86	RRAC	15	2.48	2.29	.
87	RRAC	15	2.48	2.29	.
88	RRAC	15	2.38	2.29	.

------------------- VAR=RRAC T=21 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
89	RRAC	21	2.17	2.29	.
90	RRAC	21	2.00	2.29	.
91	RRAC	21	2.06	2.29	.
92	RRAC	21	2.14	2.29	.

------------------- VAR=RRAC T=29 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
93	RRAC	29	1.92	2.29	.
94	RRAC	29	2.04	2.29	.
95	RRAC	29	1.94	2.29	.
96	RRAC	29	1.98	2.29	.

------------------- VAR=RRAC500 T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
97	RRAC500	0	2.26	2.14	.
98	RRAC500	0	2.26	2.14	.
99	RRAC500	0	2.02	2.14	.
100	RRAC500	0	2.02	2.14	.

------------------ VAR=RRAC500 T=15 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
101	RRAC500	15	2.21	2.14	.
102	RRAC500	15	2.45	2.14	.
103	RRAC500	15	2.30	2.14	.
104	RRAC500	15	2.17	2.14	.

------------------ VAR=RRAC500 T=21 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
105	RRAC500	21	2.22	2.14	.
106	RRAC500	21	2.17	2.14	.
107	RRAC500	21	2.13	2.14	.
108	RRAC500	21	2.59	2.14	.

------------------ VAR=RRAC500 T=29 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
109	RRAC500	29	2.06	2.14	.
110	RRAC500	29	2.30	2.14	.
111	RRAC500	29	1.80	2.14	.
112	RRAC500	29	2.10	2.14	.

------------------ VAR=RRANSS T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
113	RRANSS	0	2.23	2.24	50000
114	RRANSS	0	2.25	2.24	50000
115	RRANSS	0	2.23	2.24	500000
116	RRANSS	0	2.25	2.24	500000
117	RRANSS	0	2.23	2.24	30000
118	RRANSS	0	2.25	2.24	30000
119	RRANSS	0	2.23	2.24	60000
120	RRANSS	0	2.25	2.24	60000

------------------ VAR=RRANSS T=10 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
121	RRANSS	10	2.44	2.24	50000
122	RRANSS	10	2.37	2.24	500000
123	RRANSS	10	2.23	2.24	30000
124	RRANSS	10	2.02	2.24	60000

------------------ VAR=RRANSS T=15 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
125	RRANSS	15	2.15	2.24	50000

126	RRANSS	15	2.28	2.24	500000
127	RRANSS	15	2.11	2.24	30000
128	RRANSS	15	2.09	2.24	60000

------------------ VAR=RRANSS T=21 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
129	RRANSS	21	2.12	2.24	50000
130	RRANSS	21	2.13	2.24	500000
131	RRANSS	21	2.06	2.24	30000
132	RRANSS	21	2.08	2.24	60000

------------------ VAR=RRANSS T=29 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
133	RRANSS	29	1.97	2.24	50000
134	RRANSS	29	2.19	2.24	500000
135	RRANSS	29	1.93	2.24	30000
136	RRANSS	29	1.85	2.24	60000

------------------- VAR=RRAT T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
137	RRAT	0	2.08	2.05	15000
138	RRAT	0	2.02	2.05	15000
139	RRAT	0	2.08	2.05	14000
140	RRAT	0	2.02	2.05	14000
141	RRAT	0	2.08	2.05	20000
142	RRAT	0	2.02	2.05	20000
143	RRAT	0	2.08	2.05	29000
144	RRAT	0	2.02	2.05	29000

------------------- VAR=RRAT T=10 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
145	RRAT	10	2.35	2.05	15000
146	RRAT	10	2.15	2.05	14000
147	RRAT	10	2.55	2.05	20000
148	RRAT	10	2.36	2.05	29000

------------------- VAR=RRAT T=15 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
149	RRAT	15	2.00	2.05	15000
150	RRAT	15	2.27	2.05	14000
151	RRAT	15	2.15	2.05	20000
152	RRAT	15	2.18	2.05	29000

------------------- VAR=RRAT T=21 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
153	RRAT	21	2.06	2.05	15000
154	RRAT	21	2.01	2.05	14000
155	RRAT	21	2.12	2.05	20000
156	RRAT	21	2.07	2.05	29000

------------------- VAR=RRAT T=29 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
157	RRAT	29	1.85	2.05	15000
158	RRAT	29	1.86	2.05	14000
159	RRAT	29	1.89	2.05	20000
160	RRAT	29	1.82	2.05	29000

------------------- VAR=RRATA T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
161	RRATA	0	2.04	2.26	7000
162	RRATA	0	2.50	2.26	11000
163	RRATA	0	2.25	2.26	14000
164	RRATA	0	2.23	2.26	14000

------------------ VAR=RRATA T=10 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
165	RRATA	10	0.1	2.26	7000
166	RRATA	10	2.3	2.26	11000
167	RRATA	10	0.1	2.26	14000
168	RRATA	10	0.1	2.26	14000

------------------ VAR=RRAT500 T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
169	RRAT500	0	2.46	2.46	34000
170	RRAT500	0	2.46	2.46	65000
171	RRAT500	0	2.46	2.46	46000
172	RRAT500	0	2.46	2.46	34000

----------------- VAR=RRAT500 T=10 -----------------

OBS	VAR	T	CONC	INIT	BIOMASS
173	RRAT500	10	3.23	2.46	34000
174	RRAT500	10	2.76	2.46	65000
175	RRAT500	10	2.92	2.46	46000
176	RRAT500	10	3.20	2.46	34000

----------------- VAR=RRAT500 T=15 -----------------

OBS	VAR	T	CONC	INIT	BIOMASS
177	RRAT500	15	3.20	2.46	34000
178	RRAT500	15	2.73	2.46	65000
179	RRAT500	15	2.76	2.46	46000
180	RRAT500	15	2.54	2.46	34000

------------------ VAR=RRAT500 T=21 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
181	RRAT500	21	0.10	2.46	34000
182	RRAT500	21	2.86	2.46	65000
183	RRAT500	21	0.10	2.46	46000
184	RRAT500	21	2.51	2.46	34000

------------------ VAR=RRAT500 T=29 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
185	RRAT500	29	0.1	2.46	34000
186	RRAT500	29	0.1	2.46	65000
187	RRAT500	29	0.1	2.46	46000
188	RRAT500	29	0.1	2.46	34000

------------------ VAR=RRAT500A T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
189	RRAT500A	0	2.12	2.09	14000
190	RRAT500A	0	2.31	2.09	9100
191	RRAT500A	0	2.12	2.09	9000
192	RRAT500A	0	2.06	2.09	14000

------------------ VAR=RRAT500A T=10 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
193	RRAT500A	10	0.1	2.09	14000
194	RRAT500A	10	0.1	2.09	9100
195	RRAT500A	10	0.1	2.09	9000
196	RRAT500A	10	0.1	2.09	14000

-------------------- VAR=RRA2T T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
197	RRA2T	0	2.80	2.95	84000
198	RRA2T	0	3.09	2.95	84000
199	RRA2T	0	2.80	2.95	32000
200	RRA2T	0	3.09	2.95	32000
201	RRA2T	0	2.80	2.95	16000
202	RRA2T	0	3.09	2.95	16000

203	RRA2T	0	2.80	2.95	53000
204	RRA2T	0	3.09	2.95	53000

------------------- VAR=RRA2T T=1 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
205	RRA2T	1	2.65	2.95	84000
206	RRA2T	1	2.78	2.95	32000
207	RRA2T	1	2.57	2.95	16000
208	RRA2T	1	2.68	2.95	53000

------------------- VAR=RRA2T T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
209	RRA2T	2	2.12	2.95	84000
210	RRA2T	2	2.10	2.95	32000
211	RRA2T	2	1.98	2.95	16000
212	RRA2T	2	2.53	2.95	53000

------------------- VAR=RRA2T T=4 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
213	RRA2T	4	2.23	2.95	84000
214	RRA2T	4	2.10	2.95	32000
215	RRA2T	4	2.43	2.95	16000
216	RRA2T	4	2.00	2.95	53000

------------------- VAR=RRA2T T=5 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
217	RRA2T	5	2.31	2.95	84000
218	RRA2T	5	2.49	2.95	32000
219	RRA2T	5	2.48	2.95	16000

------------------- VAR=RRA2T T=12 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
220	RRA2T	12	2.51	2.95	84000
221	RRA2T	12	2.16	2.95	32000
222	RRA2T	12	2.26	2.95	16000
223	RRA2T	12	2.14	2.95	53000

------------------- VAR=RRA2T T=20 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
224	RRA2T	20	2.30	2.95	84000
225	RRA2T	20	2.06	2.95	32000
226	RRA2T	20	2.27	2.95	16000

227	RRA2T	20	2.42	2.95	53000

------------------- VAR=RRA2T T=33 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
228	RRA2T	33	0.1	2.95	84000
229	RRA2T	33	2.6	2.95	32000
230	RRA2T	33	2.4	2.95	16000
231	RRA2T	33	2.4	2.95	53000

------------------- VAR=RRA2T T=40 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
232	RRA2T	40	0.1	2.95	84000
233	RRA2T	40	0.1	2.95	32000
234	RRA2T	40	0.1	2.95	16000
235	RRA2T	40	0.1	2.95	53000

------------------- VAR=RRA2TA T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
236	RRA2TA	0	2.61	2.85	31000
237	RRA2TA	0	2.84	2.85	59000
238	RRA2TA	0	2.73	2.85	43000
239	RRA2TA	0	3.21	2.85	37000

------------------- VAR=RRA2TA T=1 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
240	RRA2TA	1	1.55	2.85	31000
241	RRA2TA	1	2.52	2.85	59000
242	RRA2TA	1	2.21	2.85	43000
243	RRA2TA	1	2.34	2.85	37000

------------------- VAR=RRA2TA T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
244	RRA2TA	2	0.10	2.85	31000
245	RRA2TA	2	1.95	2.85	59000
246	RRA2TA	2	1.27	2.85	43000
247	RRA2TA	2	1.54	2.85	37000

------------------- VAR=RRA2TA T=4 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
248	RRA2TA	4	0.10	2.85	31000
249	RRA2TA	4	2.21	2.85	59000
250	RRA2TA	4	1.04	2.85	43000

251	RRA2TA	4	1.84	2.85	37000

------------------- VAR=RRA2TA T=5 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
252	RRA2TA	5	0.10	2.85	31000
253	RRA2TA	5	2.40	2.85	59000
254	RRA2TA	5	0.75	2.85	37000

------------------- VAR=RRA2TA T=7 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
255	RRA2TA	7	0.10	2.85	31000
256	RRA2TA	7	2.14	2.85	59000
257	RRA2TA	7	0.94	2.85	43000
258	RRA2TA	7	0.75	2.85	37000

------------------- VAR=RRA2TA T=12 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
259	RRA2TA	12	0.1	2.85	31000
260	RRA2TA	12	0.1	2.85	59000
261	RRA2TA	12	0.1	2.85	43000
262	RRA2TA	12	0.1	2.85	37000

------------------- VAR=RRA2T5A T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
263	RRA2T5A	0	2.88	2.68	15000
264	RRA2T5A	0	2.58	2.68	39000
265	RRA2T5A	0	2.58	2.68	37000

------------------- VAR=RRA2T5A T=1 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
266	RRA2T5A	1	2.03	2.68	62000
267	RRA2T5A	1	1.47	2.68	15000
268	RRA2T5A	1	2.23	2.68	39000
269	RRA2T5A	1	1.61	2.68	37000

------------------- VAR=RRA2T5A T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
270	RRA2T5A	2	0.58	2.68	62000
271	RRA2T5A	2	0.10	2.68	15000
272	RRA2T5A	2	1.22	2.68	39000
273	RRA2T5A	2	0.10	2.68	37000

------------------ VAR=RRA2T5A T=4 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
274	RRA2T5A	4	0.10	2.68	62000
275	RRA2T5A	4	0.10	2.68	15000
276	RRA2T5A	4	0.44	2.68	39000
277	RRA2T5A	4	0.10	2.68	37000

------------------ VAR=RRA2T5A T=5 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
278	RRA2T5A	5	0.1	2.68	62000
279	RRA2T5A	5	0.1	2.68	15000
280	RRA2T5A	5	0.1	2.68	39000
281	RRA2T5A	5	0.1	2.68	37000

------------------ VAR=RRA2T500 T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
282	RRA2T500	0	3.16	3.13	37000
283	RRA2T500	0	3.09	3.13	37000
284	RRA2T500	0	3.16	3.13	21000
285	RRA2T500	0	3.09	3.13	21000
286	RRA2T500	0	3.16	3.13	94000
287	RRA2T500	0	3.09	3.13	94000
288	RRA2T500	0	3.16	3.13	160000
289	RRA2T500	0	3.09	3.13	160000

------------------ VAR=RRA2T500 T=1 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
290	RRA2T500	1	2.79	3.13	37000
291	RRA2T500	1	2.66	3.13	21000
292	RRA2T500	1	2.65	3.13	94000
293	RRA2T500	1	2.84	3.13	160000

------------------ VAR=RRA2T500 T=2 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
294	RRA2T500	2	1.98	3.13	37000
295	RRA2T500	2	2.08	3.13	21000
296	RRA2T500	2	2.45	3.13	94000
297	RRA2T500	2	2.56	3.13	160000

------------------ VAR=RRA2T500 T=4 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
298	RRA2T500	4	2.17	3.13	37000

299	RRA2T500	4	2.29	3.13	21000
300	RRA2T500	4	2.27	3.13	160000

------------------ VAR=RRA2T500 T=5 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
301	RRA2T500	5	2.45	3.13	37000
302	RRA2T500	5	3.17	3.13	21000
303	RRA2T500	5	2.51	3.13	94000
304	RRA2T500	5	2.55	3.13	160000

------------------ VAR=RRA2T500 T=12 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
305	RRA2T500	12	2.40	3.13	37000
306	RRA2T500	12	2.38	3.13	21000
307	RRA2T500	12	2.30	3.13	94000
308	RRA2T500	12	2.44	3.13	160000

------------------ VAR=RRA2T500 T=20 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
309	RRA2T500	20	1.78	3.13	37000
310	RRA2T500	20	2.13	3.13	21000
311	RRA2T500	20	2.34	3.13	94000

------------------ VAR=RRA2T500 T=33 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
312	RRA2T500	33	1.78	3.13	37000
313	RRA2T500	33	1.80	3.13	21000
314	RRA2T500	33	0.10	3.13	94000
315	RRA2T500	33	0.10	3.13	160000

------------------ VAR=RRA2T500 T=40 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
316	RRA2T500	40	0.1	3.13	37000
317	RRA2T500	40	0.1	3.13	21000
318	RRA2T500	40	0.1	3.13	94000
319	RRA2T500	40	0.1	3.13	160000

------------------- VAR=RR1C T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
320	RR1C	0	2.63	2.61	.
321	RR1C	0	2.57	2.61	.
322	RR1C	0	2.48	2.61	.

323	RR1C	0	2.41	2.61	.
324	RR1C	0	2.61	2.61	.
325	RR1C	0	2.81	2.61	.
326	RR1C	0	2.52	2.61	.
327	RR1C	0	2.82	2.61	.

-------------------- VAR=RR1C T=8 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
328	RR1C	8	2.73	2.61	.
329	RR1C	8	2.66	2.61	.
330	RR1C	8	2.64	2.61	.
331	RR1C	8	2.73	2.61	.
332	RR1C	8	2.67	2.61	.
333	RR1C	8	2.73	2.61	.
334	RR1C	8	2.67	2.61	.
335	RR1C	8	2.65	2.61	.
336	RR1C	8	2.48	2.61	.
337	RR1C	8	2.46	2.61	.

-------------------- VAR=RR1C T=24 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
338	RR1C	24	2.47	2.61	.
339	RR1C	24	2.58	2.61	.
340	RR1C	24	2.49	2.61	.
341	RR1C	24	2.44	2.61	.
342	RR1C	24	2.35	2.61	.
343	RR1C	24	2.42	2.61	.

-------------------- VAR=RR1C T=31 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
344	RR1C	31	2.55	2.61	.
345	RR1C	31	2.64	2.61	.
346	RR1C	31	2.45	2.61	.
347	RR1C	31	2.46	2.61	.

------------------- VAR=RR1C500 T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
348	RR1C500	0	2.65	2.35	.
349	RR1C500	0	2.48	2.35	.
350	RR1C500	0	2.48	2.35	.
351	RR1C500	0	2.13	2.35	.
352	RR1C500	0	2.33	2.35	.
353	RR1C500	0	2.11	2.35	.
354	RR1C500	0	2.40	2.35	.
355	RR1C500	0	2.14	2.35	.

-------------------- VAR=RR1C500 T=8 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
356	RR1C500	8	2.59	2.35	.
357	RR1C500	8	2.53	2.35	.
358	RR1C500	8	2.62	2.35	.
359	RR1C500	8	2.66	2.35	.
360	RR1C500	8	2.60	2.35	.
361	RR1C500	8	2.56	2.35	.
362	RR1C500	8	2.64	2.35	.
363	RR1C500	8	2.68	2.35	.
364	RR1C500	8	2.73	2.35	.

-------------------- VAR=RR1C500 T=24 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
365	RR1C500	24	2.28	2.35	.
366	RR1C500	24	2.27	2.35	.
367	RR1C500	24	2.33	2.35	.
368	RR1C500	24	2.46	2.35	.
369	RR1C500	24	2.24	2.35	.
370	RR1C500	24	2.57	2.35	.

-------------------- VAR=RR1C500 T=31 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
371	RR1C500	31	2.66	2.35	.
372	RR1C500	31	2.45	2.35	.
373	RR1C500	31	2.38	2.35	.
374	RR1C500	31	2.38	2.35	.
375	RR1C500	31	2.60	2.35	.

-------------------- VAR=RR1SS T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
376	RR1SS	0	2.40	2.49	19000
377	RR1SS	0	2.58	2.49	19000
378	RR1SS	0	2.40	2.49	10000
379	RR1SS	0	2.58	2.49	10000
380	RR1SS	0	2.40	2.49	17000
381	RR1SS	0	2.58	2.49	17000
382	RR1SS	0	2.40	2.49	150000
383	RR1SS	0	2.58	2.49	150000

-------------------- VAR=RR1SS T=4 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
384	RR1SS	4	2.37	2.49	19000
385	RR1SS	4	2.36	2.49	19000

386	RR1SS	4	2.35	2.49	10000
387	RR1SS	4	2.21	2.49	10000
388	RR1SS	4	2.48	2.49	17000
389	RR1SS	4	2.52	2.49	17000
390	RR1SS	4	2.75	2.49	150000
391	RR1SS	4	2.46	2.49	150000

------------------- VAR=RR1SS T=8 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
392	RR1SS	8	2.38	2.49	19000
393	RR1SS	8	2.31	2.49	19000
394	RR1SS	8	2.52	2.49	10000
395	RR1SS	8	2.56	2.49	10000
396	RR1SS	8	2.39	2.49	17000
397	RR1SS	8	2.33	2.49	17000
398	RR1SS	8	2.57	2.49	150000
399	RR1SS	8	2.51	2.49	150000

------------------- VAR=RR1SS T=11 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
400	RR1SS	11	2.48	2.49	19000
401	RR1SS	11	2.38	2.49	19000
402	RR1SS	11	2.37	2.49	10000
403	RR1SS	11	2.44	2.49	10000
404	RR1SS	11	2.55	2.49	17000
405	RR1SS	11	2.58	2.49	17000
406	RR1SS	11	2.34	2.49	150000
407	RR1SS	11	2.46	2.49	150000

------------------- VAR=RR1SS T=15 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
408	RR1SS	15	2.54	2.49	19000
409	RR1SS	15	2.43	2.49	19000
410	RR1SS	15	1.81	2.49	10000
411	RR1SS	15	1.57	2.49	10000
412	RR1SS	15	2.41	2.49	17000
413	RR1SS	15	2.39	2.49	17000
414	RR1SS	15	2.44	2.49	150000
415	RR1SS	15	2.45	2.49	150000

------------------- VAR=RR1SS T=18 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
416	RR1SS	18	2.74	2.49	19000
417	RR1SS	18	2.84	2.49	19000
418	RR1SS	18	0.28	2.49	10000
419	RR1SS	18	0.31	2.49	10000

420	RR1SS	18	2.69	2.49	17000
421	RR1SS	18	2.73	2.49	17000
422	RR1SS	18	0.51	2.49	150000
423	RR1SS	18	0.49	2.49	150000

------------------ VAR=RR1SS T=24 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
424	RR1SS	24	2.32	2.49	19000
425	RR1SS	24	0.10	2.49	10000
426	RR1SS	24	0.10	2.49	17000
427	RR1SS	24	0.10	2.49	150000

------------------ VAR=RR1SS T=31 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
428	RR1SS	31	2.31	2.49	19000
429	RR1SS	31	0.10	2.49	10000
430	RR1SS	31	0.10	2.49	17000
431	RR1SS	31	0.10	2.49	150000

------------------ VAR=RR1SS T=35 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
432	RR1SS	35	2.0	2.49	19000
433	RR1SS	35	0.1	2.49	10000
434	RR1SS	35	0.1	2.49	17000
435	RR1SS	35	0.1	2.49	150000

------------------ VAR=RR1T T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
436	RR1T	0	2.57	2.74	24000
437	RR1T	0	2.61	2.74	24000
438	RR1T	0	3.05	2.74	24000
439	RR1T	0	2.57	2.74	35000
440	RR1T	0	2.61	2.74	35000
441	RR1T	0	3.05	2.74	35000
442	RR1T	0	2.57	2.74	29000
443	RR1T	0	2.61	2.74	29000
444	RR1T	0	3.05	2.74	29000
445	RR1T	0	2.57	2.74	19000

------------------ VAR=RR1T T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
446	RR1T	0	2.61	2.74	19000
447	RR1T	0	3.05	2.74	19000

------------------- VAR=RR1T T=4 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
448	RR1T	4	3.00	2.74	24000
449	RR1T	4	2.93	2.74	24000
450	RR1T	4	2.45	2.74	35000
451	RR1T	4	2.50	2.74	35000
452	RR1T	4	2.94	2.74	29000
453	RR1T	4	2.90	2.74	29000
454	RR1T	4	2.36	2.74	19000
455	RR1T	4	2.48	2.74	19000

------------------- VAR=RR1T T=8 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
456	RR1T	8	2.54	2.74	24000
457	RR1T	8	2.64	2.74	24000
458	RR1T	8	2.70	2.74	35000
459	RR1T	8	2.72	2.74	35000
460	RR1T	8	2.73	2.74	29000
461	RR1T	8	2.72	2.74	29000
462	RR1T	8	2.37	2.74	19000
463	RR1T	8	2.40	2.74	19000

------------------- VAR=RR1T T=11 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
464	RR1T	11	2.32	2.74	24000
465	RR1T	11	2.40	2.74	24000
466	RR1T	11	2.50	2.74	35000
467	RR1T	11	2.48	2.74	35000
468	RR1T	11	2.43	2.74	29000
469	RR1T	11	2.53	2.74	29000
470	RR1T	11	2.44	2.74	19000
471	RR1T	11	2.40	2.74	19000

------------------- VAR=RR1T T=15 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
472	RR1T	15	2.65	2.74	24000
473	RR1T	15	2.60	2.74	24000
474	RR1T	15	2.33	2.74	35000
475	RR1T	15	2.28	2.74	35000
476	RR1T	15	2.36	2.74	29000
477	RR1T	15	2.41	2.74	29000
478	RR1T	15	2.44	2.74	19000
479	RR1T	15	2.45	2.74	19000

------------------- VAR=RR1T T=18 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
480	RR1T	18	2.90	2.74	24000
481	RR1T	18	2.90	2.74	24000
482	RR1T	18	2.93	2.74	35000
483	RR1T	18	2.89	2.74	35000
484	RR1T	18	2.77	2.74	29000
485	RR1T	18	2.59	2.74	29000
486	RR1T	18	0.60	2.74	19000
487	RR1T	18	0.59	2.74	19000

------------------- VAR=RR1T T=24 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
488	RR1T	24	2.13	2.74	24000
489	RR1T	24	2.39	2.74	24000
490	RR1T	24	2.28	2.74	35000
491	RR1T	24	2.22	2.74	35000
492	RR1T	24	1.18	2.74	29000
493	RR1T	24	1.16	2.74	29000
494	RR1T	24	0.21	2.74	19000
495	RR1T	24	0.21	2.74	19000

------------------- VAR=RR1T T=31 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
496	RR1T	31	2.44	2.74	24000
497	RR1T	31	2.49	2.74	35000
498	RR1T	31	0.10	2.74	29000
499	RR1T	31	0.10	2.74	19000

------------------- VAR=RR1T T=35 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
500	RR1T	35	2.15	2.74	24000
501	RR1T	35	2.23	2.74	35000
502	RR1T	35	0.10	2.74	29000
503	RR1T	35	0.10	2.74	19000

------------------- VAR=RR1T500 T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
504	RR1T500	0	3.14	2.95	12000
505	RR1T500	0	2.63	2.95	12000
506	RR1T500	0	3.26	2.95	12000
507	RR1T500	0	3.01	2.95	12000
508	RR1T500	0	2.63	2.95	12000
509	RR1T500	0	3.14	2.95	52000
510	RR1T500	0	2.63	2.95	52000
511	RR1T500	0	3.26	2.95	52000

512	RR1T500	0	3.01	2.95	52000
513	RR1T500	0	2.63	2.95	52000
514	RR1T500	0	3.14	2.95	77000
515	RR1T500	0	2.63	2.95	77000
516	RR1T500	0	3.26	2.95	77000
517	RR1T500	0	3.01	2.95	77000
518	RR1T500	0	2.63	2.95	77000
519	RR1T500	0	3.14	2.95	28000
520	RR1T500	0	2.63	2.95	28000
521	RR1T500	0	3.26	2.95	28000
522	RR1T500	0	3.01	2.95	28000
523	RR1T500	0	2.63	2.95	28000

------------------ VAR=RR1T500 T=4 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
524	RR1T500	4	2.67	2.95	12000
525	RR1T500	4	2.62	2.95	12000
526	RR1T500	4	2.80	2.95	52000
527	RR1T500	4	2.62	2.95	52000
528	RR1T500	4	2.85	2.95	77000
529	RR1T500	4	2.82	2.95	77000
530	RR1T500	4	2.77	2.95	28000
531	RR1T500	4	2.68	2.95	28000

------------------ VAR=RR1T500 T=8 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
532	RR1T500	8	2.40	2.95	12000
533	RR1T500	8	2.41	2.95	12000
534	RR1T500	8	2.25	2.95	52000
535	RR1T500	8	2.27	2.95	52000
536	RR1T500	8	2.36	2.95	77000
537	RR1T500	8	2.32	2.95	77000
538	RR1T500	8	2.46	2.95	28000
539	RR1T500	8	2.48	2.95	28000

------------------ VAR=RR1T500 T=11 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
540	RR1T500	11	2.48	2.95	12000
541	RR1T500	11	2.52	2.95	12000
542	RR1T500	11	2.48	2.95	52000
543	RR1T500	11	2.47	2.95	52000
544	RR1T500	11	2.46	2.95	77000
545	RR1T500	11	2.52	2.95	77000
546	RR1T500	11	2.49	2.95	28000
547	RR1T500	11	2.48	2.95	28000

------------------ VAR=RR1T500 T=15 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
548	RR1T500	15	2.54	2.95	12000
549	RR1T500	15	2.57	2.95	12000
550	RR1T500	15	2.42	2.95	52000
551	RR1T500	15	2.43	2.95	52000
552	RR1T500	15	2.44	2.95	77000
553	RR1T500	15	2.48	2.95	77000
554	RR1T500	15	1.08	2.95	28000
555	RR1T500	15	1.16	2.95	28000

------------------ VAR=RR1T500 T=18 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
556	RR1T500	18	2.78	2.95	12000
557	RR1T500	18	2.42	2.95	12000
558	RR1T500	18	0.39	2.95	52000
559	RR1T500	18	0.30	2.95	52000
560	RR1T500	18	1.53	2.95	77000
561	RR1T500	18	1.50	2.95	77000
562	RR1T500	18	0.28	2.95	28000
563	RR1T500	18	0.31	2.95	28000

------------------ VAR=RR1T500 T=24 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
564	RR1T500	24	0.1	2.95	12000
565	RR1T500	24	0.1	2.95	52000
566	RR1T500	24	0.1	2.95	77000
567	RR1T500	24	0.1	2.95	28000

------------------- VAR=RR2C T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
568	RR2C	0	2.19	2.2	.
569	RR2C	0	2.40	2.2	.
570	RR2C	0	2.04	2.2	.
571	RR2C	0	2.18	2.2	.

------------------- VAR=RR2C T=7 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
572	RR2C	7	2.61	2.2	.
573	RR2C	7	3.18	2.2	.
574	RR2C	7	2.74	2.2	.
575	RR2C	7	2.56	2.2	.

------------------- VAR=RR2C T=14 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS

576	RR2C	14	3.05	2.2	.
577	RR2C	14	2.44	2.2	.
578	RR2C	14	2.72	2.2	.
579	RR2C	14	2.75	2.2	.

------------------- VAR=RR2C500 T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
580	RR2C500	0	2.66	2.39	.
581	RR2C500	0	2.34	2.39	.
582	RR2C500	0	2.19	2.39	.
583	RR2C500	0	2.37	2.39	.

------------------- VAR=RR2C500 T=7 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
584	RR2C500	7	2.65	2.39	.
585	RR2C500	7	3.06	2.39	.
586	RR2C500	7	2.65	2.39	.
587	RR2C500	7	2.82	2.39	.

------------------ VAR=RR2C500 T=14 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
588	RR2C500	14	2.70	2.39	.
589	RR2C500	14	3.12	2.39	.
590	RR2C500	14	2.88	2.39	.
591	RR2C500	14	2.94	2.39	.

-------------------- VAR=RR2SS T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
592	RR2SS	0	2.24	2.34	62000
593	RR2SS	0	2.23	2.34	62000
594	RR2SS	0	2.24	2.34	130000
595	RR2SS	0	2.23	2.34	130000
596	RR2SS	0	2.24	2.34	44000
597	RR2SS	0	2.23	2.34	44000
598	RR2SS	0	2.24	2.34	23000
599	RR2SS	0	2.23	2.34	23000

------------------- VAR=RR2SS T=4 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
600	RR2SS	4	2.18	2.34	62000
601	RR2SS	4	2.27	2.34	130000
602	RR2SS	4	2.14	2.34	44000
603	RR2SS	4	2.06	2.34	23000

------------------- VAR=RR2SS T=7 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
604	RR2SS	7	2.68	2.34	62000
605	RR2SS	7	2.64	2.34	130000
606	RR2SS	7	2.81	2.34	44000
607	RR2SS	7	2.59	2.34	23000

------------------- VAR=RR2SS T=11 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
608	RR2SS	11	2.62	2.34	62000
609	RR2SS	11	0.10	2.34	130000
610	RR2SS	11	2.88	2.34	44000
611	RR2SS	11	3.10	2.34	23000

------------------- VAR=RR2SS T=16 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
612	RR2SS	16	0.10	2.34	62000
613	RR2SS	16	0.10	2.34	130000
614	RR2SS	16	0.10	2.34	44000
615	RR2SS	16	2.43	2.34	23000

------------------- VAR=RR2SS T=19 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
616	RR2SS	19	0.1	2.34	62000
617	RR2SS	19	0.1	2.34	130000
618	RR2SS	19	0.1	2.34	44000
619	RR2SS	19	0.1	2.34	23000

-------------------- VAR=RR2T T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
620	RR2T	0	2.25	2.25	26000
621	RR2T	0	2.25	2.25	26000
622	RR2T	0	2.25	2.25	75000
623	RR2T	0	2.25	2.25	75000
624	RR2T	0	2.25	2.25	32000
625	RR2T	0	2.25	2.25	32000
626	RR2T	0	2.25	2.25	38000
627	RR2T	0	2.25	2.25	38000

-------------------- VAR=RR2T T=4 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS

628	RR2T	4	1.95	2.25	26000
629	RR2T	4	2.27	2.25	75000
630	RR2T	4	2.26	2.25	32000
631	RR2T	4	2.07	2.25	38000

-------------------- VAR=RR2T T=7 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
632	RR2T	7	2.44	2.25	26000
633	RR2T	7	2.95	2.25	75000
634	RR2T	7	2.53	2.25	32000
635	RR2T	7	2.52	2.25	38000

-------------------- VAR=RR2T T=11 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
636	RR2T	11	3.15	2.25	26000
637	RR2T	11	2.94	2.25	75000
638	RR2T	11	0.16	2.25	32000
639	RR2T	11	0.10	2.25	38000

-------------------- VAR=RR2T T=16 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
640	RR2T	16	0.1	2.25	26000
641	RR2T	16	3.6	2.25	75000
642	RR2T	16	0.1	2.25	32000
643	RR2T	16	0.1	2.25	38000

-------------------- VAR=RR2T T=19 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
644	RR2T	19	0.10	2.25	26000
645	RR2T	19	1.91	2.25	75000
646	RR2T	19	0.10	2.25	32000
647	RR2T	19	0.10	2.25	38000

-------------------- VAR=RR2T T=23 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
648	RR2T	23	0.1	2.25	26000
649	RR2T	23	0.1	2.25	75000
650	RR2T	23	0.1	2.25	32000
651	RR2T	23	0.1	2.25	38000

-------------------- VAR=RR2T500 T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS

652	RR2T500	0	2.27	2.34	23000
653	RR2T500	0	2.40	2.34	23000
654	RR2T500	0	2.27	2.34	45000
655	RR2T500	0	2.40	2.34	45000
656	RR2T500	0	2.27	2.34	38000
657	RR2T500	0	2.40	2.34	38000
658	RR2T500	0	2.27	2.34	125000
659	RR2T500	0	2.40	2.34	125000

------------------ VAR=RR2T500 T=4 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
660	RR2T500	4	2.03	2.34	23000
661	RR2T500	4	2.25	2.34	45000
662	RR2T500	4	2.17	2.34	38000
663	RR2T500	4	2.15	2.34	125000

------------------ VAR=RR2T500 T=7 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
664	RR2T500	7	3.03	2.34	23000
665	RR2T500	7	2.95	2.34	45000
666	RR2T500	7	2.79	2.34	38000
667	RR2T500	7	2.63	2.34	125000

------------------ VAR=RR2T500 T=11 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
668	RR2T500	11	3.07	2.34	23000
669	RR2T500	11	0.10	2.34	45000
670	RR2T500	11	3.22	2.34	38000
671	RR2T500	11	0.12	2.34	125000

------------------ VAR=RR2T500 T=16 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
672	RR2T500	16	3.26	2.34	23000
673	RR2T500	16	0.10	2.34	45000
674	RR2T500	16	3.34	2.34	38000
675	RR2T500	16	0.10	2.34	125000

------------------ VAR=RR2T500 T=19 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
676	RR2T500	19	3.13	2.34	23000
677	RR2T500	19	0.10	2.34	45000
678	RR2T500	19	3.30	2.34	38000
679	RR2T500	19	0.10	2.34	125000

------------------ VAR=RR2T500 T=23 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
680	RR2T500	23	0.31	2.34	23000
681	RR2T500	23	0.10	2.34	45000
682	RR2T500	23	0.10	2.34	38000
683	RR2T500	23	0.10	2.34	125000

------------------ VAR=RR2T500 T=33 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
684	RR2T500	33	0.1	2.34	23000
685	RR2T500	33	0.1	2.34	45000

------------------ VAR=RR2T500 T=39 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
686	RR2T500	39	0.1	2.34	38000
687	RR2T500	39	0.1	2.34	125000

-------------------- VAR=TRAT T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
688	TRAT	0	2.85	2.875	70000
689	TRAT	0	2.90	2.875	70000
690	TRAT	0	2.85	2.875	270000
691	TRAT	0	2.90	2.875	270000
692	TRAT	0	2.85	2.875	35000
693	TRAT	0	2.90	2.875	35000
694	TRAT	0	2.85	2.875	27000
695	TRAT	0	2.90	2.875	27000

-------------------- VAR=TRAT T=1 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
696	TRAT	1	2.30	2.875	70000
697	TRAT	1	2.07	2.875	270000
698	TRAT	1	2.04	2.875	35000
699	TRAT	1	2.67	2.875	27000

-------------------- VAR=TRAT T=3 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
700	TRAT	3	2.62	2.875	70000
701	TRAT	3	2.08	2.875	270000
702	TRAT	3	2.53	2.875	35000
703	TRAT	3	2.31	2.875	27000

------------------- VAR=TRAT T=4 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
704	TRAT	4	2.00	2.875	70000
705	TRAT	4	2.18	2.875	35000
706	TRAT	4	2.21	2.875	27000

------------------- VAR=TRAT T=6 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
707	TRAT	6	0.1	2.875	70000
708	TRAT	6	0.1	2.875	270000
709	TRAT	6	0.1	2.875	35000
710	TRAT	6	0.1	2.875	27000

------------------- VAR=TRATA T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
711	TRATA	0	2.39	2.385	110000
712	TRATA	0	2.35	2.385	110000
713	TRATA	0	2.36	2.385	190000
714	TRATA	0	2.44	2.385	97000

------------------- VAR=TRATA T=1 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
715	TRATA	1	1.70	2.385	110000
716	TRATA	1	2.05	2.385	110000
717	TRATA	1	2.30	2.385	190000
718	TRATA	1	1.89	2.385	97000

------------------- VAR=TRATA T=3 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
719	TRATA	3	0.1	2.385	110000
720	TRATA	3	0.1	2.385	110000
721	TRATA	3	0.1	2.385	190000
722	TRATA	3	0.1	2.385	97000

------------------- VAR=TRAT500 T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
723	TRAT500	0	3.72	3.48	100000
724	TRAT500	0	3.24	3.48	100000
725	TRAT500	0	3.72	3.48	140000
726	TRAT500	0	3.24	3.48	140000
727	TRAT500	0	3.72	3.48	43000
728	TRAT500	0	3.24	3.48	43000

729	TRAT500	0	3.72	3.48	82000
730	TRAT500	0	3.24	3.48	82000

------------------ VAR=TRAT500 T=1 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
731	TRAT500	1	2.30	3.48	100000
732	TRAT500	1	2.47	3.48	140000
733	TRAT500	1	2.51	3.48	43000
734	TRAT500	1	3.54	3.48	82000

------------------ VAR=TRAT500 T=3 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
735	TRAT500	3	2.26	3.48	100000
736	TRAT500	3	2.38	3.48	140000
737	TRAT500	3	2.11	3.48	43000
738	TRAT500	3	2.39	3.48	82000

------------------ VAR=TRAT500 T=4 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
739	TRAT500	4	2.47	3.48	100000
740	TRAT500	4	1.71	3.48	140000
741	TRAT500	4	2.11	3.48	43000
742	TRAT500	4	2.54	3.48	82000

------------------ VAR=TRAT500 T=6 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
743	TRAT500	6	0.1	3.48	100000
744	TRAT500	6	0.1	3.48	140000
745	TRAT500	6	0.1	3.48	43000
746	TRAT500	6	0.1	3.48	82000

------------------ VAR=TRAT500A T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
747	TRAT500A	0	2.33	2.46	180000
748	TRAT500A	0	2.26	2.46	150000
749	TRAT500A	0	2.70	2.46	180000
750	TRAT500A	0	2.55	2.46	190000

------------------ VAR=TRAT500A T=1 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
751	TRAT500A	1	1.83	2.46	180000
752	TRAT500A	1	2.00	2.46	150000

753	TRAT500A	1	1.78	2.46	180000
754	TRAT500A	1	2.04	2.46	190000

------------------- VAR=TRAT500A T=3 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
755	TRAT500A	3	0.1	2.46	180000
756	TRAT500A	3	0.1	2.46	150000
757	TRAT500A	3	0.1	2.46	180000
758	TRAT500A	3	0.1	2.46	190000

---------------------- VAR=TRC T=0 ----------------------

OBS	VAR	T	CONC	INIT	BIOMASS
759	TRC	0	2.56	2.18	.
760	TRC	0	2.08	2.18	.
761	TRC	0	1.96	2.18	.
762	TRC	0	2.13	2.18	.

---------------------- VAR=TRC T=9 ----------------------

OBS	VAR	T	CONC	INIT	BIOMASS
763	TRC	9	2.76	2.18	.
764	TRC	9	2.62	2.18	.
765	TRC	9	2.42	2.18	.

--------------------- VAR=TRC T=16 ---------------------

OBS	VAR	T	CONC	INIT	BIOMASS
766	TRC	16	2.12	2.18	.
767	TRC	16	2.39	2.18	.
768	TRC	16	2.66	2.18	.
769	TRC	16	2.52	2.18	.

--------------------- VAR=TRC T=35 ---------------------

OBS	VAR	T	CONC	INIT	BIOMASS
770	TRC	35	3.28	2.18	.
771	TRC	35	2.79	2.18	.

-------------------- VAR=TRC500 T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
772	TRC500	0	1.64	1.86	.
773	TRC500	0	1.96	1.86	.
774	TRC500	0	1.64	1.86	.
775	TRC500	0	2.20	1.86	.

------------------- VAR=TRC500 T=9 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
776	TRC500	9	2.15	1.86	.
777	TRC500	9	2.13	1.86	.
778	TRC500	9	2.19	1.86	.
779	TRC500	9	3.00	1.86	.

------------------- VAR=TRC500 T=16 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
780	TRC500	16	2.66	1.86	.
781	TRC500	16	2.17	1.86	.
782	TRC500	16	2.05	1.86	.
783	TRC500	16	2.55	1.86	.

------------------- VAR=TRC500 T=35 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
784	TRC500	35	2.83	1.86	.
785	TRC500	35	2.61	1.86	.
786	TRC500	35	2.53	1.86	.
787	TRC500	35	2.86	1.86	.

------------------- VAR=TRNSS T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
788	TRNSS	0	1.97	2.29	400000
789	TRNSS	0	2.06	2.29	400000
790	TRNSS	0	1.97	2.29	290000
791	TRNSS	0	2.06	2.29	290000
792	TRNSS	0	1.97	2.29	850000
793	TRNSS	0	2.06	2.29	850000
794	TRNSS	0	1.97	2.29	470000
795	TRNSS	0	2.06	2.29	470000

------------------- VAR=TRNSS T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
796	TRNSS	2	2.35	2.29	400000
797	TRNSS	2	2.04	2.29	290000
798	TRNSS	2	2.29	2.29	470000

------------------- VAR=TRNSS T=6 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
799	TRNSS	6	2.07	2.29	400000
800	TRNSS	6	1.93	2.29	290000

801	TRNSS	6	1.40	2.29	850000
802	TRNSS	6	1.94	2.29	470000

------------------- VAR=TRNSS T=9 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
803	TRNSS	9	2.35	2.29	400000
804	TRNSS	9	2.90	2.29	290000
805	TRNSS	9	2.47	2.29	850000

------------------- VAR=TRNSS T=13 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
806	TRNSS	13	2.38	2.29	400000
807	TRNSS	13	2.64	2.29	290000
808	TRNSS	13	2.55	2.29	850000

------------------- VAR=TRNSS T=21 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
809	TRNSS	21	2.81	2.29	400000
810	TRNSS	21	2.62	2.29	290000
811	TRNSS	21	2.98	2.29	850000
812	TRNSS	21	2.94	2.29	470000

------------------- VAR=TRNSS T=35 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
813	TRNSS	35	2.80	2.29	400000
814	TRNSS	35	2.90	2.29	290000
815	TRNSS	35	2.74	2.29	850000
816	TRNSS	35	2.90	2.29	470000

------------------- VAR=TRSS T=0 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
817	TRSS	0	2.24	2.43	110000
818	TRSS	0	2.62	2.43	110000
819	TRSS	0	2.24	2.43	63000
820	TRSS	0	2.62	2.43	63000
821	TRSS	0	2.24	2.43	94000
822	TRSS	0	2.62	2.43	94000
823	TRSS	0	2.24	2.43	72000
824	TRSS	0	2.62	2.43	72000

------------------- VAR=TRSS T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS

OBS	VAR	T	CONC	INIT	BIOMASS
825	TRSS	2	2.09	2.43	110000
826	TRSS	2	1.95	2.43	63000
827	TRSS	2	2.04	2.43	72000

-------------------- VAR=TRSS T=6 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
828	TRSS	6	0.1	2.43	110000
829	TRSS	6	0.1	2.43	63000
830	TRSS	6	0.1	2.43	94000
831	TRSS	6	0.1	2.43	72000

-------------------- VAR=TRT T=0 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
832	TRT	0	1.66	2.08	260000
833	TRT	0	2.05	2.08	260000
834	TRT	0	1.66	2.08	380000
835	TRT	0	2.05	2.08	380000
836	TRT	0	1.66	2.08	150000
837	TRT	0	2.05	2.08	150000
838	TRT	0	1.66	2.08	190000
839	TRT	0	2.05	2.08	190000

-------------------- VAR=TRT T=2 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
840	TRT	2	1.87	2.08	260000
841	TRT	2	1.91	2.08	380000
842	TRT	2	2.05	2.08	150000
843	TRT	2	1.93	2.08	190000

-------------------- VAR=TRT T=6 --------------------

OBS	VAR	T	CONC	INIT	BIOMASS
844	TRT	6	0.1	2.08	260000
845	TRT	6	0.1	2.08	380000
846	TRT	6	0.1	2.08	150000
847	TRT	6	0.1	2.08	190000

------------------ VAR=TRT500 T=0 ------------------

OBS	VAR	T	CONC	INIT	BIOMASS
848	TRT500	0	2.35	2.02	44000
849	TRT500	0	1.87	2.02	44000
850	TRT500	0	1.90	2.02	44000
851	TRT500	0	2.35	2.02	48000
852	TRT500	0	1.87	2.02	48000
853	TRT500	0	1.90	2.02	48000

854	TRT500	0	2.35	2.02	80000
855	TRT500	0	1.87	2.02	80000
856	TRT500	0	1.90	2.02	80000
857	TRT500	0	2.35	2.02	220000
858	TRT500	0	1.87	2.02	220000
859	TRT500	0	1.90	2.02	220000

------------------- VAR=TRT500 T=2 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
860	TRT500	2	2.09	2.02	44000
861	TRT500	2	2.01	2.02	48000
862	TRT500	2	1.88	2.02	80000
863	TRT500	2	1.82	2.02	220000

------------------- VAR=TRT500 T=6 -------------------

OBS	VAR	T	CONC	INIT	BIOMASS
864	TRT500	6	0.1	2.02	44000
865	TRT500	6	0.1	2.02	48000
866	TRT500	6	0.1	2.02	80000
867	TRT500	6	0.1	2.02	220000

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