THE EFFECT OF MALATHION ON A BACTERIAL POPULATION

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

William Wesley Barrow

May 1972

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This thesis is dedicated to my beloved and devoted wife, Kathy, whose understanding and encouragement has enabled me to achieve this goal.

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ABSTRACT

An organophosphate insecticide, malathion (technical grade, 95%), which is used for mosquito fogging operations by the Harris County Mosquito Control (Houston, Texas), was found to be stimulatory or inhibitory to the bacterial population of Hermann Park Lake. The greatest stimulatory effect was observed with 0.5% and 1.0% concentration of malathion. Marked inhibition was observed with 5.0% concentration of malathion.

The growth of Houston Isolates #1, #2, and #6 was stimulated by 0.5% and 1.0% concentrations of technical grade malathion (95%). Houston Isolate #10 was inhibited by 0.5 % and 1.0% concentrations. All pure culture isolates were inhibited by 5.0% concentration.

Houston Isolates #1, #2, and #6 utilized malathion monoacid to support growth. Houston Isolate #11 utilized malathion and malathion monoacid to support its growth. The growth of Houston Isolate #11 was inhibited by malathion diacid.

The effect of a commonly used pesticide on certain microorganisms was revealed in this investigation. The fact that bacteria utilized malathion and its products is strongly suggestive that these bacteria can degrade this pesticide.

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INTRODUCTION

In recent years man has become aware of the ever increasing problem of pollution and its ultimate effect on him and his descendants. A significant contributing factor to this pollution problem is the increasing use of pesticides. It is vital and necessary for man to use certain pesticides to relieve unfavorable consequences of nature. Certain insects are serious threats to maximum agriculture production, and certain others are important vectors in the transmission of dreaded communicable diseases. Agricultural products and prevention of communicable diseases are essential to maintain a viable life system, yet the application of pesticides must be kept under strict control to prevent the nuisance of creating a pollution problem.

Among those insecticides that have received the most attention by investigators are the organophosphates (Metcalf, 1955). Parathion, diazinon, dibrom, fenthion, dursban, and malathion are several organophosphates that have been used (Pimentel, 1971). Malathion has a relatively low toxicity to mammals, therefore it is one of the most desirable organophosphates to use as an insecticide (Metcalf, 1955).

Malathion is toxic to insects, yet the immediate cause of death

is not known (O'Brien, 1967). In reporting certain findings Mengle and Casida (1958), O'Brien (1961b), and O'Brien (1967) suggested that the main cause of death in insects, caused by organophosphate poisoning, is due to cholinesterase inhibition. Malathion has been shown to be toxic to several other animals, such as: grass shrimp and sand shrimp (Eisler, 1969); tadpoles (Sanders, 1970); a variety of fishes (Macek and McAllister, 1970); and birds (Tucker and Crabtree, 1970). Ben-Dyke, et al. (1970), observed that malathion was toxic for rats. Toxicity of malathion in mammals was due to the inhibition of cholinesterase (Mitchell, 1966; and O'Brien, 1959).

Limited studies have described the toxicity of malathion to microorganisms. Steelman, et al. (1967), found several insecticides, including malathion, to be toxic to the bacterial population of waste disposal lagoons.

The degradation of malathion has been studied extensively in mammalian and insect systems. In mammals the major means of degradation is by carboxyesterase action, in which malathion monoacid and malathion diacid are products (Krueger and O'Brien, 1959; Knaak and O'Brien, 1960; O'Brien, et al., 1961a; and Chen, et al., 1969). In insects the major degradation products are those resulting from phosphatase activity (Krueger and O'Brien, 1959).

Limited studies concerned with the degradation of malathion by

microorganisms have been made. Yasuno, et al. (1965), reported an inactivation of malathion by bacteria in polluted water, but did not give any means of degradation. Matsumura and Boush (1966) isolated a soil fungus, Trichoderma viride, and a soil bacteria, Pseudomonas sp., which degraded malathion. In their survey of a number of Trichoderma viride variants, the breakdown of malathion was attributed to the action of carboxylesterases. Halvorson, et al. (1971), revealed that malathion was quickly metabolized by resting cell suspensions of sewage lagoon bacteria. Metabolic breakdown of malathion by Bacillus thuringiensis var. thuringiensis was reported by Sutter, et al. (1971).

Although malathion has been shown to be degraded by various microorganisms, no studies have revealed malathion to be stimulatory or utilized to support bacterial growth.

Theoretical Considerations

Insecticides are aimed at insects (target species), however, certain other animals (non-target species) are affected by insecticides (Pimentel, 1971). A major group of non-target species are the microorganisms which are inhabitants of air, water, and soil. Microorganisms are important in nature in that they participate in the "food chain" cycle by decomposing organic waste, thereby returning the once-living substance to the earth and the atmosphere as carbon dioxide, ammonia, hydrogen

sulfide, nitrate, and other minerals (Stanier, 1963). The fixation of nitrogen is carried out by Azotobacter and symbiotic bacteria (Stanier, 1963). As a result, "Without microbial activity, life on earth would gradually be choked off" (Pelczar and Reid, 1965). It is therefore apparent that to destroy such microorganisms in our environment will serve a serious blow to the natural equilibrium of the biosphere. It is then important to determine if certain insecticides will affect bacterial growth.

Since malathion is one of several insecticides used in mosquito fogging operations by the Harris County Mosquito Control, Houston, Texas, this investigation was initiated to determine the effects of malathion on the bacterial population of a fresh-water reservoir. Hermann Park Lake, which is located in Houston, Texas, was chosen as the source of the bacterial population, because it is publicly located and would therefore be exposed to mosquito fogging operations.

When preliminary observations revealed that certain concentrations of technical grade malathion (labeled 95%) did affect the growth of the bacterial population in Hermann Park Lake, the research plan was expanded to obtain data to answer additional questions: (1) what is the effect of malathion on the growth of pure culture isolates, members of the Hermann Park Lake bacterial population? (2) what malathion products support bacterial growth? and (3) can any of the bacterial strains degrade malathion?

Routine bacteriological methods were used in these studies. Viable cell counts were used to quantitate growth in those experiments designed to test the effect of malathion on the bacterial population or pure culture isolates. This study was limited to those bacteria that grew on a peptone-enriched medium, aerobically, and at 37^{o}C. Thin-layer chromatography was used to identify malathion and its products.

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains used in this study were isolated from Hermann Park Lake in Houston, Texas. Growth conditions were such that bacteria which grew on a peptone-enriched media (Steelman, et al., 1967), aerobically, and at 37°C were detectable. Several bacterial strains, obtained in pure culture, were designated Houston Isolates #1, #2, #6, #10, and #11, and are described in Table 2.

Media and Chemicals

The peptone-enriched media used for growth studies, as described by Steelman, et al. (1967), consisted of the following:

Chemical	Gram/liter
Peptone	5.0
Tryptone	5.0
Beef Extract	3.0
Yeast Extract	3.0
Dextrose	1.0
Agar	15.0
Distilled Water (1.0 liter)	

The media was adjusted to pH 7 with 1N NaOH before sterilizing by steam for 15 minutes, under 15 pounds of pressure.

Technical grade malathion (labeled 95%) was obtained from the Harris County Mosquito Control District, Houston, Texas. Analytical grade malathion (99%), malathion (95%), malaoxon, malathion half-ester (monoacid), and malathion dicarboxylic acid (diacid) were obtained from the American Cyanamid Company, Princeton, New Jersey. All other chemicals used were analytical reagent grade, unless otherwise indicated, and were obtained from local commercial suppliers.

Growth Studies

Growth studies were carried out primarily to quantitate the stimulatory or inhibitory actions of malathion and its products. These growth responses were monitored by determining viable cell counts at prescribed intervals of time, as described below:

a. Sampling Techniques

Fresh water samples were taken from the Hermann Park Lake, aseptically, in a sterile glass container. The location of sampling was approximately two feet from shore and at a depth of two to five inches.

b. Viable Cell Count

Viable cell counts of a culture were determined at daily intervals, for three to seven days, by transferring 1.0 ml of the culture to 9.0 ml of sterile physiological saline solution (0.9%). Subsequent serial

dilutions were made in physiological saline solution. From the appropriate dilutions, 0.1 ml was transferred to the surface of a peptone-enriched agar plate and spread with a sterile glass rod. The plates were incubated at 37°C for 24 hours, unless otherwise indicated. Plates which had colonies between 30 to 300 were counted.

c. Effect of Malathion on the Bacterial Population

A fresh-water sample was transferred aseptically to each of several 250-ml Erlenmeyer flasks upon arrival in the laboratory. Various amounts of malathion or its products were added to the appropriate culture flasks, to yield the desired concentrations. These flasks were then agitated for aeration on a Gyrotory shaker (New Brunswick Scientific Co.), at 25°C (room temperature). Viable cell counts were used to quantitate bacterial growth. Culture plates were incubated at 37°C for 24 to 48 hours before counting with an electronic colony counter (New Brunswick Scientific Co.).

d. Preparation of Inoculum and Culture

Pure culture isolates were maintained on peptone-enriched media at 4°C. Prior to each growth study, each pure culture isolate was streaked on a peptone-enriched media plate and incubated at 37°C overnight. A turbid suspension of bacterial cells was prepared in 10 ml of sterile lake water. Fifty milliliters of sterile lake water, in 125-ml Erlenmeyer flasks, were inoculated with the bacterial

suspension to yield a culture containing 10^4 to 10^6 cells per ml.

Various amounts of malathion, or its products, were added to the appropriate culture flasks, to yield the desired concentrations. These cultures were agitated, with aeration, on a Gyrotory shaker, at room temperature. At 24-hour intervals 1.0-ml samples were removed from each culture to determine the amount of bacterial growth by viable cell counts.

Thin-Layer Chromatography

Thin-layer chromatography, as described by Kadoum (1970), was used to identify malathion and its products. Glass plates (20 cm x 20 cm) were spread with Silica Gel-G to a thickness of 0.25 mm, using a Stahl-Desaga apparatus (Brinkman Instruments). The plates were air-dried and then activated at 110°C for 30 minutes (Stahl, 1965). Samples were spotted, at the origin, 15 mm from the lower edge of the plate. The solvent system used to prepare one-dimensional thin-layer chromatographs was benzene-acetic acid (80:20) or benzene-hexane-acetic acid (40:40:20). The solvent systems used for two-dimensional thin-layer chromatography were: benzene-hexane-acetic acid (40:40:20), first dimension; and hexane-acetic acid-ether (75:15:10), second dimension. The solvent front was 15 cm from the origin. The spray reagent used to detect malathion and its products was 2, 6-dibromo-N-chloro-p-quinoneimine (DCQ). A solution of DCQ was made fresh daily, with redistilled acetone, in a 0.5%

concentration. The chromatographs were sprayed, then heated at 110°C for 20 minutes.

The DCQ spray reagent is specific for sulfur-containing organophosphates (Menn, 1957).

Extraction Method

An extraction procedure, described by Mount and Stephan (1967), was used to determine the amount of malathion present in Hermann Park Lake.

Water samples (300 ml) were triply extracted with 30 ml portions of dichloromethane. The total amount of solvent (90 ml) was then reduced, under vacuum, to 0.2 ml. This was then examined, using thin-layer chromatography, for the presence of malathion.

RESULTS

The primary purpose of this research problem was to determine the effect of malathion on the bacterial population present in a fresh-water reservoir (Hermann Park Lake).

Bacterial Population of Hermann Park Lake

To initiate this study it was necessary to quantitate the bacterial population of the Hermann Park Lake. This data was obtained by doing viable cell counts on each water sample, upon arrival in the laboratory.

A variety of colony types was observed, as demonstrated by the photograph in Figure 1. Several types of bacteria were characterized, according to cellular and colonial morphology, and summarized in Table 1.

There was a variation in the number of bacteria present in Hermann Park Lake when observed for several months. This variation is shown in Figure 2. The bacterial population varied from 1.0×10^3 cells per ml to a maximum of 1.7×10^4 cells per ml, throughout this investigation. The significance of this variation, which is not related to seasonal changes, is not known.

The Presence of Malathion in Hermann Park Lake

Before studying the effect of a chemical agent on bacterial growth,

FIGURE I

Photograph of Colony Types

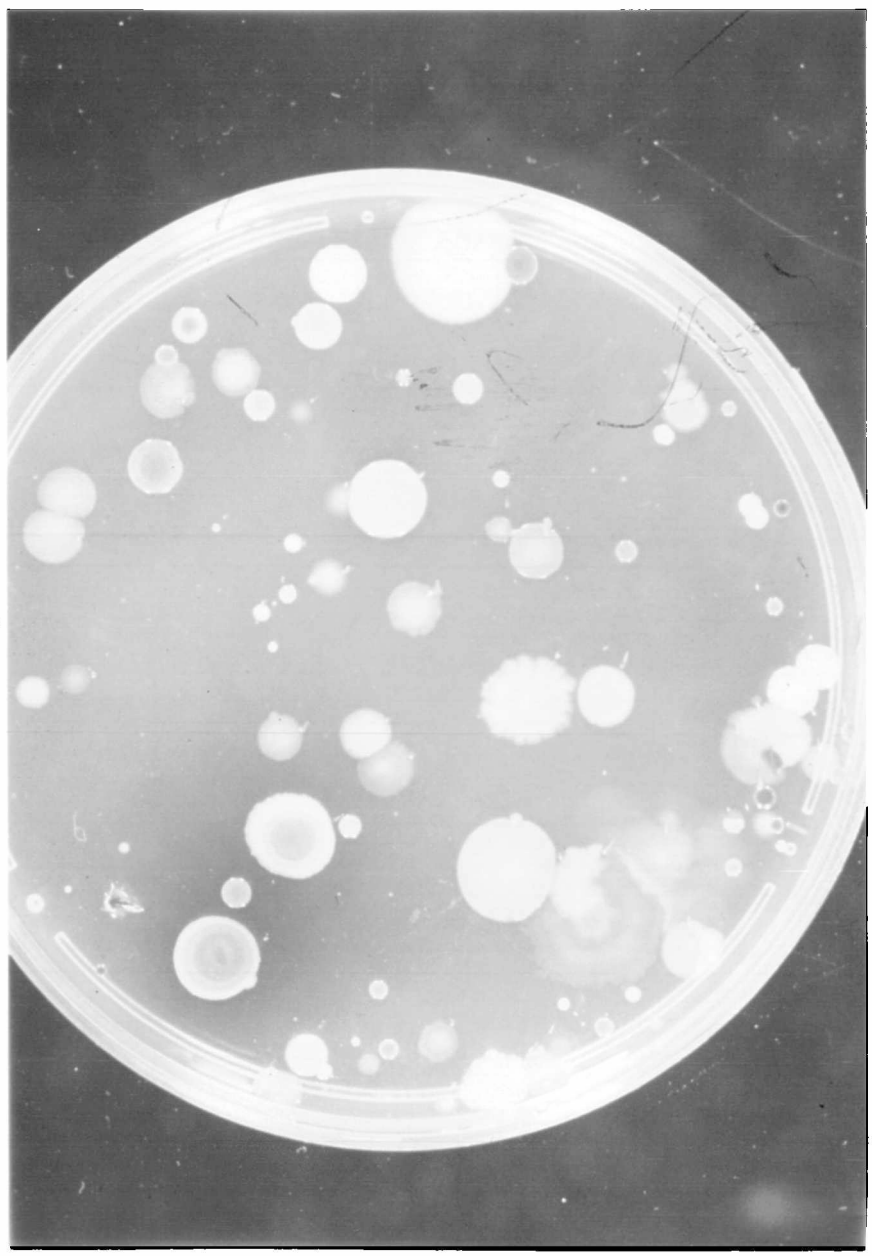


Table 1

COLONY AND CELLULAR CHARACTERISTICS OF BACTERIAL TYPES IN HERMANN LAKE

Colony Type	Colony Characteristics	Gram Stain Reaction	Cellular Morphology
A	Med., Circular, Undulate, Opaque	Gram (+)	Pleomorphic Shapes
B	Large, Circular, Entire, Smooth, Cream	Gram (-)	Small Rods
C	Small, Circular, Entire, Smooth, White	Gram (+)	Slender Small Rods
D	Extra Large, Circular, Undulate, Rough, Opaque	Gram (+)	Large Rods, Central Spores
E	Small, Circular, Entire, Smooth, Orange	Gram (+)	Medium Rods
F	Small, Circular, Entire, Smooth, Lt.-Orange	Gram (+)	Small Rods
G	Small, Circular, Entire, Smooth, Orange	Gram (+)	Small Rods
H	Medium, Circular, Entire, Smooth, Lemon-Yellow	Gram (+)	Pleomorphic Shapes
I	Small, Circular, Entire, Smooth, Lt.-Yellow	Gram (+)	Small Rods

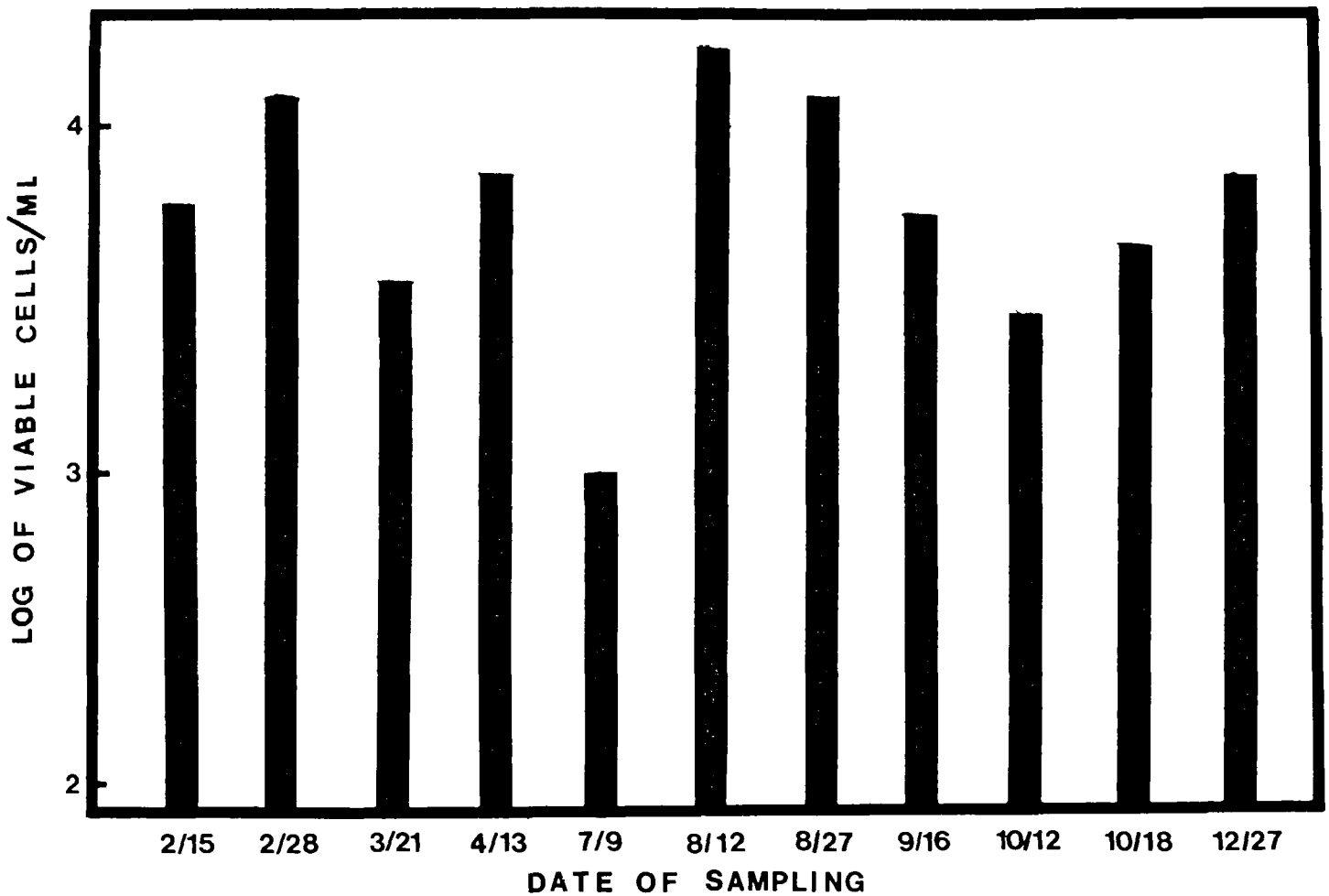
FIGURE 2

Variation of Bacterial Population

in Hermann Lake

DATE	VIABLE CELL NUMBER
2/15	5.9×10^3
2/28	1.2×10^4
3/21	3.5×10^3
4/13	7.2×10^3
7/19	1.0×10^3
8/12	1.7×10^4
8/27	1.2×10^4
9/16	5.4×10^3
10/12	3.8×10^3
10/18	4.5×10^3
12/27	7.1×10^3

VARIATION OF THE BACTERIAL POPULATION IN HERMANN LAKE



one must determine if this chemical is present in the water samples under natural conditions. To detect if malathion or its products were present in the water samples from Hermann Park Lake, an extraction procedure described by Mount and Stephan (1967) was used. This extraction procedure could detect quantities equal to or greater than 0.01% concentration of malathion in aqueous solution. No malathion was detectable in the water sample from Hermann Park Lake using this extraction procedure. If malathion was present, the quantity was not significant enough to interfere with the growth studies, since preliminary observation revealed no growth stimulation or inhibition with 0.01% concentration of technical grade malathion (95%).

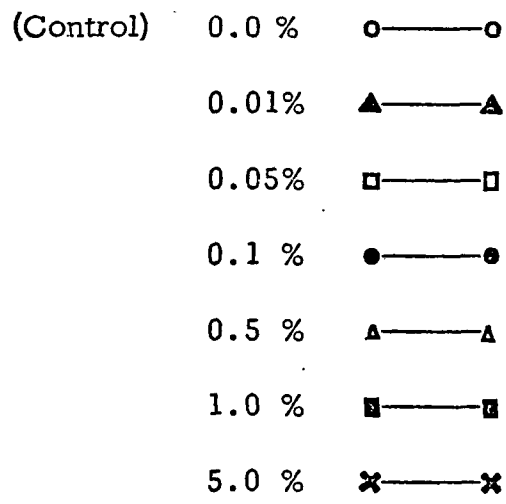
Effect of Malathion on Bacterial Population

The effects of malathion on the bacterial population in Hermann Park Lake was determined by adding known quantities of technical grade malathion (95%) to 100 ml of fresh-water samples, such that the following concentrations were obtained: 0.01%, 0.05%, 0.1%, 0.5%, 1.0%, and 5.0%. The viable cell count of each culture was made at 24 hour intervals, for a period of seven days. The growth response of the bacterial population to various concentrations of malathion is illustrated in Figure 3.

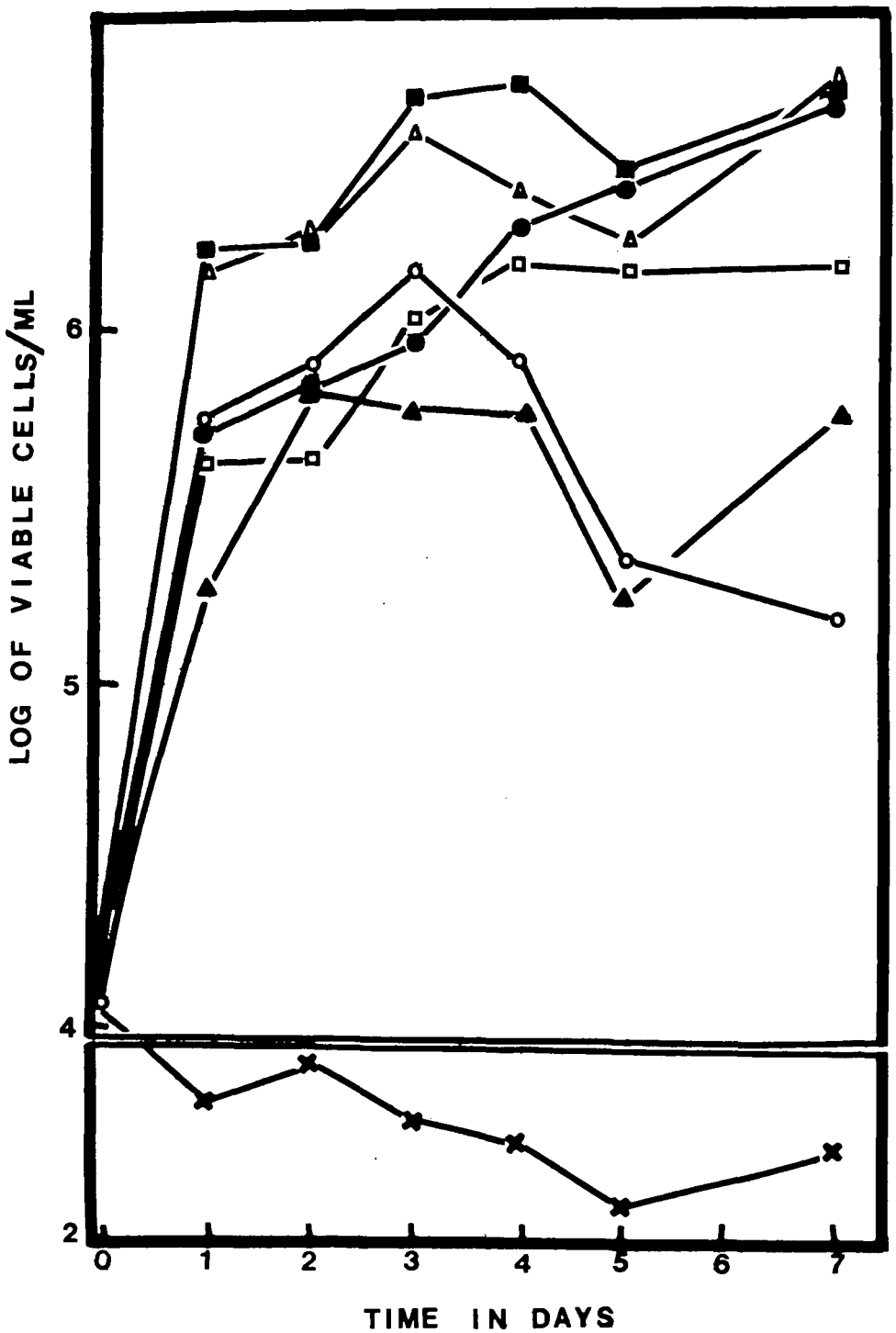
A significant decrease in the number of viable cells was observed in 5.0% concentration of malathion after one day. This marked inhibition

FIGURE 3

The Effect of Malathion on Bacterial Population



THE EFFECT OF MALATHION
ON BACTERIAL POPULATION



approximately a six-fold reduction, was observed throughout seven days of incubation.

Malathion in 0.5% and 1.0% concentrations stimulated the growth of the bacterial population after 24 hours. With the 0.5% concentration, a maximum growth response of 5.3×10^6 cells per ml was obtained after seven days. With the 1.0% concentration a maximum growth response of 5.1×10^6 cells per ml was obtained after four days.

A stimulatory effect was observed, after three days, with 0.05% and 0.1% concentrations, but not as great as detected in the 0.5% and 1.0% concentrations. The maximum growth response in the 0.05% and 0.1% concentrations was 1.6×10^6 cells per ml in four days and 4.4×10^6 cells per ml in seven days, respectively.

There was no significant change in the viable cell number observed in 0.01% concentration, when compared to that of the control, throughout the seven days.

It was observed in all cultures, including the control, that the viable cells increased significantly during the first 24 hours. Experiments were designed to determine the significance of this initial increase. Altering certain physical factors, including aeration (by shaking) vs. non-aeration and presence and absence of direct sunlight, failed to account for this initial increase in viable cells.

Isolation of Bacterial Strains

Several bacterial strains were isolated from colonies which had developed in 1.0% concentration of malathion. These bacterial strains, whose colony types were more predominant, were obtained in pure culture and designated stimulatory isolates (Houston Isolates #1, #2, and #6). Two bacterial strains were isolated from colonies which represented survival in 5.0% concentration of malathion. These strains were designated inhibitory isolates (Houston Isolates #10 and #11).

The colony and cellular characteristics of Houston Isolates #1, #2, #6, #10, and #11 are summarized in Table 2. The gram-stain reaction, presence of spores, and motility were used to characterize bacterial strains (Pelczar, 1965). The Indole, Methyl Red, Voges-Proskauer, Citrate, Nitrate, Litmus Milk tests, and the Triple Sugar Iron Agar (TSI) were also used to characterize certain bacterial strains (Pelczar, 1965).

All three Houston Isolates (#1, #2, and #6) gave negative reactions for the Indole, Methyl Red, and Voges-Proskauer tests; and a positive reaction for the Citrate test. Only Houston Isolate #2 was able to reduce nitrate to nitrite. Houston Isolates #1 and #6 produced an acid reaction in Litmus Milk and Houston Isolate #2 produced an alkaline reaction in Litmus Milk. Houston Isolates #1, #2, and #6 all produced negative TSI slants, indicating that they were not able to attack dextrose, lactose or sucrose and were not able to liberate sulfides.

TABLE 2

COLONY AND CELLULAR CHARACTERISTICS OF HOUSTON ISOLATES

Type	Size	Color	Colony Morphology	Gram Stain RX	Cellular Morphology	Spore	Motile
#1	Small	White	Circular, Umbonate, Entire, Smooth	+ *	Cocci in pairs or bunches	-	+
#2	Medium	Yellow-green	Irregular, Raised, Filamentous, Smooth	-	Medium slender rods	-	+
#6	Small	White	Circular, Umbonate, Entire, Smooth	+ *	Cocci in pairs or bunches	-	+
#10	Medium	Light tan	Circular, Raised, Entire, Rough	+	Medium thick rods	+	+
#11	Small	Dark Yellow	Circular, Raised, Filamentous, Rough	+	Medium slender rods (chains)	-	+

*Initially stained gram-negative.

From morphological characteristics and limited biochemical tests, Houston Isolate #2 appears to be a Pseudomonas species (Breed, et al., 1957). Houston Isolates #1 and #2 were initially gram-negative rods but later changed to gram-positive cocci. This characteristic is indicative of bacteria comprising the genus Arthrobacter. However, certain biochemical tests did not correspond with specific species of Arthrobacter (Breed, et al., 1957).

Effect of Malathion on Pure Culture Isolates

As described elsewhere, technical grade malathion (95%) in 0.5%, 1.0%, and 5.0% concentrations was added to 50 ml of sterile fresh-water samples, which had been reseeded with a designated Houston Isolate. The cultures were incubated at 25°C, for three days. One-ml quantities were removed at 24 hour intervals and analyzed for the number of viable cells.

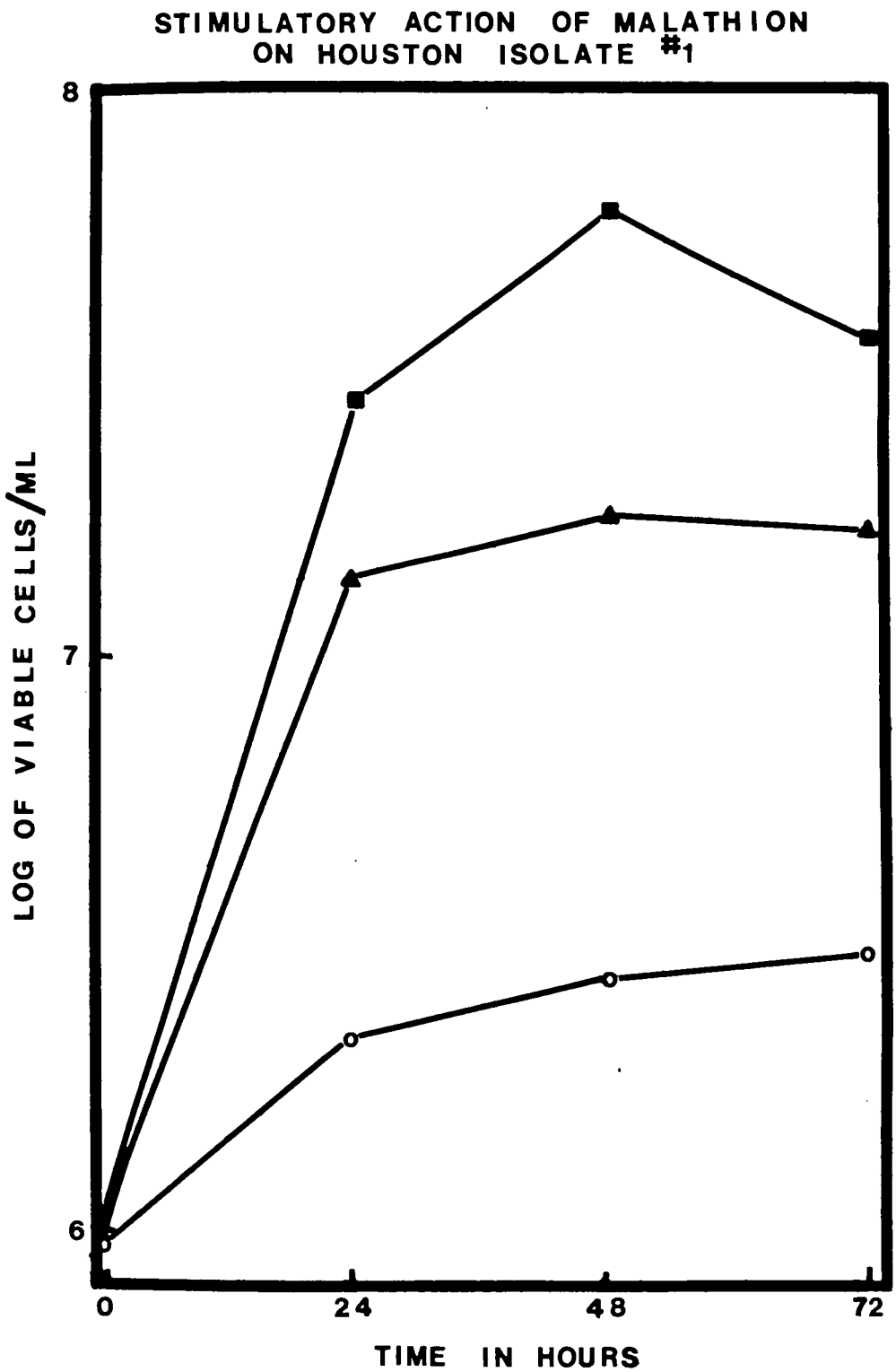
a. Growth Response of Stimulatory Isolates

The growth response of Houston Isolate #1 is illustrated in Figure 4. Malathion in 0.5% and 1.0% concentrations stimulated the growth of Houston Isolate #1 after 24 hours. This growth stimulation was observed throughout the incubation period. A maximum growth response of 1.8×10^7 and 6.0×10^7 cells per ml to 0.5% and 1.0% concentrations, respectively, was obtained after 48 hours. There was growth inhibition by the 5.0% concentration, to the extent that no growth was observed using dilutions which would detect 100 cells per ml.

FIGURE 4

Stimulatory Action of Malathion on Houston

Isolate #1


STIMULATORY ACTION OF MALATHION
ON HOUSTON ISOLATE #1
8
7
6
LOG OF VIABLE CELLS/ML
0
24
48
72
TIME IN HOURS

The growth response of Houston Isolate #2, to malathion, is illustrated in Figure 5. A maximum growth response of 1.7×10^7 cells per ml was obtained with 1.0% concentration of malathion after 48 hours. This growth stimulation was followed by a marked inhibition to 1.0×10^2 cells per ml at 72 hours. No significant growth response was observed in 0.5% concentration although there was a slight growth inhibition of the 0.5% concentration throughout 72 hours, indicative of its inhibitory action.

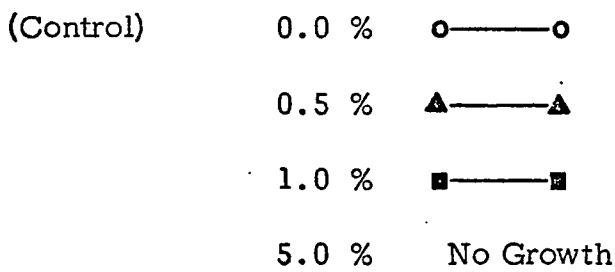
The marked inhibition of growth, in the 1.0% concentration, after 48 hours could be caused by the presence of a toxic compound. It is known that malathion monoacid, which is present in technical grade malathion (95%), will degrade to malathion diacid. As will be reported later, malathion diacid was toxic for certain bacteria, and therefore could be this toxic compound which accumulates after 48 hours.

The growth response of Houston Isolate #6 is illustrated in Figure 6. Malathion, in 0.5% and 1.0% concentrations, stimulated the growth of Houston Isolate #6 after 24 hours. In the 0.5% concentration the maximum growth response of 3.2×10^7 cells per ml was obtained after 24 hours. An inhibitory effect, similar to that observed with Houston Isolate #2, occurred after 24 hours with Houston Isolate #6 in the 1.0% concentration. This inhibition of the bacterial population, from 1.8×10^7 go 5.4×10^5 cells per ml, continued throughout 72 hours. The absence of growth with 5.0% concentration, after 24 hours, was indicative of its

FIGURE 5

Stimulatory Action of Malathion on Houston

Isolate #2



STIMULATORY ACTION OF MALATHION
ON HOUSTON ISOLATE #2

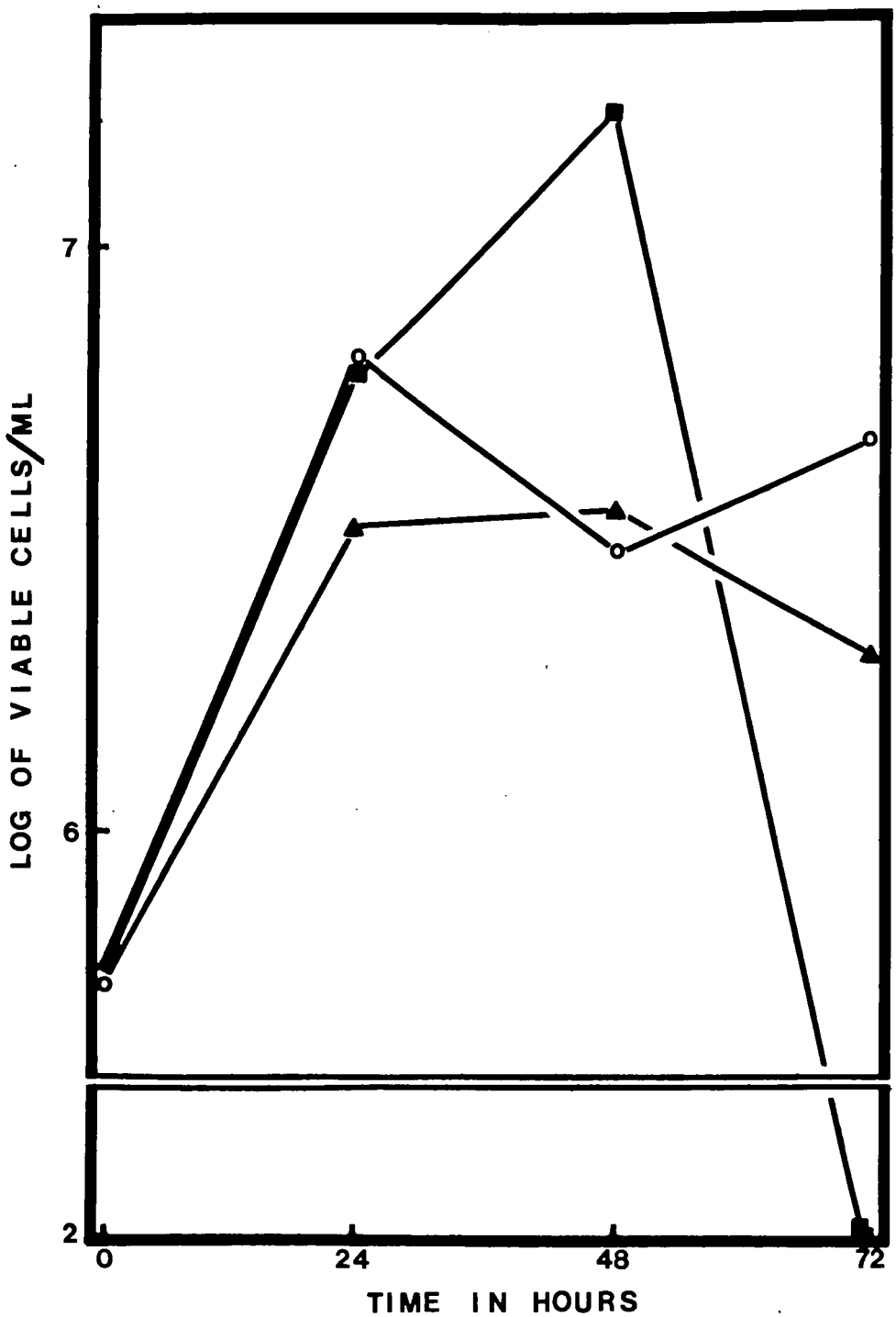
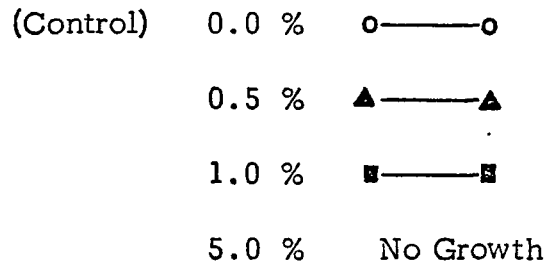


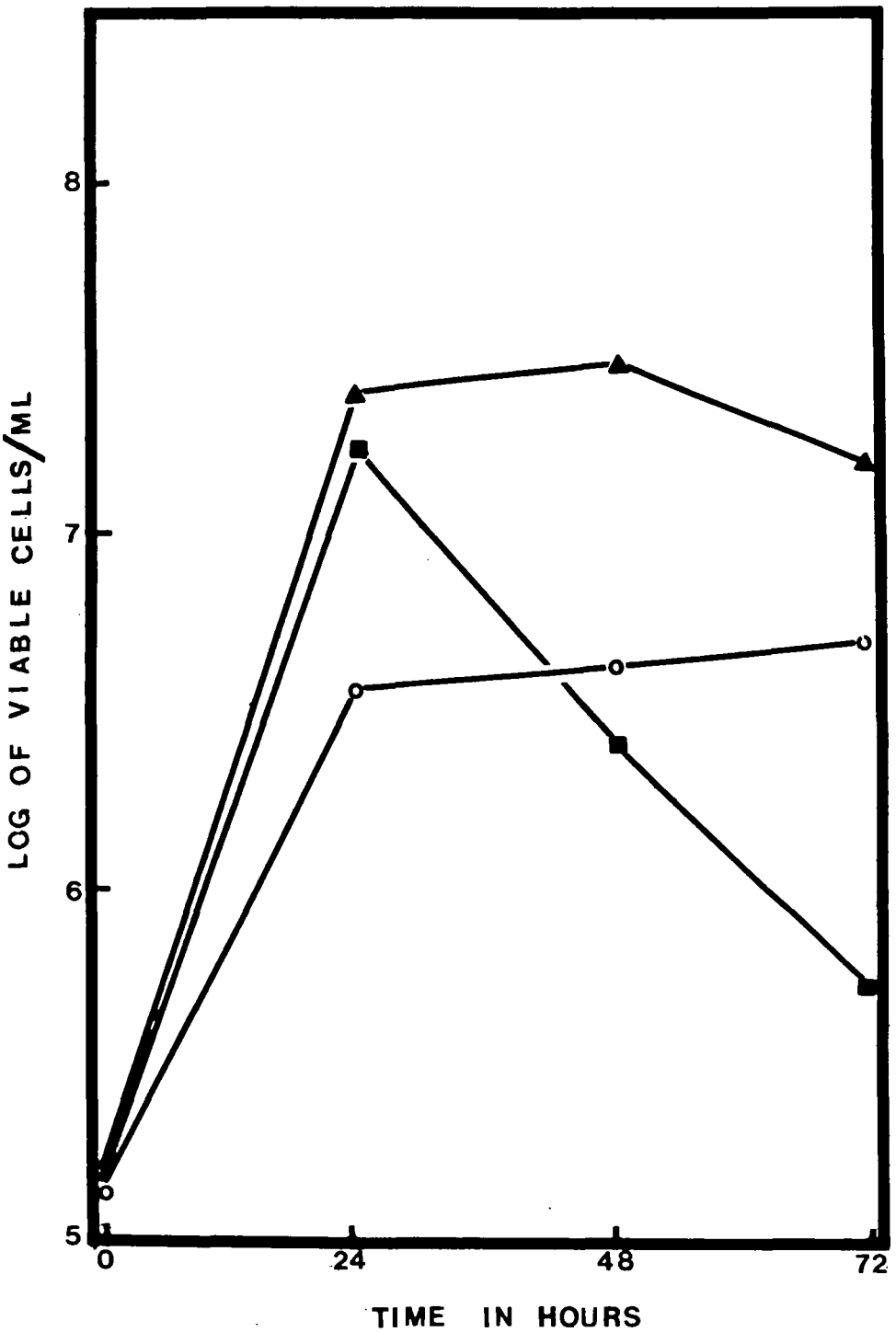
FIGURE 6

Stimulatory Action of Malathion on Houston

Isolate #6



STIMULATORY ACTION OF MALATHION
ON HOUSTON ISOLATE #6



marked inhibitory action.

The inhibition of growth for Houston Isolate #6, in the 1.0% concentration, resembled that seen for Houston Isolate #2, but to a lesser extent. This inhibition was probably due to the presence of a toxic compound, such as malathion diacid.

b. Growth Response of Inhibitory Isolate

The inhibitory action of malathion on Houston Isolate #10 is illustrated in Figure 7. Malathion in 0.5% and 1.0% concentrations, inhibited the growth of Houston Isolate #10 throughout 72 hours, but not to the extent seen with the 5.0% concentration. Unlike Houston Isolate #1, #2, and #6, the growth of this organism failed to be stimulated by either 0.5% or 1.0% concentrations of malathion, indicating that it could not utilize malathion or its products, present in technical grade malathion (95%), for growth.

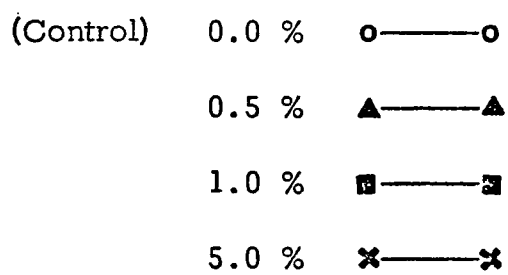
Utilization of Malathion

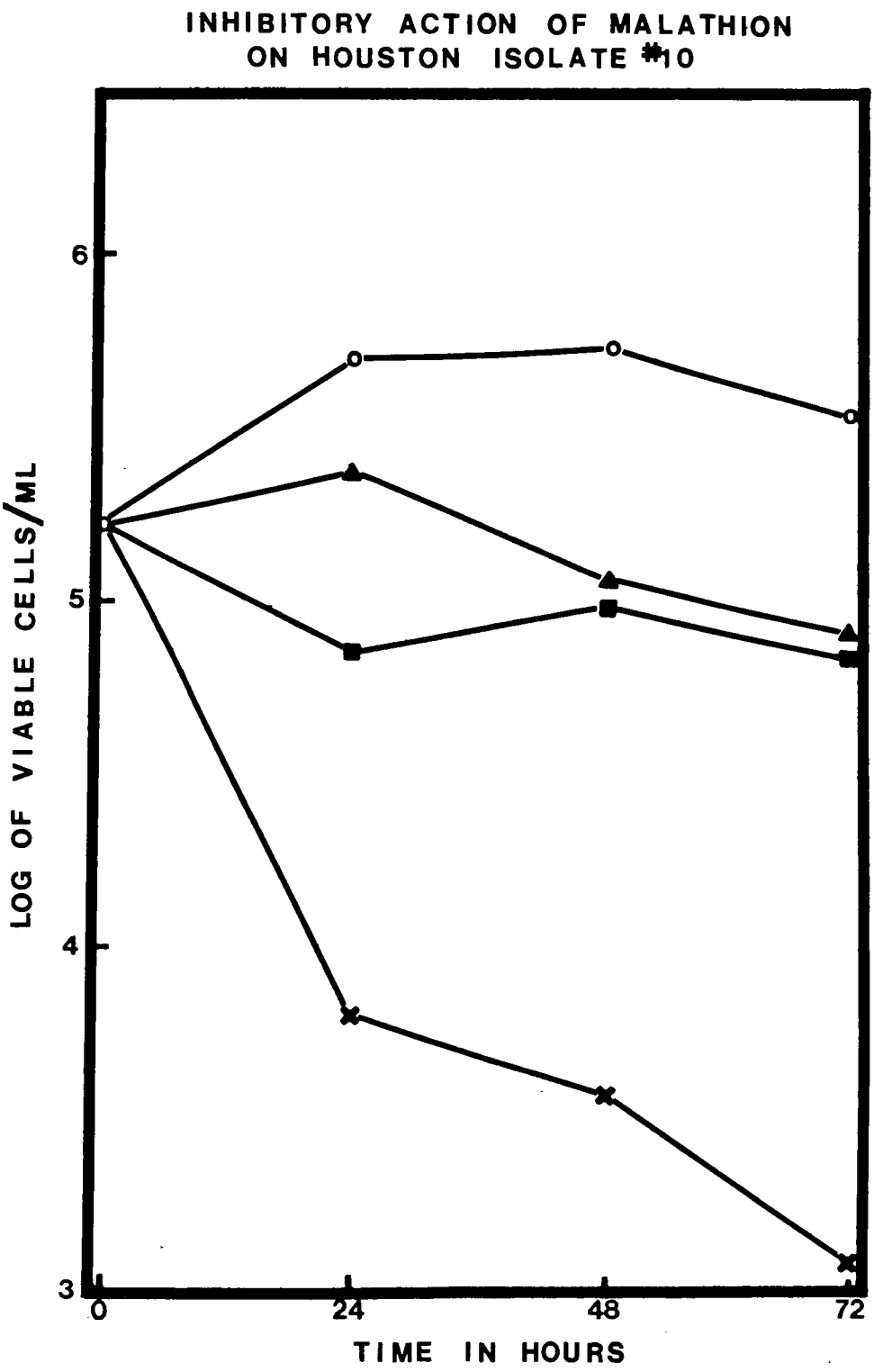
Since certain concentrations of technical grade malathion (95%) supports and stimulates bacterial growth, experiments were designed to determine what products of malathion stimulated growth. Bacterial types (Houston Isolates #1, #2, and #6), which had shown growth stimulation with certain concentrations of malathion, were chosen for this investigation.

FIGURE 7

Inhibitory Action of Malathion on Houston

Isolate #10




INHIBITORY ACTION OF MALATHION
ON HOUSTON ISOLATE #10
LOG OF VIABLE CELLS/ML
6
5
4
3
0
24
48
72
TIME IN HOURS

The chemical structures of several malathion products are shown in Figure 8.

Malathion and its products were analyzed using thin-layer chromatography. A one-dimensional thin-layer chromatograph of these products, using the solvent system benzene-acetic acid (80:20), is illustrated in Figure 9. It was observed that malathion technical grade (95%) and malathion analytical grade (95%) contained three major components as revealed by thin-layer chromatography. Malathion analytical grade (99%), malathion monoacid, and malathion diacid contained only one component, as revealed by one spot with a characteristic R_f value (Kadoum, 1970), using thin-layer chromatography. It was revealed that two of the three major spots (designated "B" and "C") observed from the malathion technical grade and analytical grade (95%) were identical to certain known organophosphates. Spot "C" corresponded to the malathion monoacid and spot "B" corresponded to malathion. Spot "A", which had the greatest R_f values of those components observed for malathion and its products using two different solvent systems, benzene-acetic acid (80:20); and benzene-hexane, acetic acid (40:40:20).

Houston Isolates #1, #2, and #6 were cultured, separately, in 50-ml volumes of sterile lake water with 1.0% concentrations of technical grade malathion (95%). The control flask contained 1.0% concentration of technical grade malathion in 50 ml of sterile lake water. The nonaqueous

FIGURE 8

Chemical Structure of Malathion Products

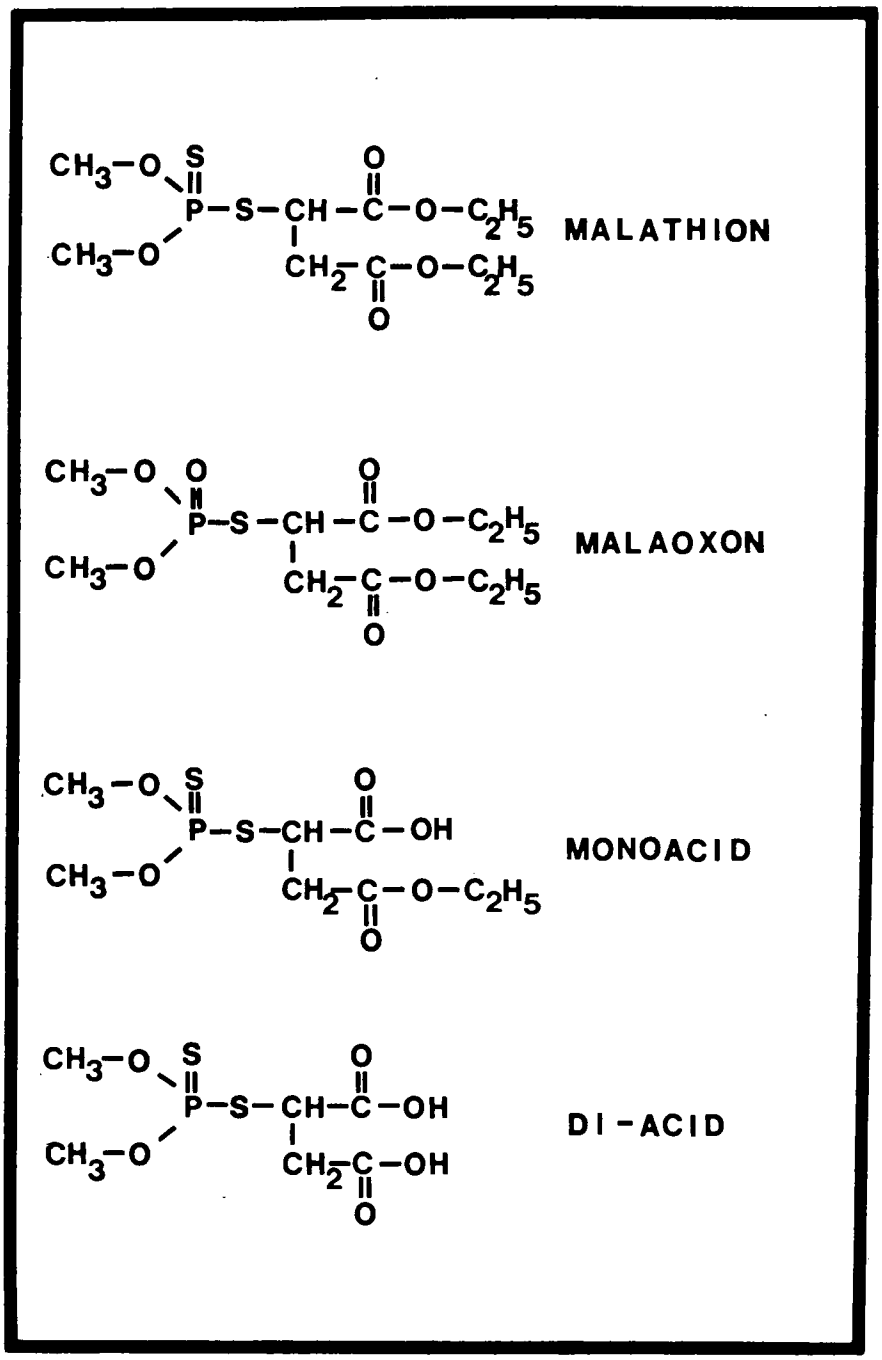

MALATHION
MALAOXON
MONOACID
DI-ACID

FIGURE 9

One-Dimensional Thin-Layer Chromatography of

Malathion and its Products

Solvent system: benzene (80): acetic acid (20)

POSITION

1 -- malathion technical grade (labeled 95%)

2 -- malathion analytical grade (labeled 95%)

3 -- malathion analytical grade (labeled 99%)

4 -- malathion monoacid

5 -- malathion diacid

SPOT A: Unknown

SPOT B: Malathion

SPOT C: Malathion monoacid

SPOT D: Malathion Diacid

ONE-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY
OF MALATHION AND ITS PRODUCTS

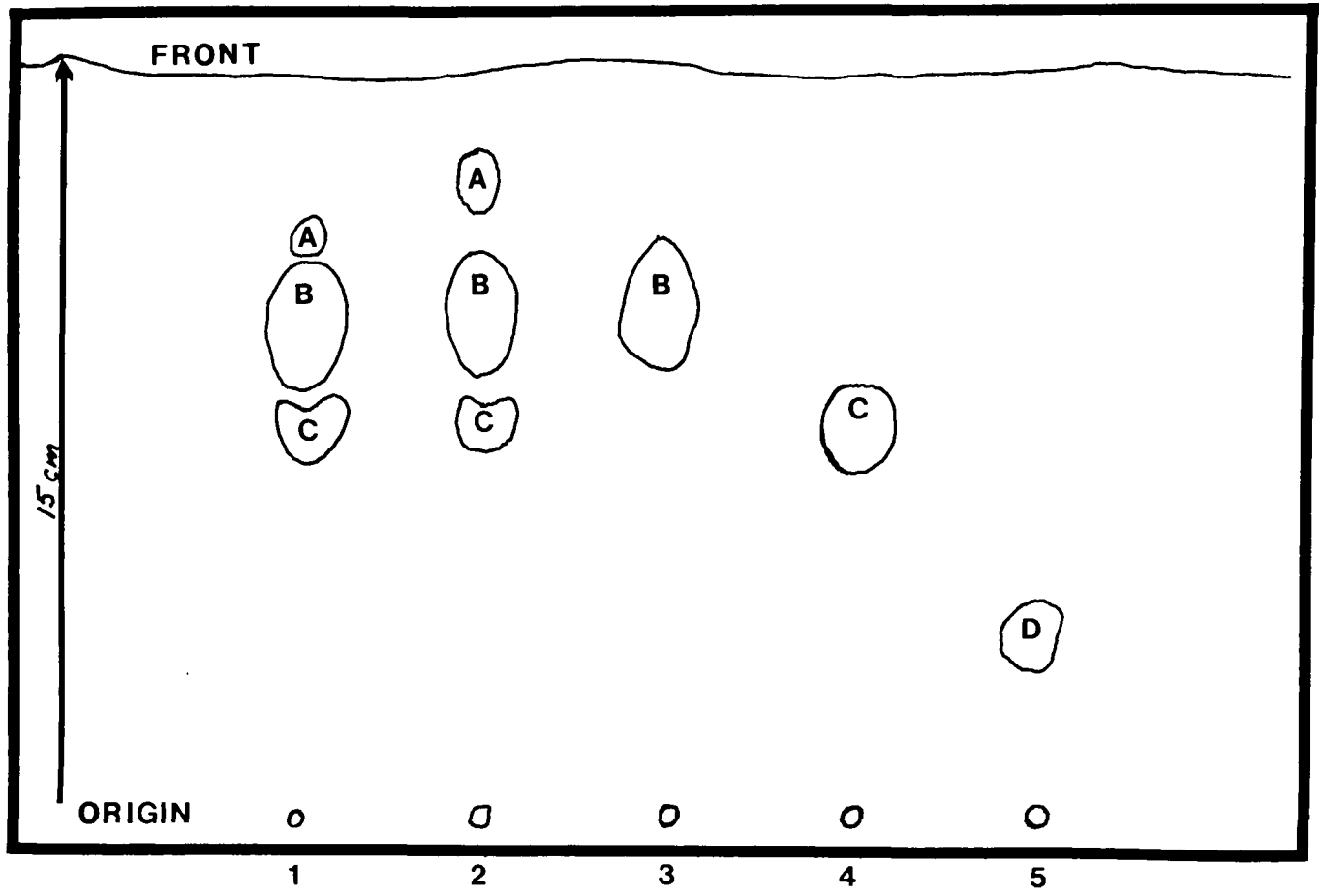


TABLE 3

R_f VALUES FOR MALATHION AND ITS PRODUCTS USING TWO SOLVENT SYSTEMS

	Spot	Benzene:Acetic Acid (80:20)	Benzene:Hexane: Acetic Acid (40:40:20)
*Malathion (95%)	A	.85	Not Present
	B	.74	.88
	C	.62	.77
**Malathion (95%)	A	.90	Not Present
	B	.77	.87
	C	.63	.76
**Malathion (99%)	B	.78	.86
**Malathion half-ester (monoacid)	C	.63	.75
**Malathion dicarboxylic acid (diacid)	D	.40	.53

*Technical grade: Harris County Mosquito Control

**Analytical grade: American Cyanamid Co.

layer was monitored, using thin-layer chromatography, at 0 hours, 2 hours, 6 hours, 8 hours, 24 hours, and 48 hours. Spot "C", which disappeared, corresponded in R_f value to the malathion monoacid in both solvent systems. Since this spot did not disappear in the control flask, the disappearance of spot "C", along with an increase in growth of the stimulatory isolates, permits one to conclude that these bacterial strains could utilize the malathion monoacid to support growth.

Degradation of Malathion

One important aspect of this research problem was to detect if bacteria could degrade malathion and its products.

Houston Isolate #11, which resisted 5.0% concentration of malathion, was chosen to determine if bacteria could utilize and degrade malathion and its products. An experiment was designed such that an inoculum of Houston Isolate #11 was incubated in 10 ml of sterile freshwater samples to which known quantities of malathion and its products were added. The growth responses of Houston Isolate #11 to the various malathion products are illustrated in Figure 10. The greatest growth stimulation of Houston Isolate #11 was observed with malathion monoacid in 0.1% concentration. There was an initial stimulation after one day. This stimulation continued, to a maximum of 5.9×10^5 cells per ml, for six days. Malathion (99%) in 1.0% concentration stimulated the growth of Houston Isolate #11 after one day. A maximum growth response of 1.1 x

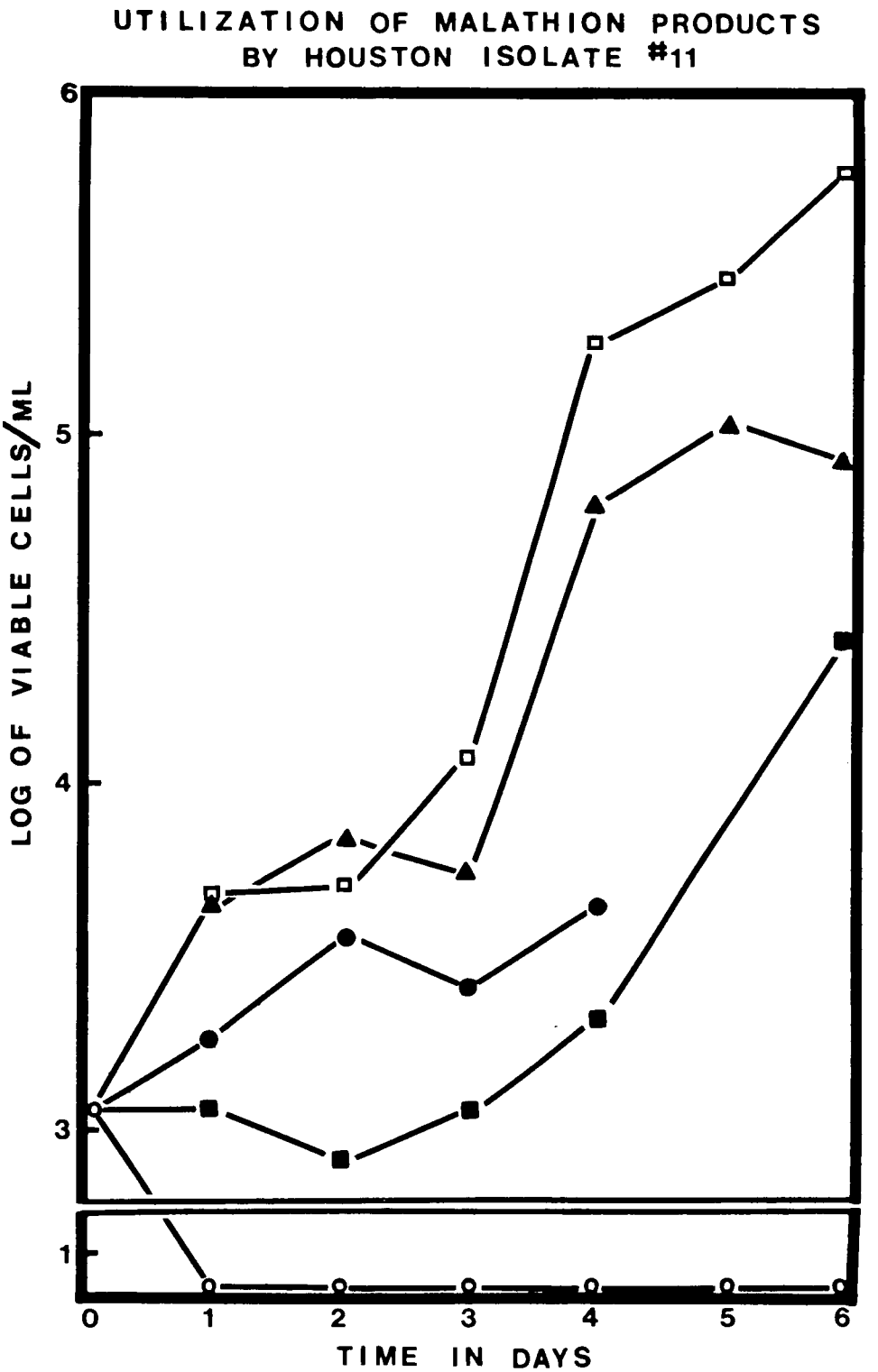
FIGURE 10

Utilization of Malathion Products by Houston

Isolate #11

(Control)	0.0 %	o———o
Malathion (99%)	1.0 %	▲———▲
Malathion (95%)	1.0 %	■———■
Monoacid	0.1 %	□———□
Malaoxon	0.1 %	●———●
Diacid	No Growth	

(All compounds listed above are analytical grade obtained from American Cyanamid Co.)


UTILIZATION OF MALATHION PRODUCTS
BY HOUSTON ISOLATE #11
LOG OF VIABLE CELLS/ML
6
5
4
3
1
0
1
2
3
4
5
6
TIME IN DAYS

10^5 cells per ml was obtained after five days. Malathion (95%) in 1.0% concentration stimulated the growth of Houston Isolate #11 after four days. A maximum growth response of 2.6×10^4 cells per ml was obtained after six days. The observed growth response by Houston Isolate #11 to malathion (99%) and malathion monoacid is indicative that these compounds are used to support and stimulate growth, thus they are probably degraded to some extent. The extent of degradation was not determined.

The significant decrease in the number of viable cells in the control culture, from 1.2×10^3 cells per ml to less than 10^1 cells per ml, after one day was not expected. Several experiments were carried out to determine if this initial decrease of viable cells had any bacteriological basis. It was observed that this initial decrease in viable cell number occurred in repeated experiments. Another experiment revealed that the decrease in viable cell number was greater when the culture contained 10^5 cells per ml as compared to a culture containing 10^6 cells per ml. The ability of Houston Isolate #11 to recover from this initial decrease in viable cells was dependant on the inoculum size. It was also observed that a clumbing of cells, at 24 hours, appeared in all cultures containing Houston Isolate #11. This clumping probably contributed to a fewer number of viable cells than would be expected in the control of experiments similar to that illustrated in Figure 10. The reason for this clumping may be attributed to this rod-shaped organism's ability to remain intact after cell division, thereby forming a chain. This chain formation was observed microscopically.

DISCUSSION

Since pesticides are of ecological significance as pollutants and the pesticide malathion is used routinely in mosquito fogging operations, there is great value in studying the effect of malathion on bacteria in nature.

Several questions were asked at the beginning of this investigation. What is the effect of malathion on the bacterial population present in Hermann Park Lake? Can bacterial strains degrade malathion?

When viable cell counts were used to measure growth response of bacteria caused by the presence of malathion, significant results were obtained. Initially, it was observed that certain concentrations of technical grade malathion (labeled 95%) were inhibitory or stimulatory to the bacterial population, which was composed of a variety of bacteria, present in fresh-water. Stimulatory effects were observed using 0.1%, 0.5%, and 1.0% concentrations of malathion. Malathion has not been described in the literature as being stimulatory to bacterial growth. It is conceivable that certain pathogenic enteric bacilli; such as Escherichia coli, Shigella species, Salmonella species, and Pseudomonas species; could accidently gain entrance to a fresh-water source and be exposed to low concentrations (1.0% or less) of malathion. This could cause an increase in their number and present a public health hazard.

Marked inhibition, of bacterial growth, was observed using 5.0% concentration of malathion. If the concentration of malathion is permitted to accumulate to 5.0% or greater, growth inhibition of the bacterial flora in fresh-water would result. This would be of ecological significance because certain bacteria are essential to the cycles in nature, Desulfovibrio species—sulfur cycle; Pseudomonus species—oxygen and carbon cycle; and Azotobacter, nitrogen cycle (Stanier, et al., 1963). Many protozoa, which are food for invertebrates, feed on bacteria (Berger and Kimball, 1964; Ducoff, et al., 1964). Therefore destroying these bacteria would interfere with this food chain.

The studies involving pure culture isolates permitted the investigator to observe the utilization of malathion and its products. It was shown that malathion and malathion monoacid, in the appropriate concentrations, stimulated the growth of certain bacteria. These two compounds probably supported bacterial growth by serving as a carbon source. When malathion is converted to malathion monoacid an ethyl fragment is released, probably as ethanol (Morrison and Boyd, 1959). When malathion monoacid is converted to malathion diacid, an ethyl fragment is also released. These ethyl fragments, if in the form of ethanol, can be utilized as a carbon source for growth (Reddy, et al., 1972). Since malathion diacid inhibited bacterial growth this is suggestive that the bacteria cannot utilize this component of the malathion molecule. The utilization of malathion and

certain of its products by bacteria indicate that these compounds are degraded to some extent. Other investigators have shown the degradation of malathion by microorganisms (Matsumura and Boush, 1966; Halvorson, et al., 1971; Sutter, et al., 1971). Additive studies are necessary to obtain bacterial strains that will degrade melathion to the extent that it or its products are no longer biological toxic.

This investigation, although exploratory in nature, provided answers to certain questions set forth in the original hypothesis. These findings should encourage a continuation of similar studies to alleviate certain consequences that may occur due to the use of malathion during routine fogging operations.

SUMMARY

This investigation revealed that technical grade malathion (95%), which is used for mosquito fogging operations by the Harris County Mosquito Control District, was stimulatory or inhibitory to the bacterial population of Hermann Park Lake. The greatest stimulatory effect was observed with 0.5% and 1.0% concentrations of malathion. Marked inhibition was observed with 5.0% concentration of malathion.

The stimulatory isolates (Houston Isolates #1, #2 and #6), when incubated with 0.5% and 1.0% concentrations of technical grade malathion (95%), were stimulated. An inhibitory isolate (Houston Isolate #10) was inhibited by 0.5% and 1.0% concentrations. All pure culture isolates were inhibited by 5.0% concentration.

The stimulatory isolates were found to utilize malathion monoacid to support growth. Houston Isolate #11 utilized malathion and malathion monoacid to support its growth. Malathion diacid inhibited the growth of Houston Isolate #11.

This investigation is significant in that it revealed the effect of a commonly used pesticide on certain microorganisms. The utilization of malathion and certain of its products is strongly suggestive that bacteria can degrade this pesticide.

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