EFFECTS OF ALDRIN, A CHLORINATED HYDROCARBON
INSECTICIDE, ON SOIL MICROORGANISMS AND
THEIR ACTIVITIES RELATED TO SOIL FERTILITY

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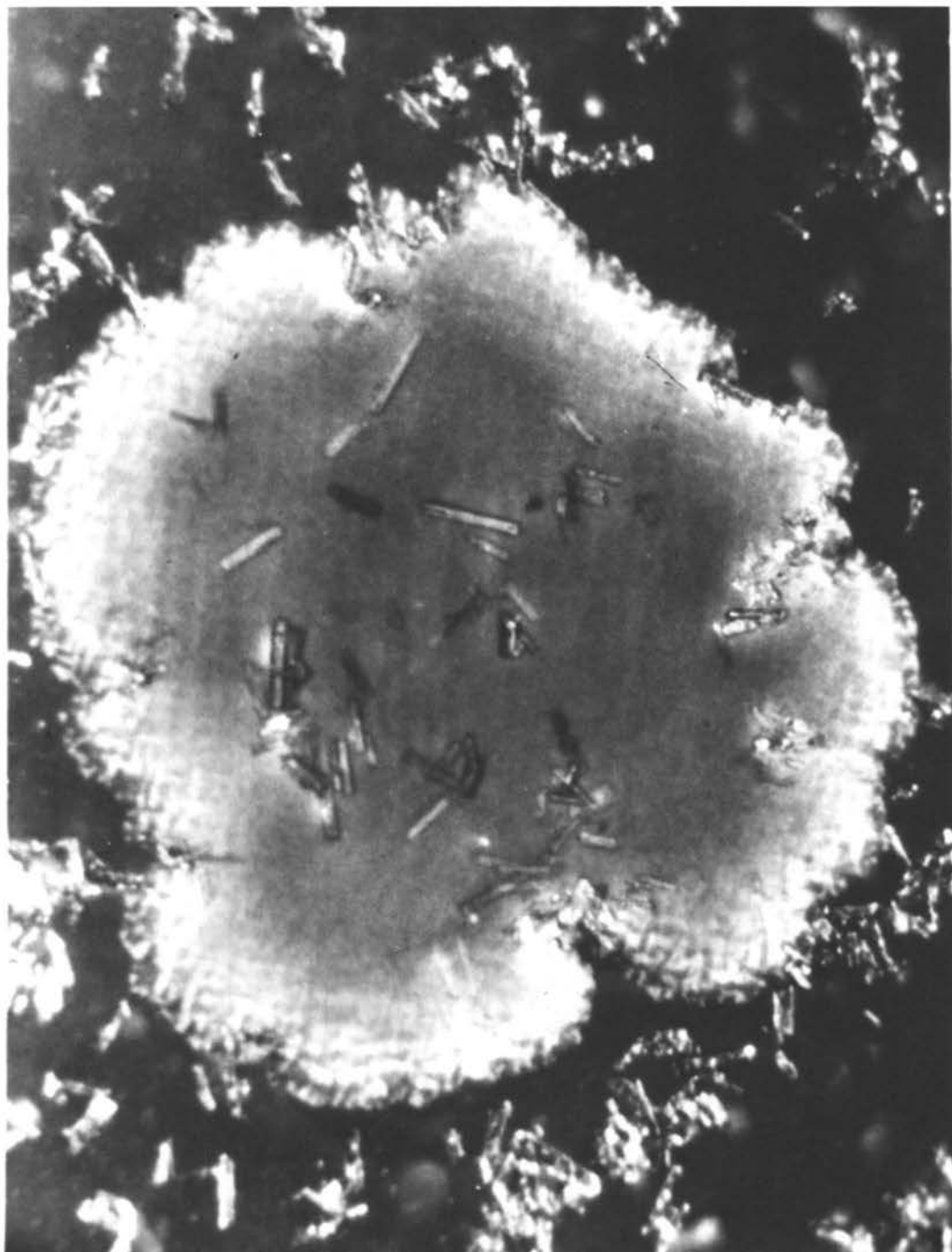
Photomicrograph of a colony of an aldrin contaminant, identified as
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INTRODUCTION

Recently introduced compounds designed for the control of soil insects are demanding more and more attention to determine possible effects of these biological poisons upon the microbial activity in the soil and resulting influences on soil fertility. It was the purpose of this experimentation to determine the effect of aldrin upon the ammonification and nitrification of added peptone, also its effect upon the numbers and certain groups of soil microorganisms, and its effect upon the liberation of carbon dioxide from the soil.

Soil bacteriology is concerned with the microorganisms which make up the soil population and their relationship to soil fertility. The soil may be considered a culture medium sustaining the growth and reproduction of both microorganisms and higher plants. Soil microbes do not exist in separate communities, but are variously intermixed, so that even minute portions of soil contain representatives of the various groups. The activity of a single microorganism is extremely complex, and the complexity of the activities of the intermixed population results in an intricate symbiosis.

The physiological activity of soil microorganisms represent the potential fertility of the soil. These microorganisms render organic residues as well as inorganic substances and gaseous nitrogen available to plants as plant food. Additions of carbohydrate, protein, or other readily soluble organic compounds to the soil

disappear rapidly with the concommitant formation of the humus-
complex. Humus is the absorption complex of dead microbial proto-
plasm and organic plant and animal residues. Humus represents
an available store of plant food; and due to its resistant nature,
it is rendered available to the plants only very slowly. Thus,
it tends to maintain the carbon-nitrogen balance of the soil.

Soil microorganisms represent two basically distinct types
of nutrition from the standpoint of biological economy. The min-
eral feeders, autotrophes, which are beneficial, non-parasitic, and
generally considered producers. The chlorophyll-containing algae,
the photosynthetic and chemosynthetic bacteria of which the nitri-
fiers are an extremely important group of organisms, make up this
type of nutritional classification. The second nutritional type
of microorganisms are the biological feeders, heterotrophes, which
are considered destructive and parasitic. The heterotrophes en-
compass a large portion of the soil microbes which include the
molds, most of the bacteria, and the protozoa.

Before one may gain an insight to the complex nature of
soil physiology, it is necessary that a general understanding of
the cycles of carbon and nitrogen be salient. In the carbon cycle,
low energy-containing carbon dioxide from the atmosphere is conver-
ted by the autotrophic microorganisms into complex organic com-
pounds of higher energy content. The photosynthetic bacteria and
algae are capable of converting the sun's energy, incident light
energy, to chemical energy. The autotrophic microorganisms de-
rive energy by the oxidation of certain soil minerals such as

sulfur and hydrogen. Some obtain energy from the oxidation of such simple compounds as ammonia, nitrate, methane, hydrogen sulfide, and carbon monoxide. The heterotrophic microorganisms utilize the organic compounds created by the autotrophic bacteria, and/or plant or animal residues as a source of energy. The end products of these manifold reactions are in general carbon dioxide and energy and thus carbon dioxide reenters the carbon cycle.

Up until fairly recently, carbon dioxide was considered as a poisonous principle and was referred to as Spiritus lethalis. This conception was largely due to the reasoning that if an excessive concentration of carbon dioxide were present it would replace the oxygen and suffocation would result. Since the work of Wood and Werkman (60, pp. 1263-1271), it is known that carbon dioxide is essential to the living cell and enters into several reactions of the assimilative cycle. At present there is a tendency to refer to carbon dioxide as the Spiritus vitalis--the spirit of life.

The nitrogen cycle consists largely of five rather distinct phases: ammonification, nitrosofication, nitrification, nitrogen fixation, and denitrification. Nitrogen metabolism in soil encompasses a vast sequence of chemical and physical reactions manifest by the nitrogen molecule in biological processes taking place in soil, from its initial fixation until its final liberation into the atmosphere. The transformations in soil of organic nitrogen compounds arising from all forms of biological material into ammonium ions is termed ammonification. Most heterotrophic bacteria,

molds, and Streptomyces are capable of this hydrolysis or diges-
tion of protein with the subsequent formation of ammonia. The
liberation of ammonia is limited by the carbon:nitrogen ratio of
the soil organic matter. If this ratio of carbon to nitrogen does
not greatly exceed a range of from 10 or 20:1, the nitrogen com-
pounds in plant residues will be decomposed to form ammonia. The
breakdown of protein is not a simple one-step reaction, but one
in which there are several continuous and simultaneous reactions
which ultimately yield amino acids. These amino acids may yield
ammonia by the exercise of oxidase systems of either resting or
proliferating microorganisms. Ammonia thus becomes available as
a source of energy for the autotrophic organisms. Ammonium cation
is oxidized to nitrite and then to nitrate anions by these auto-
trophic nitrosofying and nitrifying bacteria. This conversion is
accomplished largely by two groups of organisms, Nitrosomonas
(also Nitrosocystis and Nitrosospira), which forms nitrite ion
from ammonium ions, and Nitrobacter (also Nitrocystis and Bactaderma),
which converts nitrite ions into nitrate ions. The nitrate as well
as ammonium ion are utilized by plants and converted to protein
through a reversal of the metabolic process, which thus completes
the cycle.

Another segment of the nitrogen cycle, which is of utmost
importance in the maintenance of nitrogen economy, is nitrogen fixa-
tion. This process is the transformation of atmospheric nitrogen
by soil microorganisms into compounds which nurture the heterotrophic

microbe and plant. The ability to reduce or fix atmospheric nitro-
gen is limited to but a few bacteria, which are heterotrophic, the
mixotrophic purple bacteria, and certain blue-green algae. However,
the process is accomplished largely by two sets of microorganisms,
one consisting of the root nodule bacteria living symbiotically
in the nodules of certain leguminous host plants, and the other,
consisting of microorganisms living independently in the soil.
Non-symbiotic nitrogen fixation is effected by Azotobacter and
certain species of Clostridium. The nitrogen fixed by Azotobacter
is converted directly to ammonia which may be assimilated and in-
corporated in the cell protoplasm. Upon the death of these micro-
organisms the protoplasmic nitrogen is then made available to other
heterotrophic organisms. Symbiotic nitrogen fixation is accomplished
by species of Rhizobium living symbiotically in the nodules of le-
guminous plants. The method of nitrogen fixation by both symbiotic
and free living organisms is still in some question; however, it
has been established that Rhizobium does not form ammonia as an
intermediate, which is in contrast to Azotobacter metabolism
(57, p.41). The excess nitrogen thus fixed becomes available to
the host and some may diffuse into the soil. All of the nitrogen
is available only after decomposition of the host plant residue.

The conditions under which free nitrogen is lost in denitri-
fication are not well understood. There are many heterotrophic
microorganisms which are capable of breaking down nitrates to
nitrites. However, only a few are capable of the reduction with
the liberation of free nitrogen or other oxides of nitrogen. It

is known that the lack of sufficient soil aeration is conducive to these processes. The heterotrophic utilization of nitrogen oxides as a source of oxygen in the absence of adequate atmospheric oxygen is probably the major source of denitrification. However, a quantitative evaluation of this reaction in soils has not been assessed.

The extremely large and ramified animal kingdom uses the correspondingly large and complex plant kingdom as a major source of food. We know that the animals do not utilize all of the plant substance as a whole. A major portion of this is left in an undigested or partially digested state. The residual material then becomes subjected to the decomposing activities of microorganisms. As a result of the activities of these microorganisms, the elements which had originally been consumed by the plants for organic synthesis are returned to circulation, thus completing the cycle of the elements in the process of life.

Organic matter, such as plant residues, green manure, barnyard manure, and organic fertilizers, when added to the soil, under favorable conditions, are immediately subject to decomposition by soil microorganisms. The molds, aerobic bacteria, and the Streptomyces initially act on these relatively simple and soluble compounds, such as sugars, starches, alcohols, acids, proteins, and urea. The large quantities of carbon dioxide liberated in this early phase of decomposition is important because of its solvent action on soil minerals. The products resulting from these reactions are carbon dioxide, water, ammonia, hydrogen sulfide, hydrogen, organic acids, alcohols, other incompletely oxidized substances, and energy.

The soluble organic by-products stimulate the free living nitrogen-fixing bacteria.

Further decomposition is characterized by two rather distinct phases: An autotrophic phase, wherein the autotrophic bacteria oxidize ammonia, hydrogen sulfide, methane, and hydrogen; and a heterotrophic phase wherein the organic by-products are utilized by a large number of diverse heterotrophic microorganisms. After the supply of these foods is exhausted, the more specialized soil microorganisms begin to decompose more complex starches, hemicelluloses, celluloses, and fats. The decomposition of these materials is accomplished by a variety of taxonomically and nutritionally different microorganisms. This second stage in organic matter decomposition proceeds more slowly, and weeks or months may elapse before their decomposition is completed. During this time there is a tremendous increase in the number of microorganisms originally present, and much of this initially added organic matter is now in the form of dead and living microbial protoplasm.

The added organic residues do not become completely mineralized. A certain portion of this material is somewhat immune to microbial decomposition and remains for a period of time in an unaltered or in a slightly modified state, and under certain conditions may even accumulate. This resistant material is dark brown to black in color and possesses certain characteristic physical, chemical, and colloidal properties. Decomposition in its final stage is referred to as humification and is characterized by the

gradual formation and slow continual, more or less constant, decomposition of the humus-complex.

As a result of the formation and accumulation of humus, some of the elements essential for life, especially carbon, nitrogen, phosphorous, sulfur, and potassium, become adsorbed and removed from biological circulation; thus, humus serves as a check upon plant life by storing carbon, nitrogen, available phosphorous, and potassium in an unavailable state. But since humus can undergo slow decomposition under certain favorable conditions, it tends to supply a slow but continual source of elements essential for new plant synthesis. It plays a prominent role in the formation of most soils. It exerts a wide variety of physical, chemical, and biological influences upon the soil, making the soil a favorable substrate for plant growth. The relationship between humus and soil microorganisms must be appreciated in any explanation of the development of life on this planet.

There exists an important relationship between the carbon and nitrogen content of organic materials added to the soil because of the effect this relationship has upon the general course of the microbiological activity. The situation is analogous to the feeding of animals wherein a correct balance of carbohydrate and protein must be maintained for the health and well being of the individual. This relationship is generally referred to as the carbon:nitrogen (C:N) ratio. Invaluable reactions occur in the soil when this ratio of the humus-complex does not go below a minimum of about 8:1, and does not exceed a maximum of approximately 12:1. Depending upon the

environmental factors, the optimum carbon:nitrogen ratio of humus

is considered to be 10:1, i.e., ten parts of carbon to one part of

nitrogen. When organic matter with a wide carbon:nitrogen ratio is

added to soil, there is a loss of organic matter and an almost im-

mediate utilization of soil nitrogen by soil microorganisms. This

represents a depletion of the nitrogen which otherwise would be

available for plant use.

If organic matter with a narrow carbon-nitrogen ratio is added

to the soil, there tends to be a saving of organic carbon and a re-

lease of simple nitrogenous by-products. The simple substances are

decomposed more economically and the energy derived from this pro-

cess is utilized more efficiently in the chemical transformation

of the more complex substances present.

In this introduction to the principles of soil microbiology,

there has been an effort to emphasize the complex and interreacting

nature of the factors involved in a study of the crop-producing capa-

city of the soil from the microbiological point of view. All of these

factors, as well as others not immediately concerned in this investi-

gation, are so intimately linked and interconnected that if the ac-

tivity of a single group of microorganisms is affected in any way,

it will be reflected and possibly magnified in the activity of a

closely related group; thus, the introduction of neoteric substances

(insecticides, herbicides, or fungicides) foreign to the soil may re-

sult in a partial or complete inhibition of the biological cycles which

find their inception in the soil and are necessary for continued fertility.

PREVIOUS INVESTIGATIONS

There have been some studies directed to determine the effect of insecticides such as o,p-dichloro diphenyl trichloroethane (DDT), benzene hexachloride, chlordane, zinc-lime, chlorinated camphene, lead arsenate, parathion, toxaphene, sulfur, and fermate upon soil microorganisms. Jones (19, pp.238-239) found DDT did not injure nitrifiers, ammonifiers, or sulfur-oxidizing microorganisms, at concentrations below 0.1 per cent. Jones also observed no effect of DDT upon the nitrogen-fixing bacteria in soils containing as much as 1.0 per cent of the insecticide; he could observe no injury to soil microorganisms determined by plate counts, but found instead an increase in numbers. Appleman and Seats (3, p.247) found no injury to legume nodulation when soil treatments were less than 100 pounds DDT per acre. Wilson and Choudhri (56, p 538) found no effect upon numbers, ammonifiers, or nitrifying bacteria at DDT concentrations as high as 5 per cent. While these same authors found that an application of 30.0 p.p.m. benzene hexachloride was seriously injurious to legumes, yet there was no obvious effect upon nodulation bacteria. Smith and Wenzel (40, pp.221-233) noted that DDT had no effect upon the total numbers, nitrifiers, mold, or protozoa. These investigators also found that benzene hexachloride applied at a rate of 500 pounds per acre had a great stimulatory effect upon the numbers of bacteria. However, at this concentration there was a drastic reduction in the numbers of molds present. The nitrifying organisms were even more drastically reduced. They found that the Streptomyces were apparently not affected. Chlordane added to the soil at

100 pounds per acre generally increased the numbers of organisms as
determined by the plate count method. However, the nitrifiers ap-
peared to be adversely affected; the nitrate formers were more se-
verely affected than the nitrite forming organisms. Chlorinated
camphene was definitely stimulatory to the total numbers of micro-
organisms. In this case the insecticide was assumed to be a food
source. Jones (19, p.238) found that DDT was toxic to ammonifiers
at concentrations of 0.1 per cent and that benzene hexachloride and
chlordane were injurious to nitrifiers at concentrations above
0.5 per cent. Experiments at the West Virginia Agricultural Experi-
ment Station (14, p.713) indicate that DDT, parathion, benzene hexa-
chloride, chlordane, toxaphene, zinc-lime, sulfur, and fermate as
well as 2,4-dichlorophenoxyacetic acid (2,4 D) have a stimulatory
effect upon the growth and productivity of apples and peaches when
the compounds were added at rates as high as 30 times those recom-
mended. These investigators are unable to explain the growth stimu-
lating effects of the chemicals.

A Chlorinated Hydrocarbon Insect Toxicant, Aldrin

One of the more recently introduced soil insecticides is
"aldrin," the official coined name for the alkali-stable compound
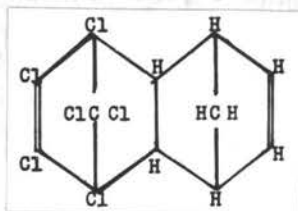
containing not less than 95 per cent of 1,2,3,4,10,10-hexachloro-
1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene. This

dimethanonaphthalene has an empirical formula $C_{12}H_8Cl_6$, and its planar structural representation is represented above.

Aldrin is freely soluble in aliphatic and aromatic hydrocarbon solvents and is sparingly soluble in methanol. It is insoluble in water (soluble to the extent of 0.05 p.p.m.)*(17, pp.3-4). Aldrin is stable in the presence of strong organic and inorganic alkalis and is unaffected by hydrated metallic chlorides. It is also unaffected by acids normally encountered in the agricultural chemical field. Owing to its chemical stability, aldrin may be used with most of the available agricultural chemicals including fertilizers, herbicides, fungicides, and insecticides. It may be used in the presence of alkaline soils, lime, lime-sulfur, and other materials of high pH. Aldrin is used in concentrations of less than 5 pounds per acre for the control of many subterranean insects, including Thrips. (17, pp.1-2). The aldrin used in this study was obtained from Julius Hyman and Company, Denver, Colorado; for the laboratory studies it was passed through a 100-mesh screen and was in a crystalline form, 99 per cent pure.

* Personal communication from L. L. Lykken.

I LABORATORY TREATED SOIL STUDIES

A. INTRODUCTION

Since aldrin has shown considerable promise for the control
of a wide variety of insects and is being widely tested in different
soils by the Entomology Department of the Oregon Agricultural Experi-
ment Station, laboratory studies were undertaken to determine its
effect on microorganisms and some of their activities in 12 repre-
sentative field plot samples. Table 1, page 14, lists the sampled
soils by location, description, class, and type. Each sample was
made from composites collected randomly from the designated areas.
The samples were stored in air-tight metal containers and were not
opened until they were to be prepared for laboratory use. To prepare
the soil it was spread out in flat wooden trays and allowed to air
dry until friable, after which it was passed through a 10-mesh screen.
The screened sample was replaced in the air-tight container, the
moisture content and the saturation capacity determined.

One hundred-mesh aldrin was admixed at rates of 200 and 1,000
p.p.m. (400 and 2,000 pounds per acre, respectively),* with the soil
and incubated under optimum moisture and temperature. At selected
intervals total numbers of microorganisms were determined by plate
counts, and conventional procedures were used to study ammonification,
nitrosofication, nitrification, and carbon dioxide evolution in the
treated and control samples.

* Based on the conventional usage of 2,000,000 pounds for the
weight of one acre of an average soil to plow depth of 6 2/3 inches.

TABLE 1

Composite Soil Samples from Representative Field Plots in Oregon

Field No.	Date Collected	Description and Location	Soil Class or Type
1	5-17-51	Schoth Garden--Corvallis, Benton Co.	Willamette Clay Loam
2	5-17-51	Entomology Farm--Corvallis, Benton Co.	Willamette Sandy Loam
3	4-16-51	H. H. White Farm--West Stayton, Benton Co.	Willamette Silty Clay Loam
4	4-10-51	Hathaway Farm--Kiger Island, Benton Co.	Willamette Clay Loam
5	4-14-51	Kurth Farm--Labish Center, Marion Co.	Labish Peat
6	5-7-51	Chambers Farm--Deaver, Linn Co.	Silty Clay Loam
7	4-14-51	Stayton Farm--Hermiston, Umatilla Co.	Fine Sand
8	5-15-51	Oregon State College Potato Plots Corvallis, Benton Co.	Willamette Clay Loam
9	3-22-51	Hartley Ranch--Oyhee Dam, Malheur Co.	Silty Loam
10	5-16-51	Oak Creek Field-- Corvallis, Benton Co.	Cove Clay Loam
14	5-7-51	Towery Farm--Stayton Marion County	Silty Clay Loam
16	4-25-51	Zielke Farm--Roberts Marion Co.	Silty Clay Loam
20	3-26-51	Pitcher Farm--Grand Island, Yamhill Co.	Silt Loam

B. EXPERIMENTAL METHODS

Soil Counts

(1) The effect of aldrin added to dilution blanks: Twenty grams soil, water-free basis, and portions of the insecticide were weighed into 100-ml. sterile water blanks, shaken for five minutes, and then allowed to settle for one minute. The experiment was designed to determine the effect of concentrations of 200 (20 mg./100 gm. of w-f soil) and 1,000 p.p.m. (100 mg./100 gm. w-f soil) aldrin.

Subsequent dilutions were made from the original 1:5 suspension in a like manner, in all cases pipetting the sample from half-way down in the supernatant. The insolubility of aldrin in water makes the use of a standard dilution from which aliquots could be taken, impossible.

(2) The effect of aldrin admixed with soil only or soil plus added peptone and incubated under optimum conditions.

The Willamette clay loam, fine sand, and Labish peat soils were admixed with 200 and 1,000 p.p.m. aldrin, incubated for from 0 to 31 days under optimum moisture and temperature. Periodic plate counts were made during the incubation (Table 4, pp.26-27; Figures 2, 3,4, pp.28,29,30). Aldrin-treated Willamette clay loam soil (Schoth Garden) was incubated with 1,000 p.p.m. added peptone and the plate counts determined at 0, 5, 10, and 20 days incubation.

Three plates at two dilutions for bacteria and molds were found satisfactory. For the bacteria and Streptomyces dilutions 1 to 50,000 and 1 to 500,000 were used; for the molds, 1 to 500 and

1 to 5,000. Sodium albuminate agar was used as the medium for the bacteria and Streptomyces; peptone-glucose acid (PGA) agar was used for the molds. All plates were incubated at 28°C. The mold plates were counted after 3 or 4 days and the bacterial plates after 5 to 7 days. Only plates showing 50 to 300 bacterial colonies or 20 to 100 mold colonies were counted.

Ammonification

Table 2, p. 17, indicates the way in which the experiment was set up. One hundred gram (w-f basis) samples were used throughout the experiment--the peptone, (Difco), which had been passed through 100-mesh screen, and the aldrin, were added to the soil samples and mixed thoroughly. Since the aldrin and the peptone tended to adhere, aldrin was mixed with the soil first; the peptone was then admixed. Care was taken to break up clumps when they were discovered. The treated soils were then carefully placed in pint milk bottles which were of appropriate capacity to facilitate the preparation of a 1 to 4 soil suspension for later analysis.

Soil in each bottle was made up to 60 per cent saturation and incubated at 28°C. One set of the samples was analyzed after five days, other sets at ten and twenty days. All samples were maintained as closely as possible to 60 per cent saturation during the incubation period. After incubation, ammonia was determined by distillation with phosphate buffer, pH 7.4 (29, p.311); nitrites were determined colorimetrically by the sulfanilic acid--alpha naphthylamine method (1, p.18); and nitrate by the phenodisulfonic acid method.(2, p.24).

TABLE 2

Ammonification: Experimental Set Up

Number of Samples	Treatment
6	100 grams soil*
6	100 grams soil* plus 200 p.p.m. aldrin
6	100 grams soil* plus 1,000 p.p.m. aldrin
6	100 grams soil* plus 200 p.p.m. aldrin plus 1,000 p.p.m. N as peptone
6	100 grams soil* plus 1,000 p.p.m. aldrin plus 1,000 p.p.m. N as peptone

* Water-free basis.

Two samples from each set were analyzed after 5, 10, and 20 days incubation.

Nitrification

The concentrations of nitrites and nitrates after 20 days incubation in the ammonification experiment were indicative of nitrification. For a more direct study of nitrification, ammonium chloride and sodium nitrite were added at rates of 500 p.p.m. N to 100 grams of soil in pint milk bottles. Two sets were treated respectively with 200 p.p.m. and 1,000 p.p.m. aldrin; a third set used as a control contained no aldrin. All were incubated at 28°C. Duplicate samples were analyzed at ten and twenty days for ammonia, nitrite, nitrate, and pH. Optimum moisture was maintained throughout the incubation period.

pH

The pH was determined with a glass electrode, using soil suspensions of 1 to 5 in distilled water.

Carbon Dioxide Evolution

Carbon dioxide evolution, plate counts, and ammonification studies were made concurrently upon the Willamette clay loam soil. The CO_2 was collected in 1 M NaOH using positive pressure methods, and determined volumetrically using the Beckman automatic titrimeter. The CO_2 is reported as milligrams carbon, per 100 grams soil, water-free basis. The experimental set up used was the same as the one used in the ammonification study (Table 2, page 17). All treatments were made in quadruplicate. The samples were incubated at 28°C.

The CO_2-free air was passed over the soil at a slight pressure necessary to cause it to bubble slowly and consistantly through a 1 by 6 inch test tube containing approximately 30 ml. of 1 M NaOH. The CO_2 absorption tubes were changed after 24 and 48 hours and 5, 10, 15, and 20 days. (Figures 15 and 16, p. 43)

TABLE 3

Analysis of Field Treated Soils*
Schoth Garden: Willamette Clay Loam
Schoth Check Composite: 1951

Chemical Analysis:

Moisture, per cent	12.1
Saturation Capacity, per cent	40.0
pH	7.0
Lime Requirement (Troug) Tons/Acre	2.5
Nitrogen	
Ammonium ion	20.0 p.p.m.
Nitrite	Trace
Nitrate	2.0 p.p.m.
Kjeldahl, per cent	0.246
Total Carbon, per cent	3.23
C:N Ratio	13:1

Microorganisms:

Molds, per gram	5,500
Penicillia, per cent	45.4
Mucors, per cent	45.4
Aspergilli, per cent	0
Trichoderma, per cent	9.0
Bacteria, per gram	25.0×10^6
Streptomyces, per cent	23.0
Azotobacter	Present

* Data expressed on water-free basis.

Nitrification by Perfusion Studies

Samples of the Willamette clay loam soil were perfused with
0.01 M NH_4Cl solution (350 ml.), in a modified Quastel-Lees perfusion
apparatus (32, p.288), as shown in Figure 1, p. 21. A vacuum of
approximately 5 pounds per square inch was maintained throughout
the experiment. The soil was treated in two ways: (1) Twenty-five
grams of crumbed soil (2-4 mm.) (water-free basis) plus crystalline
aldrin at 1,000 p.p.m. were admixed and placed in the sample tube.
The experiment was set up in triplicate, three tubes containing soil
only, three others the aldrin-treated soil. (2) Twenty-five grams
of the crumbed soil (2-4 mm.) were placed into the sample tube.
Five ml. of aldrin in 95 per cent ethyl alcohol solution were then
pipetted on to the soil. The 5 ml. of the aldrin solution contained
5 mg. per ml. of the insecticide and was equivalent to 1,000 p.p.m.
aldrin when added to the soil. Soil only and soil plus 5 ml. ethyl
alcohol were used to control the experiment.

The rate of perfusion in each of the flasks was adjusted as
closely as possible so that all samples were perfused in uniform
manner; however, some variation occurred due to unavoidable varia-
tions in internal friction; water pressure was used to actuate the
vacuum source. Perfusion rates were such so that each sample was
at all times saturated with the NH_4Cl solution, care taken not to
water log the soil at any time.
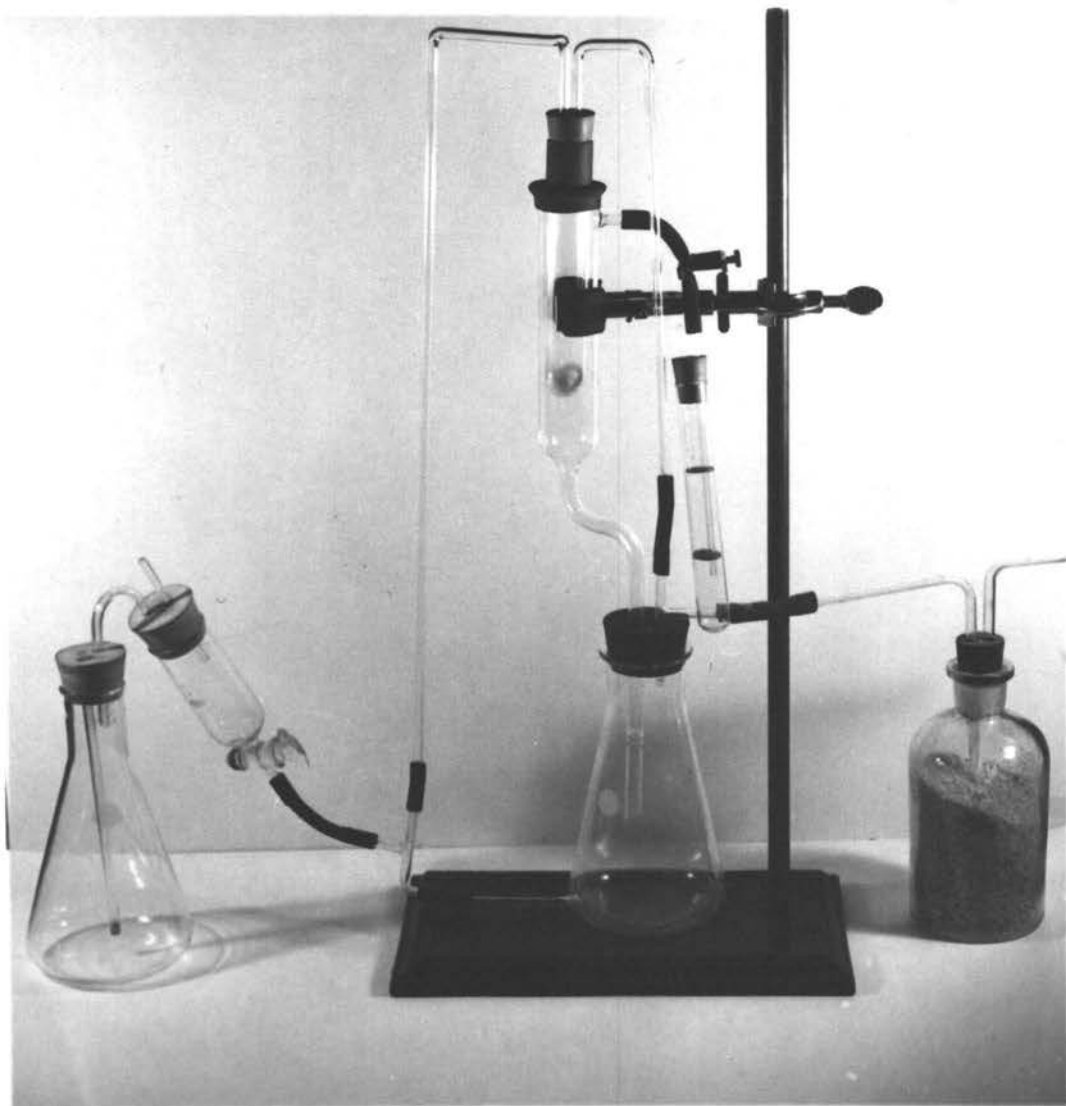
FIGURE 1: SOIL PERFUSION APPARATUS

Manometric Investigation of Ammonium Ion

Oxidation by Aldrin-Treated, Enriched Soil

Soil that has been enriched or saturated with organisms attack-
ing certain substrates may be conveniently investigated with the con-
ventional Warburg manometric technique.

A soil known to be enriched with nitrifying organisms, through
several perfusions of ammonium salts until complete nitrification
has taken place, is washed with distilled water until the washings
are free from nitrate and ammonium ions, and is pushed out of the soil
tube on to a muslin cloth which is then wrapped around the soil.
This is suspended over a sink and the soil allowed to drain over-
night (18 hours at $20^{\circ}C.$). The soil is now squeezed gently to break
up lumps and chopped with a spatula until it becomes a mass of small
discrete crumbs rather than a mud. The water content of this soil
is about 50 per cent. One and one-half grams of the soil is weighed
out and placed in a Warburg flask, care being taken to prevent soil
entering the center well or the side arm. This is done by placing
a small glass hood over the well and tilting so that the side arm is
uppermost. Two ml. of 0.01 M NH_4Cl is added to the soil, which now
becomes completely covered with fluid. Filter papers soaked in KOH
solution are placed in the center well and the Warburg manometers
are set up and shaken in the conventional manner. Experiments were
carried out at $37^{\circ}C.$, as, at this temperature, rates of oxygen uptake
are appreciably higher than those at $21^{\circ}C.$ There is no reason to
suspect that the kinetics of oxidation by the soil organisms are

adversely affected by the higher temperature. Control manometric experiments are always carried out with soils suspended in water only (33, pp.15-17).

Azotobacter by the Mud-Pie Method

To 50 grams of soil (water-free basis) was added 0.5 grams of $CaCO_3$ and 2.5 grams of soluble potato starch (36, pp.1-36). The $CaCO_3$ and starch were incorporated into the soil by thorough mixing. The soil was then placed in 3-inch petri dishes, sterile water added, and worked into a stiff paste. The surface of the soil was then glazed by pressing and smoothing with a spatula. Samples treated with 200 and 1,000 p.p.m. aldrin, and controls, were set up in duplicate. They were incubated in a moist chamber at 28°C. for 7 days. The results were recorded as relative abundance of Azotobacter colonies developing on the surface of the soil.

Stability of Aldrin in Garden Soil

Preliminary experiments with carbon dioxide production from soil and soil extracts treated with aldrin indicate that the aldrin may have been utilized as a carbon source. The following experiment was designed to determine the stability or possible microbial decomposition of the compound in Willamette clay loam garden soil: One hundred gram samples, water-free basis, were admixed with 200 p.p.m. (20 mg.) aldrin and incubated at optimum moisture and temperature. Peptone at 1,000 p.p.m. nitrogen was added with the insecticide to determine the effect of the recrystallized aldrin on

ammonification and nitrification. Chemical analyses for ammonium, nitrite, and nitrate ions were made after 0, 10, and 20 days incubation; concommittantly on a replicate experimental set-up, hexane extractions were obtained for aldrin analysis.

The extraction procedure was relatively simple and consisted of the following: Samples in toto were removed from the milk bottle containers, placed on paper, and allowed to air dry. The moisture of each of these air-dry samples was determined and recorded. Seventy-five grams of the air-dried soil weighed to ± 0.01 gram, was then placed into a 250 ml. centrifuge bottle and an equal quantity of n-hexane added. This was then mechanically shaken for 30 minutes, centrifuged at 1,500 r.p.m., and filtered through absorbent cotton. The aldrin content of these extracts was determined by the colorimetric method of Danish and Lidov (8, pp.190-197).*

* Analyses of the extracts were made in the laboratories of Julius Hyman and Company, a division of Shell Oil Company, under the direction of Dr. L. L. Lykken, Technical Service Manager.

Soil Counts

Aldrin applied at concentrations of 200 and 1,000 p.p.m.
appeared to have no injuriouseffect upon the numbers of microorganisms
as determined by the plate count method. Instead, a stimulation due
to the presence of the insecticide was noted. This stimulation was
quite marked in all cases with added peptone at 5, 10, and 20 days
(Figures 2, 3, and 4; pp. 28, 29, and 30). There were no specific
groups or species of bacteria noted in cases where this stimulation
was apparent. The Streptomyces appeared not to be effected by addi-
tion of the insecticide at either concentration (Table 4, pp. 26-27).
Mold counts indicate little or no effect of the compound (Table 4,
pp. 26-27). Plate counts obtained from the perfused soil will be
discussed elsewhere.

Ammonification

A compilation of the total p.p.m. of ammonia recovered from
the ten soils chosen from the study indicates a slight stimulation
to the ammonification process early in the stages of incubation.
This stimulation occurs at approximately 5 days; however, shortly
following, there is a definite trend toward inhibition at both
concentrations of insecticide. This inhibition is quite apparent
at 10 days incubation, as shown in Figures 5, 6, 7, and 8; pp.
32, 33, and 34, and Table 5, p. 31. Results indicate that there is
a gradual recovery from this depression and that recovery is partially
complete at approximately 20 days.

TABLE 4

The Effect of Aldrin on the Numbers of
Soil Microorganisms
Bacteria

Soil Type and Field Number	Treatment	Incubation Period (Days)	Total Numbers Per Gram X 10^6	Per Cent Streptomyces	Per Cent Apparent Stimulation or Inhibition	
					Bacteria	Streptomyces
(6) Silty Clay Loam	Soil only	0	40.4	11.1	--	--
	≠ 200 ppm Aldrin	0	48.6	9.7	≠ 20.3	- 12.7
	≠ 1000 ppm Aldrin	0	86.0	11.0	≠112.3	- 1.0
(1) Willamette Clay Loam	Soil Only	0	25.0	6.7	--	--
	≠ 200 ppm Aldrin	0	82.0	6.8	≠266.2	≠ 1.5
	≠ 1000 ppm Aldrin	0	74.0	7.3	≠173.5	≠ 8.9
	Soil Only	5	70.0^x	10.6	--	--
	≠ 1000 ppm Peptone N	5	306.0^x	4.3	--	--
	≠ 200 ppm Aldrin	5	96.0^x	6.8	≠ 35.5	- 7.5
	≠ 200 ppm Aldrin ≠ 1000 ppm Peptone N	5	294.0^x	4.1	≠ 28.6	≠ 23.1
	≠ 1000 ppm Aldrin	5	90.0^x	12.2	≠ 27.7	- 6.5
	≠ 1000 ppm Aldrin ≠ 1000 ppm Peptone N	5	668.0^x	3.1	≠118.5	≠ 57.7
	Soil Only	10	67.0^x	8.9	--	--
	≠ 1000 ppm Peptone N	10	556.0^x	12.2	--	--
	≠ 200 ppm Aldrin	10	69.4^x	8.9	--	--
	≠ 200 ppm Aldrin ≠ 1000 ppm Peptone N	10	1000.0^x	5.0	≠ 87.0	- 15.4

Soil Type and Field Number	Treatment	Incubation Period (Days)	Total Numbers Per Gram $\times 10^6$	Per Cent Streptomyces	Per Cent Apparent Stimulation or Inhibition	
					Bacteria	Streptomyces
(1) Willamette Clay Loam Con't.	+ 1000 ppm Aldrin	10	65.0[x]	9.2	- 3.7	- 10.0
	+ 1000 ppm Aldrin + 1000 ppm Peptone N	10	1080.0[x]	2.3	+102.0	- 61.5
	Soil Only	20	29.6[x]	17.0	--	--
	+ 1000 ppm Peptone N	20	270.0[x]	16.7	--	--
	+ 200 ppm Aldrin	20	42.0[x]	14.3	+ 42.3	+ 20.0
	+ 200 ppm Aldrin + 1000 ppm Peptone N	20	710.0[x]	9.8	+165.0	+ 55.6
	+ 1000 ppm Aldrin	20	57.6[x]	17.3	+105.5	+160.0
	+ 1000 ppm Aldrin + 1000 ppm Peptone N	20	400.0[x]	10.0	+ 49.1	+ 25.0
(2) Willamette Sandy Loam	Soil Only	0	360.0	8.3	--	--
	+ 200 ppm Aldrin	0	190.0	2.6	- 47.2	- 83.3
	+ 1000 ppm Aldrin	0	196.0	5.1	- 45.8	- 66.6
(16) Silty Clay Loam	Soil Only	0	125.0	14.0	--	--
	+ 200 ppm Aldrin	0	132.0	9.6	+ 4.8	- 28.8
	+ 1000 ppm Aldrin	0	122.0	16.3	- 2.0	+ 14.3
(7) Fine Sand	Soil Only	0	87.6	12.6	--	--
	+ 200 ppm Aldrin	0	76.0	9.9	- 13.1	- 31.8
	+ 1000 ppm Aldrin	0	91.0	12.1	+ 4.0	--
	Soil Only	5	55.0[x]	9.1	--	--
	+ 200 ppm Aldrin	5	72.0[x]	6.3	+ 30.9	- 20.0
	+ 1000 ppm Aldrin	5	66.4[x]	4.4	+ 22.7	- 40.0
	Soil Only	31	116.0	12.9	--	--
	+ 200 ppm Aldrin	31	152.0	8.2	+ 31.5	- 16.6
	+ 1000 ppm Aldrin	31	158.0	9.1	+ 36.6	- 3.3
(5) Labish Peat	Soil Only	0	41.6[x]	14.1	--	--
	+ 200 ppm Aldrin	0	30.0[x]	14.8	- 28.3	- 25.0
	+ 1000 ppm Aldrin	0	38.0[x]	17.1	- 10.6	+ 7.7
	Soil Only	5	58.0	5.2	--	--
	+ 200 ppm Aldrin	5	44.0	4.4	- 22.4	- 33.3
	+ 1000 ppm Aldrin	5	38.0	7.9	- 34.5	--
	Soil Only	31	54.0	12.7	--	--
	+ 200 ppm Aldrin	31	58.0	12.1	+ 5.5	--
	+ 1000 ppm Aldrin	31	76.0	15.8	+ 38.2	+ 71.4

Soil Type and Field Number	Treatment	Incubation Period (Days)	Total Numbers Per Gram X 10^6	Per Cent Streptomyces	Per Cent Apparent Stimulation or Inhibition	
					Bacteria	Streptomyces
Labish Peat (Con't.)	Soil Only	0	134.0	13.8	--	--
	╪ 200 ppm Aldrin	0	142.0	10.9	╪ 5.6	- 16.2
	╪ 1000 ppm Aldrin	0	116.0	12.5	- 13.4	- 21.6
Willamette Silty Clay Loam (3)	Soil Only	0	98.0	10.5	--	--
	╪ 200 ppm Aldrin	0	90.0	25.5	- 9.5	╪ 62.0
	╪ 1000 ppm Aldrin	0	99.0	36.5	- 3.0	╪ 86.0
Willamette Clay Loam (4)	Soil Only	0	133.4	12.4	--	--
	╪ 200 ppm Aldrin	0	137.6	13.8	╪ 3.4	╪ 15.2
	╪ 1000 ppm Aldrin	0	117.6	10.3	- 12.0	- 27.3

x Average of six replicates.

TABLE 4a

The Effect of Aldrin on the Numbers of Soil Microorganisms
Molds

Soil Type and Field Number	Treatment	Incubation Period (Days)	Total Nos. Per Gram 2x 10^{1}	% Stim. or Inhibition	% Mucor	% Penc.	% Asp.	% Demi.	% Tricho.
(6) Silty Clay Loam	Soil Only	0	1,350	--	3.7	67.6	3.7	--	9.2
	≠ 200 ppm Aldrin	0	1,550	≠ 14.8	11.3	54.9	3.2	--	4.9
	≠ 1000 ppm Aldrin	0	2,200	≠ 63.0	5.0	52.0	1.0	--	2.0
(1) Willamette Clay Loam	Soil Only	0	275	--	45.5	18.2	36.3	--	--
	≠ 200 ppm Aldrin	0	350	≠ 26.3	42.8	12.5	50.0	--	--
	≠ 1000 ppm Aldrin	0	300	≠ 9.1	66.5	16.5	33.3	--	--
	Soil Only	5	675[x]	--	36.0	36.0	--	--	--*
	≠ 1000 ppm Peptone N	5	500[x]	--	10.0	15.0	40.0	--	2.8*
	≠ 200 ppm Aldrin	5	450[x]	- 28.0	11.1	22.2	1.8	--	1.8*
	≠ 200 ppm Aldrin ≠ 1000 ppm Peptone N	5	375[x]	- 25.0	13.1	--	60.0	--	26.7*
	≠ 1000 ppm Aldrin ≠ 1000 ppm Aldrin ≠	5	450[x]	- 28.0	44.4	22.2	11.1	--	--*
	1000 ppm Peptone N	5	475[x]	- 24.0	1.9	10.5	26.3	--	--*
	Soil Only	10	(2,000)[xx]	--	50.0	12.5	12.5	--	12.5
	≠ 1000 ppm Peptone N	10	450[x]	--	16.7	--	44.4	--	5.5*
	≠ 200 ppm Aldrin	10	800[x]	--	64.5	9.4	6.2	--	--*
	≠ 200 ppm Aldrin ≠ 1000 ppm Peptone N	10	(1,500)[xx]	--	33.3	16.7	16.7	--	--*
	≠ 1000 ppm Aldrin ≠ 1000 ppm Aldrin ≠	10	325[x]	--	50.0	25.0	--	--	--*
	1000 ppm Peptone N	10	425[x]	- 5.6	23.5	11.8	11.8	--	--*
	Soil Only	20	(1,250)[xx]	--	80.0	20.0	--	--	--
	≠ 1000 ppm Peptone N	20	(750)[xx]	--	33.3	66.6	--	--	--
	≠ 200 ppm Aldrin	20	(1,500)[xx]	--	33.3	50.0	--	--	--*
	≠ 200 ppm Aldrin ≠ 1000 ppm Peptone N	20	(2,250)[xx]	--	44.4	22.2	1.1	--	--*
	≠ 1000 ppm Aldrin	20	(750)[xx]	--	33.3	33.3	--	--	--*
	≠ 1000 ppm Aldrin ≠ 1000 ppm Peptone N	20	(1,500)[xx]	--	16.7	66.6	16.7	--	--
(2) Willamette Sandy Loam	Soil Only	0	125	--	60.0	40.0	--	--	--
	≠ 200 ppm Aldrin	0	50	- 60.0	--	100.0	--	--	--
	≠ 1000 ppm Aldrin	0	50	- 60.0	50.0	50.0	--	--	--
(16) Silty Clay Loam	Soil Only	0	450	--	83.3	5.6	--	--	11.1
	≠ 200 ppm Aldrin	0	375	- 16.7	11.8	76.5	--	--	11.8
	≠ 1000 ppm Aldrin	0	775	≠ 72.7	12.9	83.9	3.2	--	--
(7) Fine Sand	Soil Only	0	350	--	--	92.8	--	--	7.2
	≠ 200 ppm Aldrin	0	375	≠ 7.1	--	86.7	--	--	6.8*
	≠ 1000 ppm Aldrin	0	575	≠ 64.3	--	91.3	--	--	9.5

Soil Type and Field Number	Treatment	Incubation Period (Days)	Total Nos. Per Gram 2 x 10^1	% Stim. or Inhibition	% Mucor	% Penc.	% Asp.	% Demi.	% Tricho.
(7) Fine Sand Con't.	Soil Only	5	750	--	33.3	33.3	10.0	--	13.3*
	/ 200 ppm Aldrin	5	575	- 23.3	30.4	47.8	8.7	--	17.4
	/ 1000 ppm Aldrin	5	750	--	13.3	80.0	--	--	10.0*
	Soil Only	31	375	--	--	86.7	20.0	--	--
	/ 200 ppm Aldrin	31	525	/ 40.0	4.8	95.0	4.8	--	--
	/ 1000 ppm Aldrin	31	500	/ 33.3	--	100.0	--	--	--
(5) Labish Peat	Soil Only	0	1,575	--	7.9	60.5	--	--	--*
	/ 200 ppm Aldrin	0	1,500	- 4.9	26.7	70.0	--	--	3.0
	/ 1000 ppm Aldrin	0	1,650	/ 4.9	7.6	9.1	--	--	1.5*
	Soil Only	5	1,050	--	74.0	21.3	--	--	2.3
	/ 200 ppm Aldrin	5	900	- 11.9	44.5	58.3	--	--	5.6
	/ 1000 ppm Aldrin	5	800	- 23.8	50.0	46.8	3.1	--	3.1
	Soil Only	31	650	--	26.9	73.2	--	--	--
	/ 200 ppm Aldrin	31	1,125	/ 73.1	13.1	82.3	--	--	2.3*
	/ 1000 ppm Aldrin	31	675	/ 3.4	33.3	62.0	--	--	--
	Soil Only	0	2,700	--	Data Not Available				
	/ 200 ppm Aldrin	0	2,100	- 22.2	Data Not Available				
	/ 1000 ppm Aldrin	0	1,750	- 35.2	Data Not Available				
(3) Willamette Silty Clay Loam	Soil Only	0	1,800	--	Data Not Available				
	/ 200 ppm Aldrin	0	3,050	/ 69.4	Data Not Available				
	/ 1000 ppm Aldrin	0	3,400	/ 88.8	Data Not Available				
(4) Willamette Clay Loam	Soil Only	0	2,830	--	Data Not Available				
	/ 200 ppm Aldrin	0	3,280	/ 13.9	Data Not Available				
	/ 1000 ppm Aldrin	0	2,520	- 10.6	Data Not Available				

x   Average of six replicates.

( )^{xx} Mold count at 1:5000 dilution--1:500 dilution overgrown with Trichoderma.

*   Plus unknown species equals 100 per cent.

FIGURE 2: CHANGES IN THE NUMBER OF BACTERIA & <u>STREPTOMYCES</u>
DURING THE DECOPOSITION OF ADDED PEPTONE IN ALDRIN TREATED
SCHOTH GARDEN SOIL (WILLAMETTE CLAY LOAM)

FIGURE 3 : CHANGES IN THE NUMBER OF BACTERIA & <u>STREPTOMYCES</u> IN ALDRIN TREATED LABISH—CENTER SOIL (LABISH PEAT)

FIGURE 4: CHANGES IN THE NUMBER OF BACTERIA & STREPTOMYCES IN ALDRIN TREATED HERMISTON SOIL (FINE SAND)
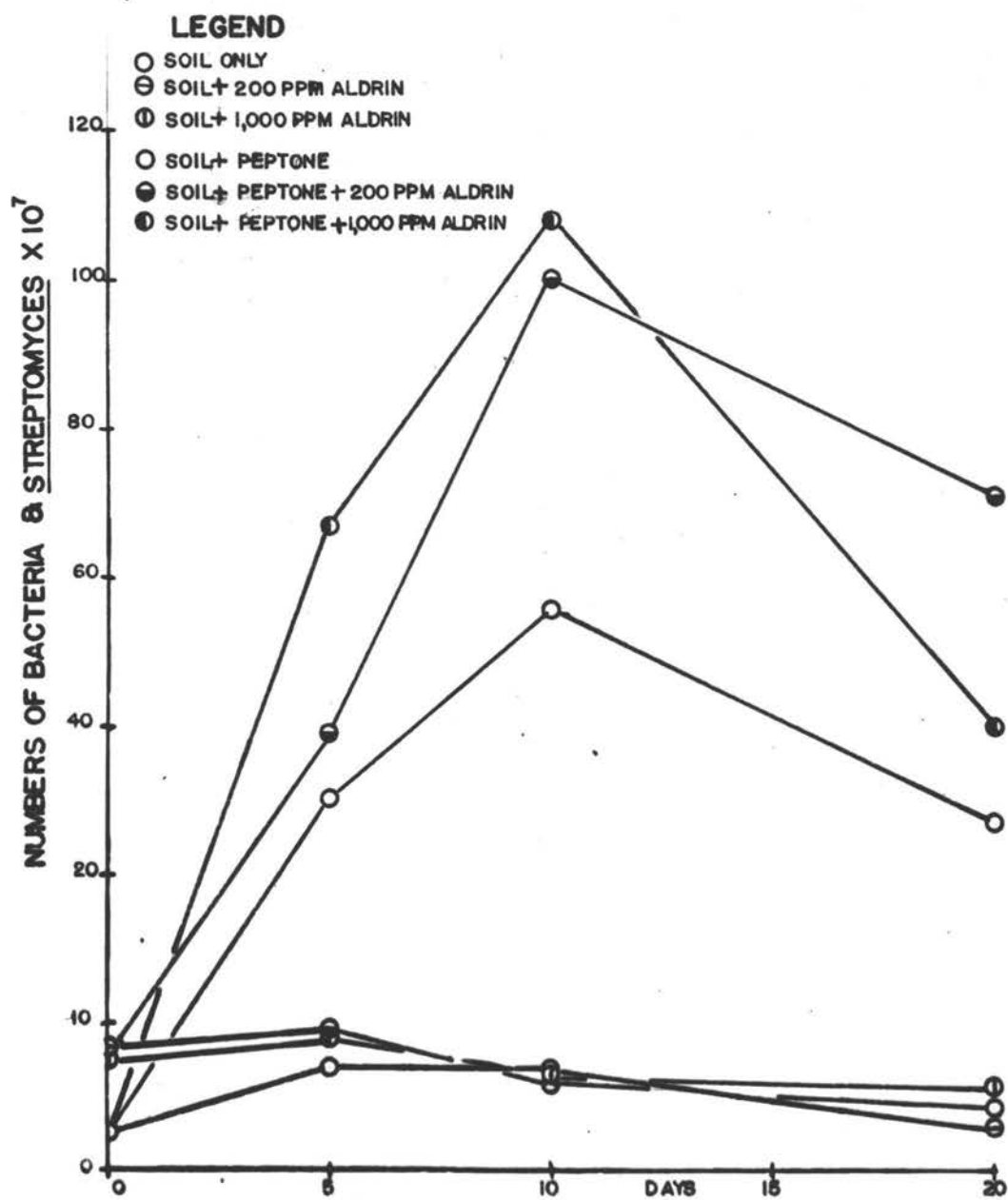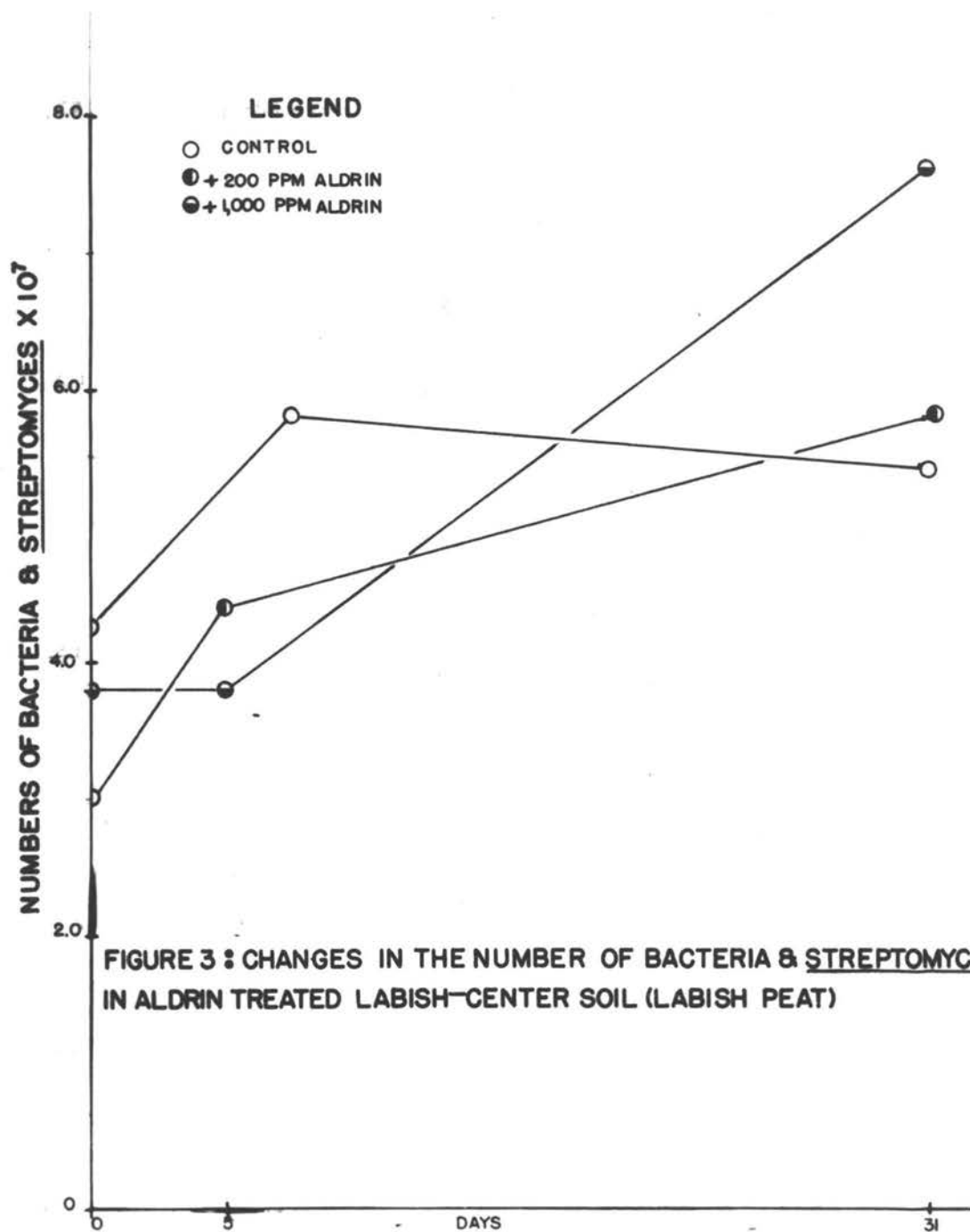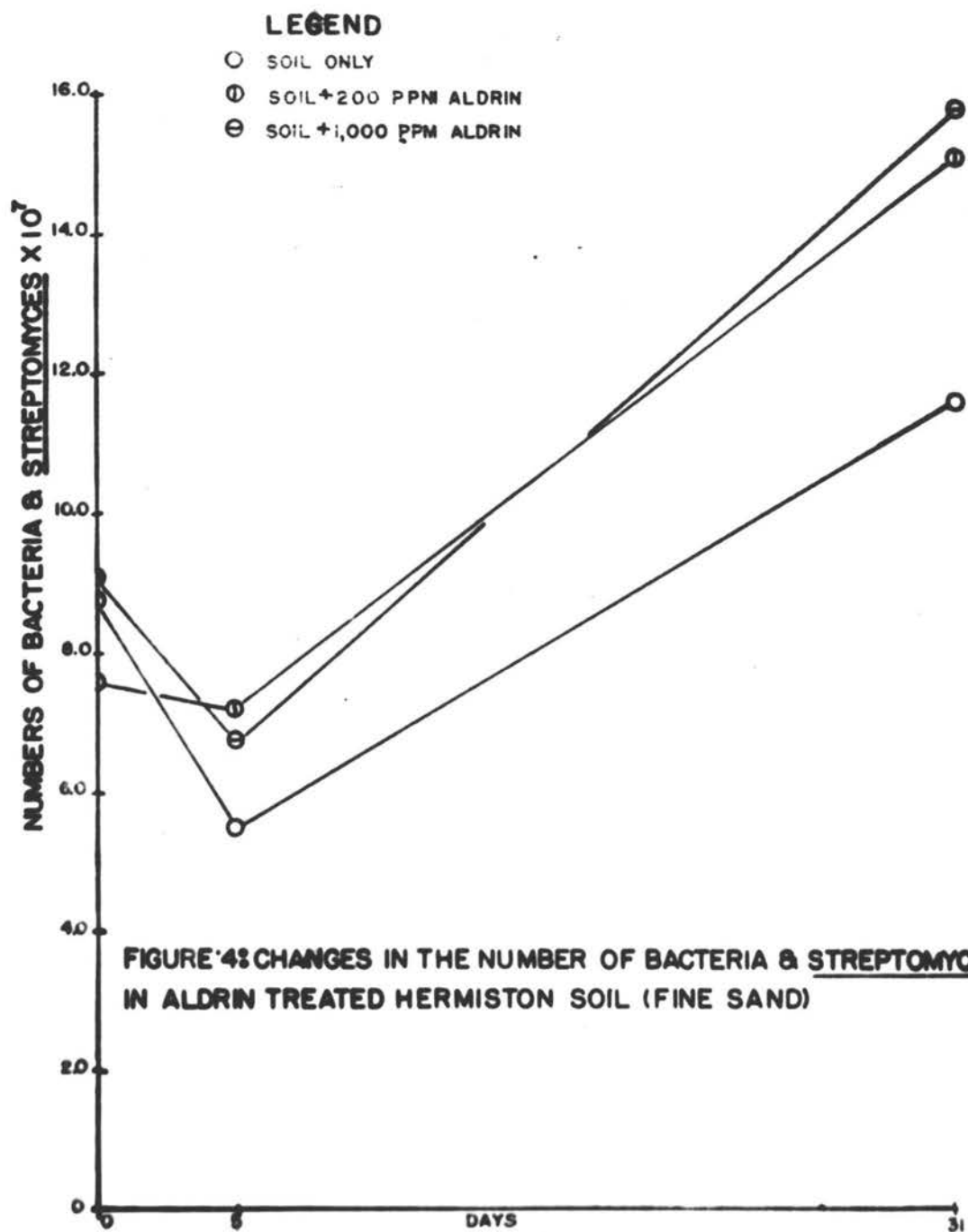
TABLE 5

Summary of Data Representing the Peptone Nitrogen Transformation in Representative Oregon Soils

	200	415	385	7	22	543	375	T	168	438	230	T	198	1395
	1000	398	390	12	-4	508	385	1	122	347	180	T	167	1252
WILLAMETTE SILTY CLAY LOAM:													
Deaver	0	359	333	T	26	362	323	T	149	448	208	0	240	1169
	200	336	335	T	2	332	255	T	77	475	228	1	248	1143
	1000	424	418	T	7	346	255	T	91	582	283	3	296	1353
Stayton	0	642	625	8	9	627	635	-5	-2	669	430	0	179	1938
	200	732	715	5	12	548	665	1	-18	641	445	0	194	1921
	1000	673	658	15	1	598	605	T	-7	629	440	0	189	1901
SANDY LOAM:													
	200	342	360	3	-21	425	345	1	80	165	140	3	22	932
	1000	359	360	2	-3	359	315	T	44	170	145	3	22	889
TOTALS:	0	5022	4930	44	36	5527	5164	112	364	4592	3418	103	1088	151,405
	200	5153	5105	50	11	5010	4571	134	315	4306	3248	111	973	144,692
	1000	5077	5279	55	-11	4707	4228	150	304	4121	3123	104	895	138,786

FIGURE 5: A COMPILATION OF THE TOTAL NITROGEN PRESENT* IN THE TEN OREGON SOILS STUDIED (SEE TABLE 5)

*VALUES FOR CONTROL SOILS SUBTRACTED

FIGURE 6: THE PERCENT PEPTONE NITROGEN AMMONIFIED (TOTAL BASIS) IN SOHOTH GARDEN SOIL TREATED WITH ALDRIN AND INCUBATED WITH 1,000 PPM NITROGEN — VALUES FOR CONTROL SOILS SUBTRACTED

FIGURE 7: THE EFFECT OF ALDRIN ON THE TOTAL AMMONIA NITROGEN PRESENT IN THE TEN OREGON SOILS STUDIED* (SEE TABLE 5)

* VALUES FOR CONTROL SOILS SUBTRACTED

LEGEND
○ CONTROL (SOIL+PEPTONE)
◐ + 200 PPM ALDRIN
● + 1,000 PPM ALDRIN

FIGURE 8: PPM AMMONIA NITROGEN PRESENT IN ALDRIN TREATED SCHOTH GARDEN SOIL INCUBATED WITH 1,000 PPM PEPTONE NITROGEN

LEGEND

○ SOIL ONLY
⊖ SOIL + 200 PPM ALDRIN
⊕ SOIL + 1,000 PPM ALDRIN

○ SOIL + PEPTONE
● SOIL + PEPTONE + 200 PPM ALDRIN
◐ SOIL + PEPTONE + 1,000 PPM ALDRIN

Nitrification

In soil samples incubated with added ammonium chloride and in the samples incubated with added peptone, the nitrosofying organisms which convert ammonia to nitrite appeared to be slightly stimulated by the insecticide at each concentration (Table 5, p. 31 and Figures 9 and 11, pp. 36 and 38). The apparent stimulation is temporary, and nitrosofication returns to control levels at the end of 20 days. It might be well to note, as it will be discussed elsewhere, a similar stimulation is indicated by the increased rate of oxygen uptake by soil which had been perfused with ammonium chloride solution. Here again the maximum effect was recorded after 10 days incubation. The insecticide at 1,000 p.p.m. gave maximum stimulation; at 200 p.p.m. the effect tended to be more prolonged and less pronounced.

The nitrifying bacteria which oxidize nitrite to nitrate were slightly depressed by the presence of the insecticide at each concentration, 1,000 p.p.m. having the greater effect (Table 5, p.31 and Figures 10 and 12, pp. 37 and 39). The inhibition in both cases was not severe.

In general, the effect based upon the overall nitrogen transformation in the ten soils appears to be one of slight inhibition; 1,000 p.p.m. aldrin exhibited the most pronounced depression. Table 5, page 31, and Figure 5, page 32, graphically demonstrate the trend.

These results are borne out in the calculations based upon the total of nitrogen metabolized by the organisms present. These calculations do not take into account the time intervals at which analyses were made, but show all the peptone nitrogen that was converted to ammonia, nitrite, or nitrate after 20 days incubation (Table 17, p.129).

FIGURE 9: TOTAL PPM NITRITE NITROGEN PRESENT IN THE TEN
OREGON SOILS STUDIED* (SEE TABLE 5)

* VALUES FOR CONTROL SOILS SUBTRACTED

FIGURE 10: THE EFFECT OF ALDRIN ON THE TOTAL NITRATE NITROGEN PRODUCED FROM ADDED PEPTONE IN THE TEN OREGON SOILS STUDIED* (SEE TABLE 5)

FIGURE II: PPM NITRITE NITROGEN PRESENT IN ALDRIN TREATED SCHOTH GARDEN SOIL INCUBATED WITH 1,000 PPM PEPTONE NITROGEN (VALUES FOR CONTROL SOILS SUBTRACTED)

FIGURE 12: PPM NITRATE NITROGEN PRODUCED IN ALDRIN
TREATED SOIL INCUBATED WITH 1,000 PPM PEPTONE
NITROGEN* (SCHOTH GARDEN SOIL)

* VALUES FOR CONTROL SOILS SUBTRACTED

LEGEND
O SOIL+PEPTONE
◐ SOIL+PEPTONE+200 PPM ALDRIN
● SOIL+PEPTONE+1,000 PPM ALDRIN
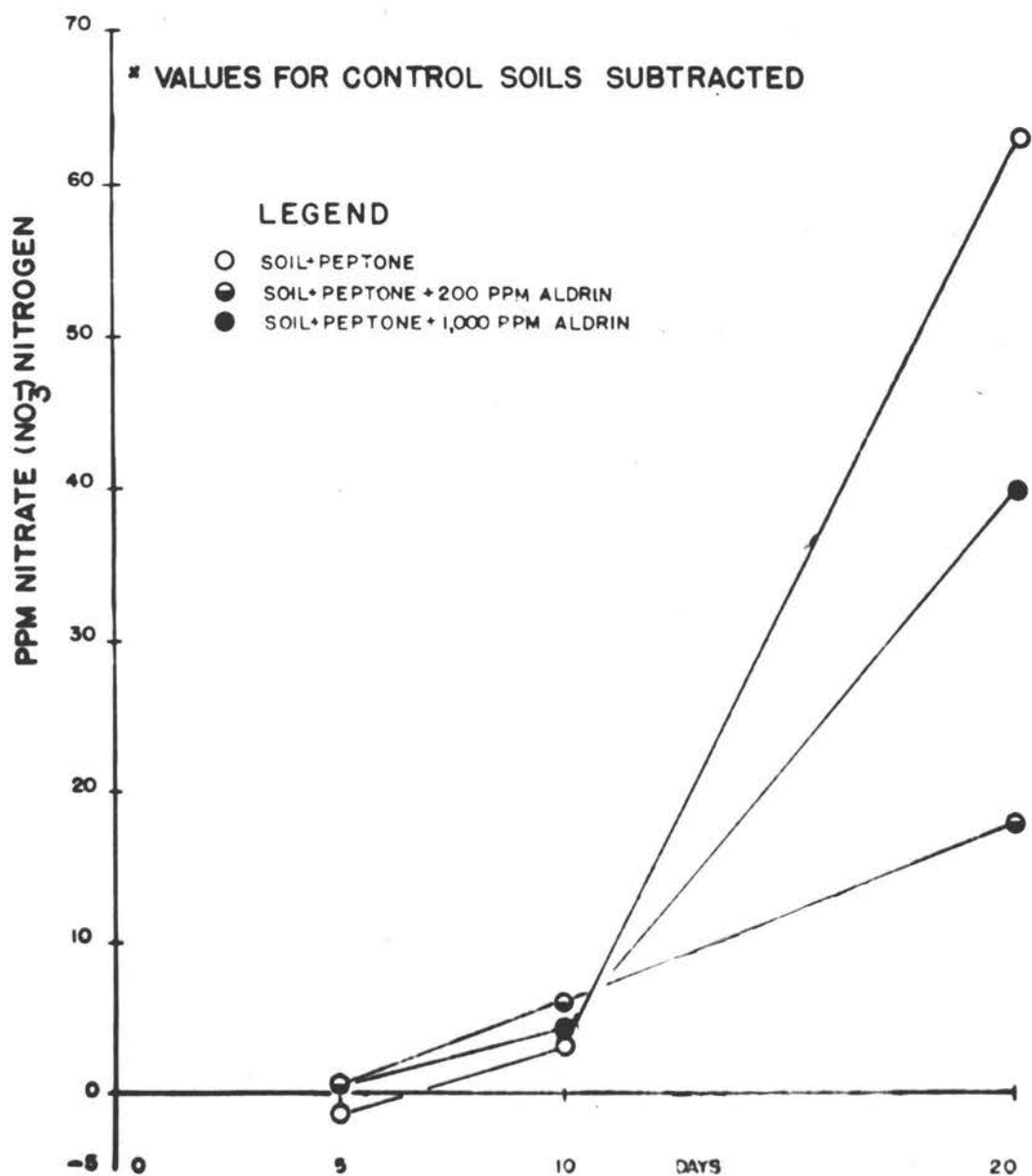
Carbon Dioxide Evolution

A slight but definite stimulation of carbon dioxide evolution from the soil resulted where aldrin was added, and the effect was similar for each concentration of the insecticide. Plate counts made at 5, 10, and 20 days respiration show corresponding increases in the numbers of microorganisms. Treated samples show a 10-fold increase in numbers, but this is not reflected to the same degree in the quantity of carbon dioxide evolved.(Figures 13 and 14, pp. 41 and 42).

There appeared significant differences in carbon dioxide production, from those samples which had no added peptone; a stimulation manifested where the insecticide had been added. The effect seems to come early in the incubation period, as it does in ammonification and nitrification experiments. Aldrin appears to increase the production of carbon dioxide by soil microorganisms.

Perfusion Studies

The perfusion technique used in this work has lacked uniformity because of mechanical difficulties; nevertheless, it yielded, when subsequently combined with Warburg studies, significant results. The "biologically saturated" soil, when placed in the reaction vessels of the Warburg respirometer containing 0.01 M NH_4Cl showed that aldrin additions result in as much as a two-fold increase in oxygen uptake. The results of the Warburg experiments are shown in Figures 17 and 18, pp. 46 and 48.
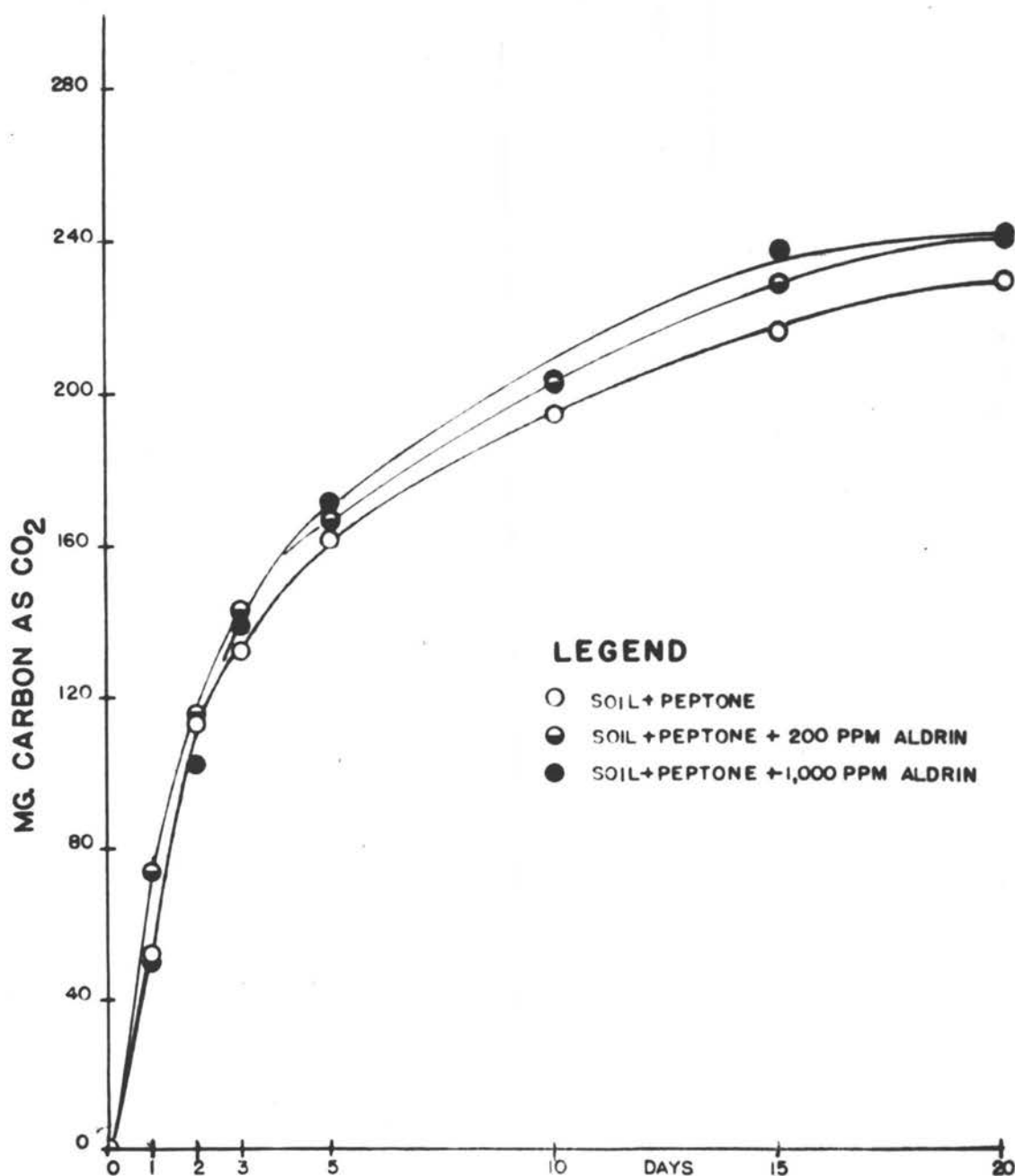
FIGURE 13 : CUMULATIVE CARBON DIOXIDE PRODUCTION FROM ALDRIN TREATED SCHOTH GARDEN SOIL INCUBATED WITH 1,000 PPM PEPTONE NITROGEN*

* SOIL ONLY AND THE ALDRIN TREATED CONTROLS SUBTRACTED
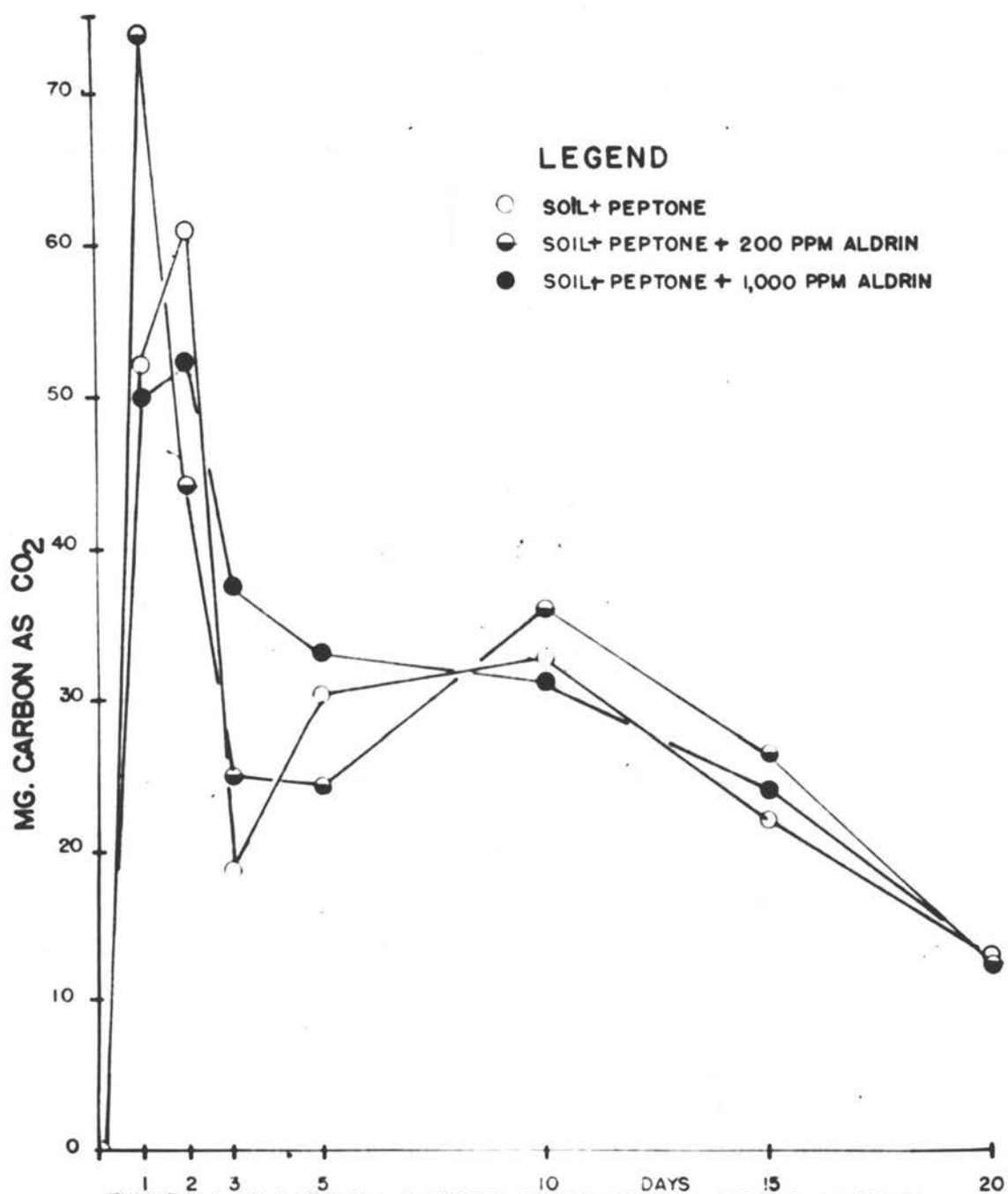
FIGURE 14: CARBON DIOXIDE PRODUCTION FROM ALDRIN TREATED SCHOTH GARDEN SOIL* (SEE FIGURE 13)

* SOIL ONLY AND THE ALDRIN TREATED CONTROLS SUBTRACTED
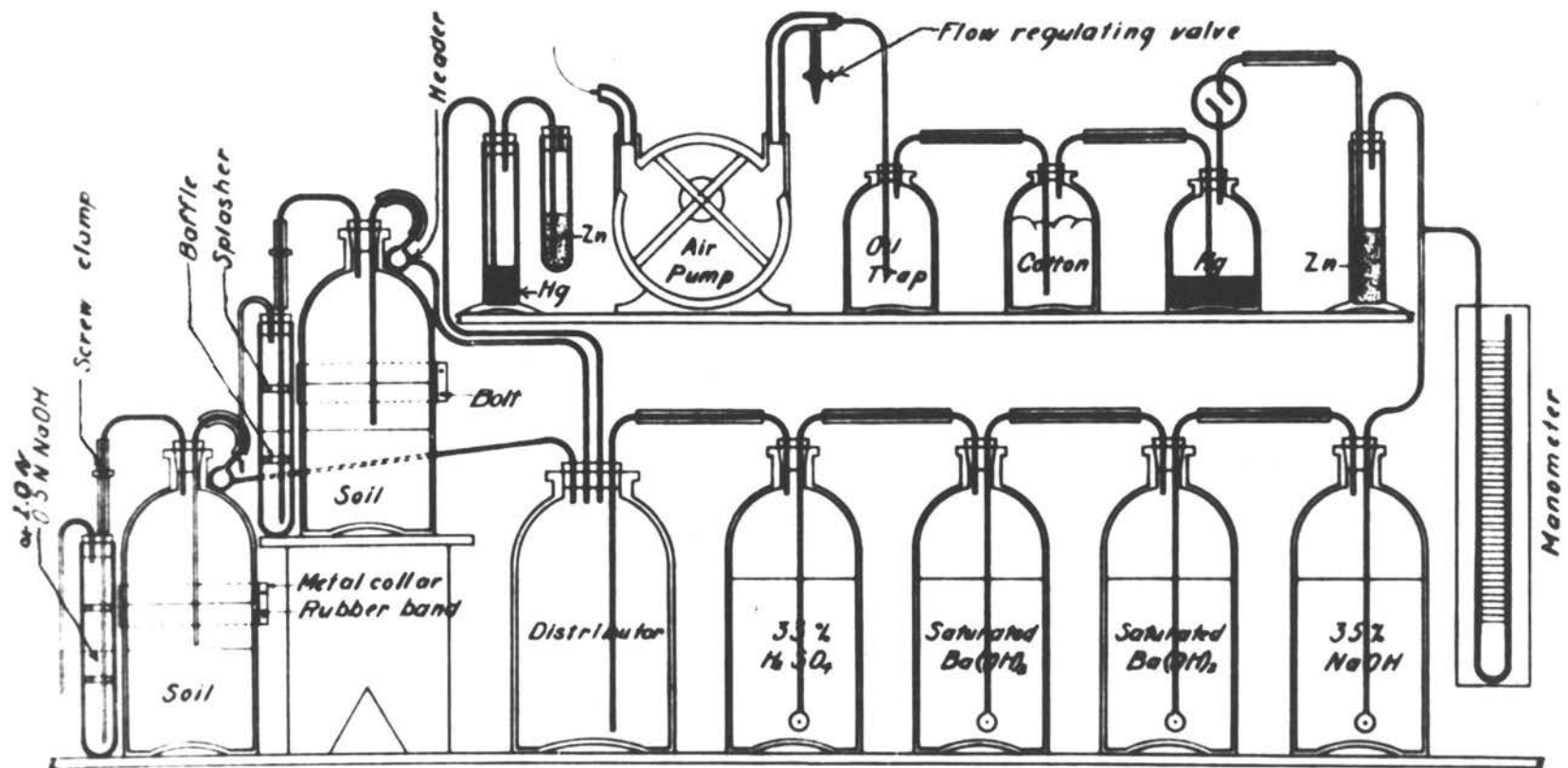
FIGURES 15 & 16 : SOIL RESPIRATION APPARATUS

TABLE 6

Warburg Respirometer Studies from Perfused Soil

Soil Treatment:

Place perfused soil on folds of cheese cloth, allow to

set 24 hours, then squeeze gently, chop up with a spatula

into fine aggregates, and assume 50 per cent H_2O content.

Cup Contents:

#1	#2	#3	#4
1.5 gm. soil A	1.5 gm. soil B (1,000 p.p.m. Aldrin)	1.5 gm. soil B	1.5 gm. soil A
1 ml. 0.01 M NH_4Cl	1 ml. 0.01 M NH_4Cl	2.0 ml. H_2O	2.0 ml. H_2O
1 ml. H_2O	1 ml. H_2O	0.2 ml. KOH	0.2 ml. KOH
0.2 ml. KOH	0.2 ml. KOH		

TABLE 7

The Effect of Aldrin on Ammonium Chloride Perfused Soil

		Oxygen Uptake in Microliters[x]				
Soil Sample* Substrate	I H_2O	I 1 ml.0.01 M NH_4Cl	III + 5 ml. EtOH 0.01 M NH_4Cl	III + 5 ml. EtOH 0.01 M NH_4Cl	V + 1000 ppm Aldrin 5ml. EtOH H_2O	V + 1000 ppm Aldrin 5ml EtOH 0.01 M NH_4Cl
Time						
15	0	0	12	14	23	26
30	3	7	24	27	50	50
45	4	7	33	39	68	68
60	5	10	43	50	84	86
90	10	12	60	68	117	117
120	13	17	77	91	149	148
150	16	17	91	103	176	178
180	19	21	102	122	208	207
240	27	35	134	151	259	258
255	26	30	136	157	273	274

[x]Average of replicate samples.

*Soil samples: Schoth garden.

FIGURE 17: CUMULATIVE OXYGEN UPTAKE BY PERFUSED ALDRIN TREATED SCHOTH GARDEN SOIL

TABLE 8

The Effect of Aldrin on Ammonium Chloride Perfused Soil

	Oxygen Uptake in Microliters[x]			
Time	Soil A 0.01 M NH_4Cl ul. O_2 Uptake	Soil A* H_2O ul. O_2 Uptake	Soil B* 0.01 M NH_4Cl ul O_2 Uptake	Soil B* H_2O ul. O_2 Uptake
0				
20	4	1	8	9
40	10	4	14	13
60	19	15	17	14
80	29	22	28	36
100	32	28	32	41
120	36	30	41	48
140	35	34	45	53
160	41	39	52	58
180	44	42	47	63
220	55	46	67	74

[x]Average of replicate samples.

*Soil sample: Schoth garden.
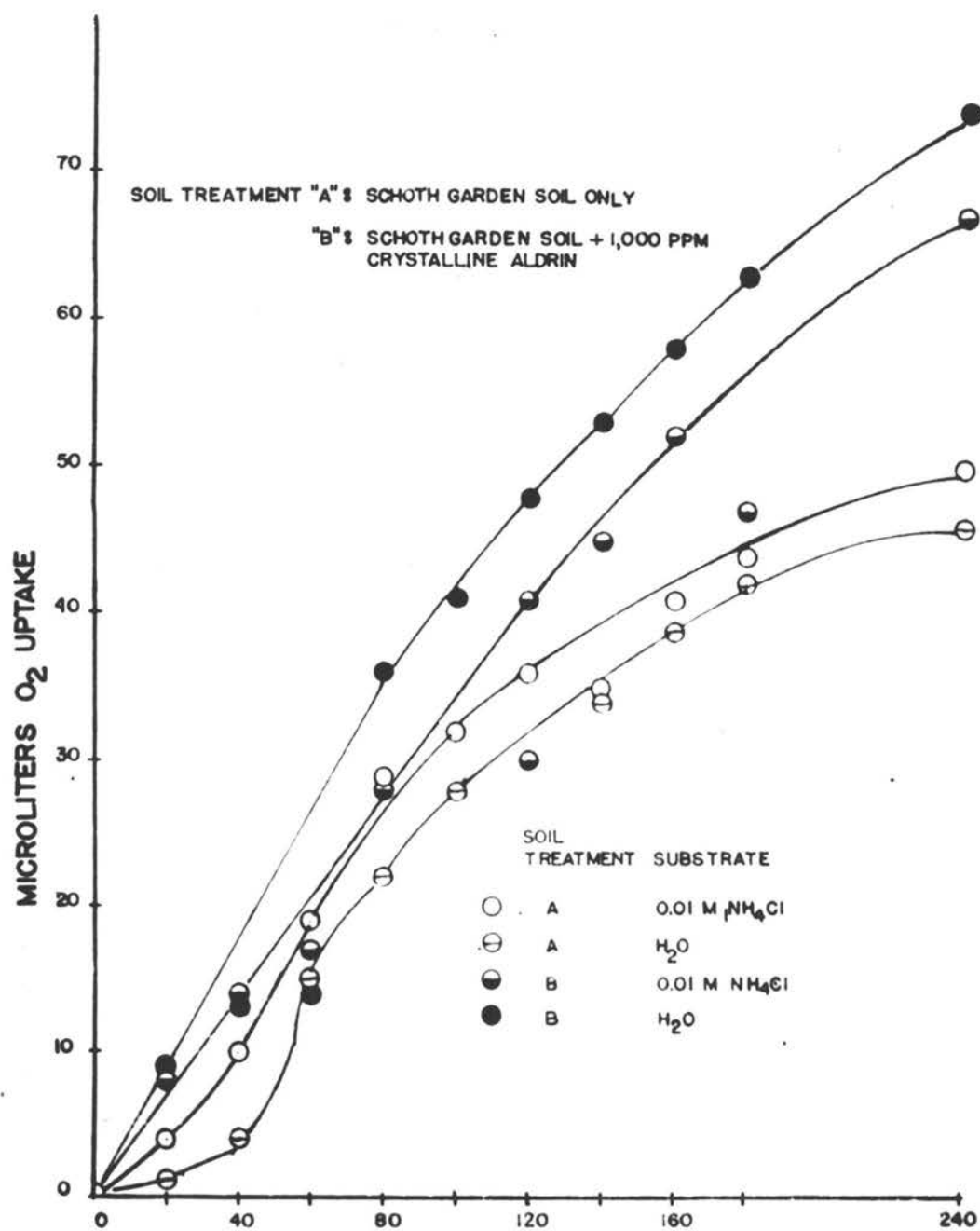
FIGURE 18: CUMULATIVE OXYGEN UPTAKE OF PERFUSED
SCHOTH GARDEN SOIL
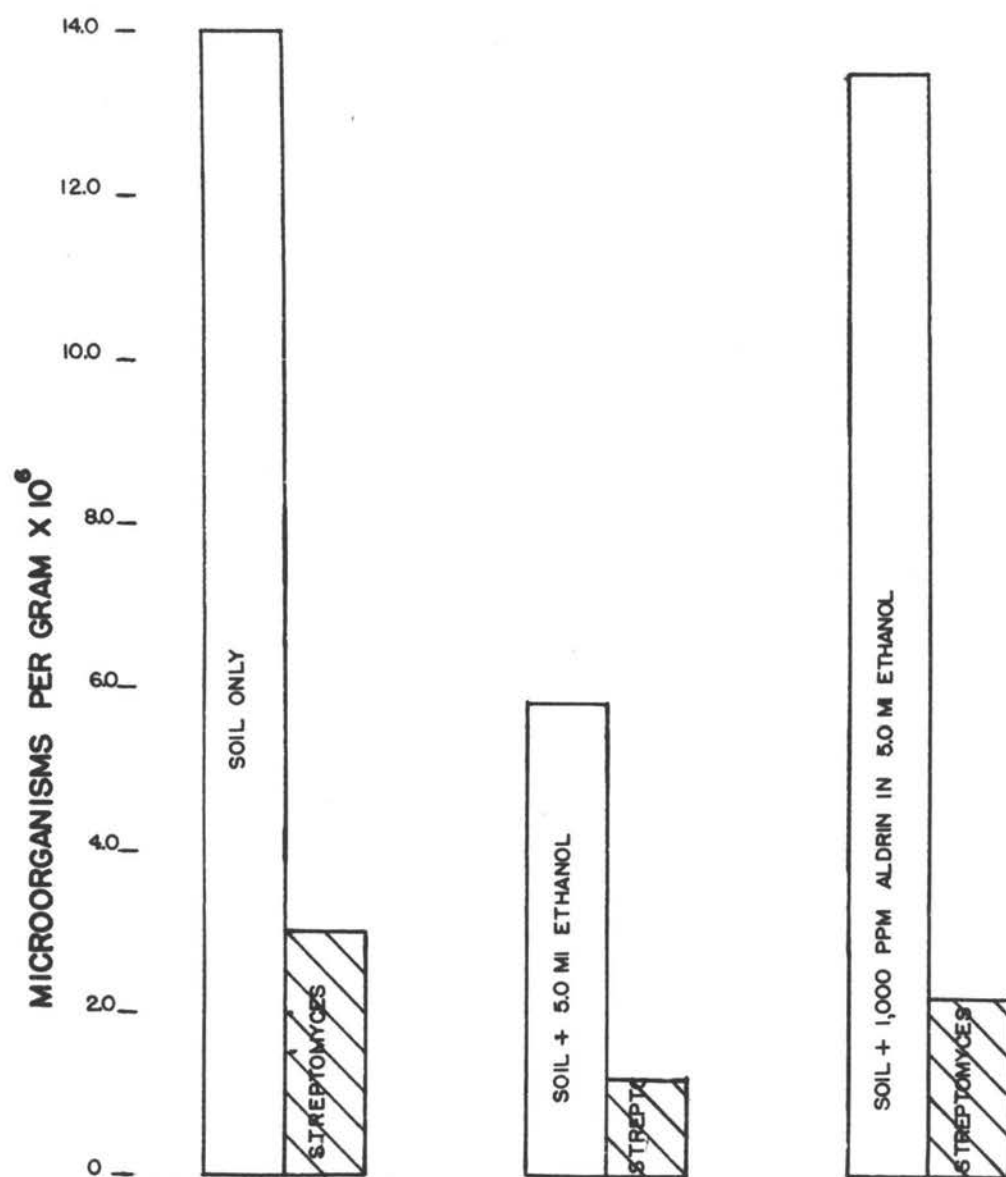
FIGURE 19: THE EFFECT OF ETHANOL AND ALDRIN ON
HETEROTROPHIC MICROORGANISMS IN ALDRIN TREATED
SOIL PERFUSED 144 HOURS WITH 0.01 M NH₄Cl
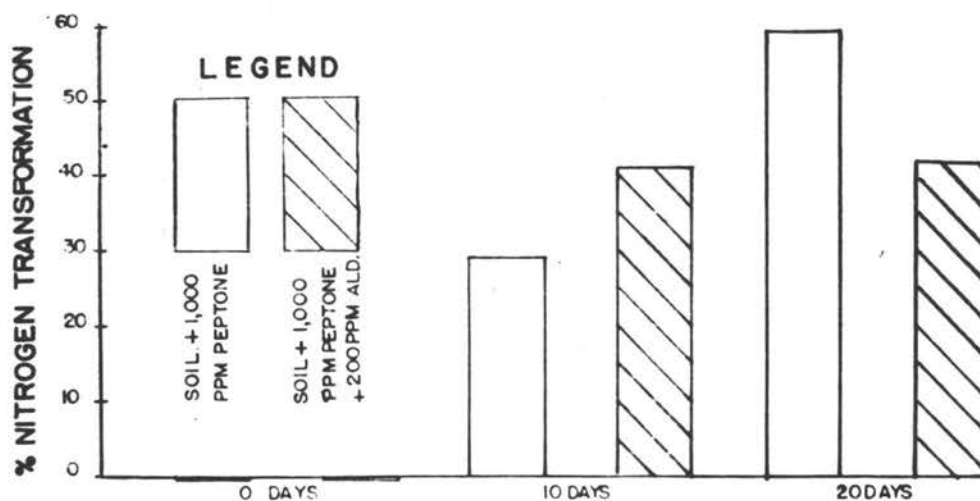
FIGURE 20: PERCENT TOTAL NITROGEN TRANSFORMATION FROM SCHOTH GARDEN SOIL TREATED WITH 200 PPM ALDRIN (SEE TABLE 9 FOR ALDRIN ANALYSES)
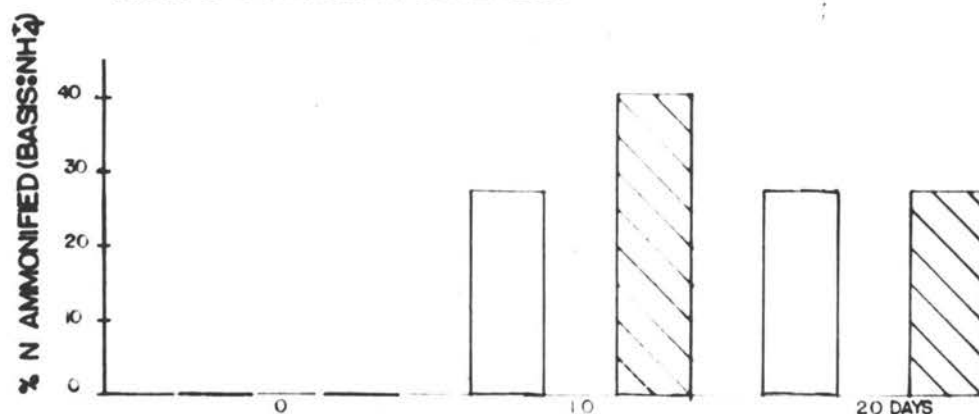
FIGURE 21: PERCENT PEPTONE NITROGEN AMMONIFIED (BASIS: NH_4^+) IN SCHOTH GARDEN SOIL TREATED WITH 200 PPM ALDRIN (SEE TABLE 9 FOR ALDRIN ANALYSES)
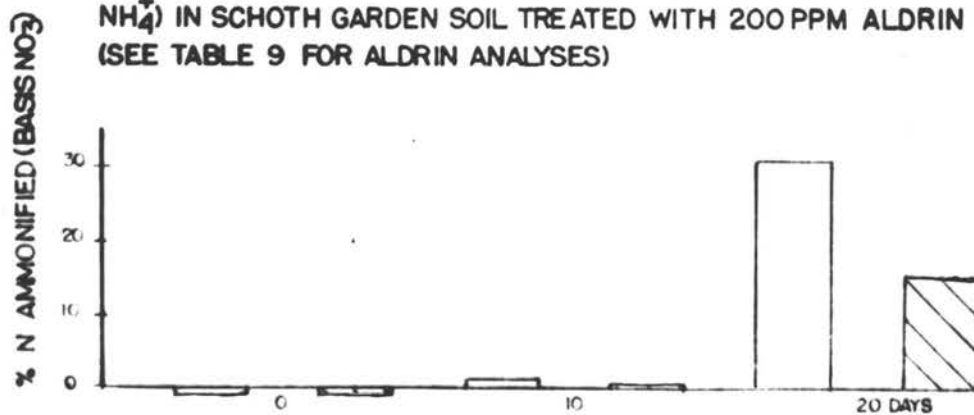
FIGURE 22: PERCENT PEPTONE NITROGEN AMMONIFIED (BASIS NO_3^-)

Stability of Aldrin in Garden Soil

The results of this experiment are given in Table 9, page 52, and show obvious losses in aldrin concentrations after 20 days incubation under optimum laboratory conditions. Results of the ammonification study made simultaneously with the stability experiment show the characteristic stimulation to ammonification after 10 days incubation, as well as the previously noted inhibition to nitrification (Figures 20, 21, and 22, p.50) after 20 days.

It is apparent that even those samples taken immediately after soil treatment show an aldrin content considerably lower than the 200 p.p.m. applied. This loss may be partially due to volatilization of the compound while air-drying the soil. The rate of volatilization of aldrin at 23°C. has been determined at 3.39 x 10^{-7} rate grams/cm.^{2}/sec. (18, p.1).

TABLE 9

Aldrin Content of Soil 0, 10, and 20 Days
after Treatment with 200 p.p.m. Aldrin

Application: Aldrin was applied to soil samples, either alone or
in combination with peptone, at the rate of 200 p.p.m.
The treated soil was incubated at 28° C. and sampled
at 0, 10, and 20 days after treatment.

Analytical Method: Phenyl azide-Colorimetric Method (8, pp.190-197).

Sensitivity of Analysis: 0.1 p.p.m.

Sample No.	Treatment (Aldrin) (p.p.m.)	Interval from Treatment and Sampling	Aldrin Found (p.p.m.)	Average (p.p.m.)	Difference (p.p.m.)
13	200	0 Days	95.0		
14	200	0 Days	104.8	99.9	
15	200	10 Days	63.8		
16	200	10 Days	63.8	63.8	36.1
17	200	20 Days	39.1		
18	200	20 Days	55.8	47.5	52.4
37	200 + 1000 p.p.m. Peptone N	0 Days	71.0	71.0	
39	"	10 Days	73.0		
40	"	10 Days	55.5	64.5	6.7
41	"	20 Days	44.9		
42	"	20 Days	62.2	53.6	17.4
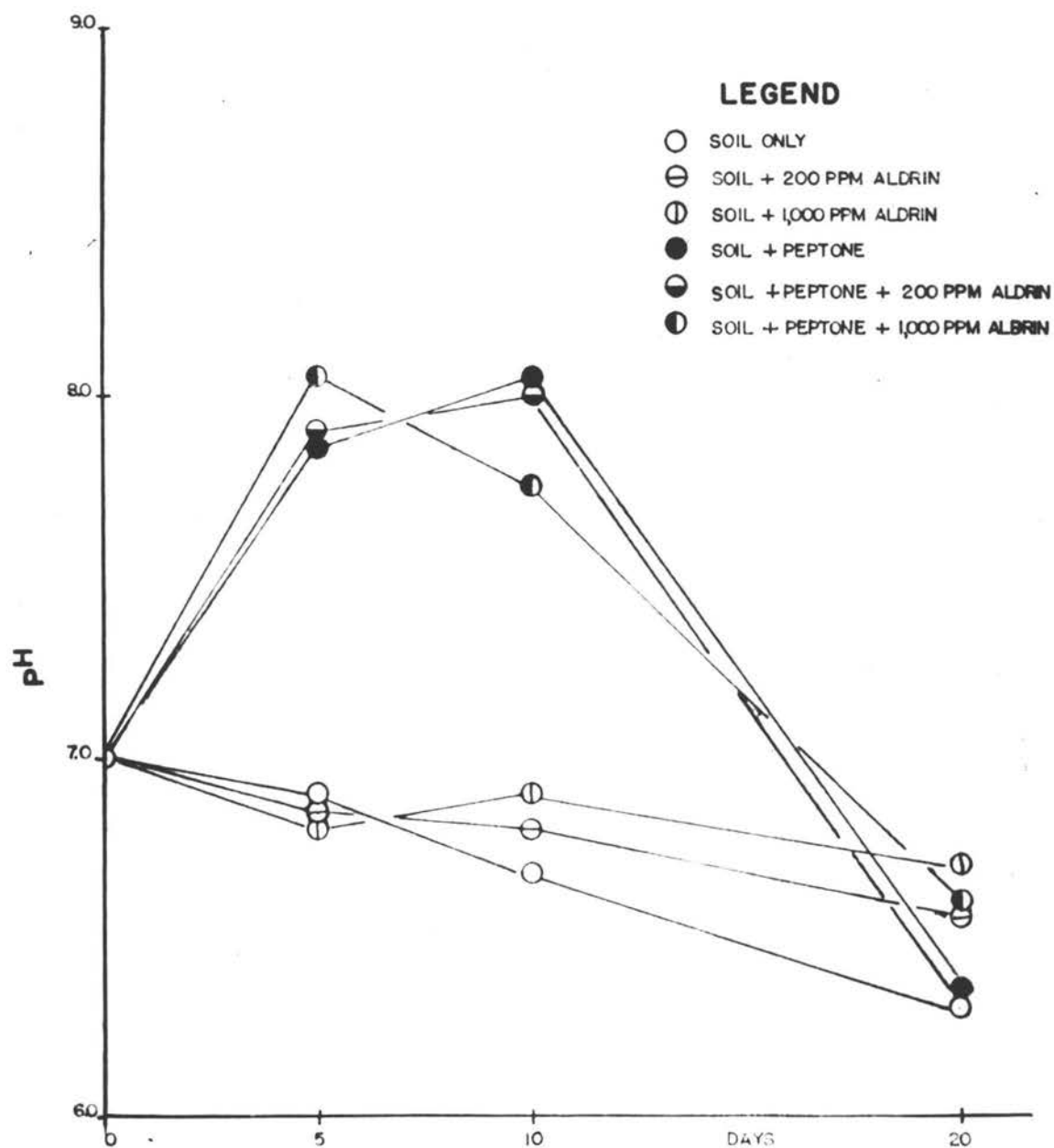
FIGURE 23: CHANGES IN pH OF ALDRIN TREATED SCHOTH GARDEN SOIL INCUBATED WITH 1,000 PPM PEPTONE NITROGEN

LEGEND

○ SOIL ONLY
⊖ SOIL + 200 PPM ALDRIN
◑ SOIL + 1,000 PPM ALDRIN
● SOIL + PEPTONE
⊖ SOIL + PEPTONE + 200 PPM ALDRIN
◑ SOIL + PEPTONE + 1,000 PPM ALDRIN

II FIELD TREATED SOIL STUDIES

A. Introduction

In cooperation with the Department of Entomology, Oregon State College, soil samples from field plots established under the direction of Dr. R. G. Rosenstiel were studied in the laboratory to determine the effectiveness of aldrin (among other soil insecticides) in controlling subterranean insects and thrips on strawberries in the Willamette Valley. Experimental plots were designated as Schoth Garden,(Willamette clay loam), Corvallis Oregon. All plots were set up and sampled randomly. The aldrin-treated soil (Table 10, p.56) contained 50 pounds per acre added as a 5 per cent dust at a rate of 1,000 pounds per acre. The insecticide-dust mixture was incorporated into the soil by rototiller technique, and immediately after treatment, young strawberry plants were set out and allowed to grow for approximately one year, at which time random samples were collected from each of the aldrin-treated plots and on equal number of control plots. A portion of all of the test plots as well as the control plot samples (Table 11, p.57) were separately combined to form composite samples.

The purpose of this study was to assess the effect of four commonly used soil insecticides upon certain soil fertility factors: Aldrin, DDT, chlordane, and heptachlor field-treated soils were studied by the previously described methods. Ammonia, nitrite, nitrate, pH, and soil counts were determined after 10 and 20 days incubation.

Although this study includes four insecticide-treated soils, only the results of the aldrin-treated soil will be included in this thesis.

B. EXPERIMENTAL METHODS

Nitrification

Duplicate samples of each of the treated soils were admixed respectively with 500 p.p.m. ammonium chloride, 500 p.p.m. sodium nitrite, and 1,000 p.p.m. peptone nitrogen. The samples were incubated at optimum moisture and temperature, and the analyses noted above were obtained after 10 and 20 days incubation.

Ammonification

Ammonification processes were illustrated by following the nitrogen transformation of the duplicate samples treated with 1,000 p.p.m. peptone nitrogen noted in the nitrification study.

Soil Counts

After 10 days incubation at 28° C. and at optimal moisture conditions, triplicate plates at dilutions of 5×10^4 and 5×10^5 for bacteria and Streptomyces, and at dilutions of 5×10^2 and 5×10^3 for molds, were poured. The bacterial plates were incubated at 28° C. for 10 days. Ammonia, nitrite, nitrate, and pH were also determined at this time. The values given in Figures 24 and 25, pp. 59 and 60, are the average of the four individual plot samples which represent the average of three replications.

TABLE 10

Analysis of Schoth Garden Soil*
(Willamette Clay Loam)
Check Composite--1952

Chemical Analysis:

Moisture, per cent	25.4
Saturation Capacity, per cent	50.1
pH	6.3
Lime Requirement (Troug), Tons/Acre	2.5

Nitrogen:

Ammonium	20.0 p.p.m.
Nitrite	Trace
Nitrate	1.0 p.p.m.
Kjeldahl, per cent	0.227
Total Carbon, per cent	2.98
C:N Ratio	13:1

Microorganisms:

Molds, per gram	12,000
Penicillia, per cent	66.6
Mucors, per cent	16.7
Aspergilli, per cent	4.2
Trichoderma, per cent	12.5
Bacteria, per gram	7.8×10^{6}
Streptomyces, per cent	47.0
Azotobacter	Present

* Data expressed on water-free basis.

TABLE 11

Analysis of Field Treated Soil*
(Schoth Garden--Willamette Clay Loam)
Aldrin Composite--1952[X]

Chemical Analysis:

Moisture, per cent	23.8
Saturation Capacity, per cent	50.1
pH	6.2
Lime Requirement (Troug), Tons/Acre	2.5

Nitrogen:

Ammonium	24.0 p.p.m.
Nitrite	Trace
Nitrate	2.0 p.p.m.
Kjeldahl, per cent	0.217

Total Carbon, per cent	2.82
C:N Ratio	13:1
Aldrin (Total Chloride)**(23,pp.781)	22.5 p.p.m.

Microorganisms:

Molds, per gram	11,500

Penicillia, per cent	65.2
Mucors, per cent	30.2
Aspergilli, per cent	0
Trichoderma, per cent	4.3

Bacteria, per gram	9.5×10^6

Streptomyces, per cent	43.0
Azotobacter	Present

* Data expressed on the water-free basis.

[X] Treated with aldrin (50 pounds per acre or 25 p.p.m.) as a 5 per cent commercial dust.

** Data expressed on the air-dry basis.

Aldrin Analysis

The method of Kobliztsky and Chisholm (23, p.781) was used
to determine the free chloride concentration after sodium decompo-
sition.

C. RESULTS

Soil Counts

Aldrin applied at a rate of 50 pounds per acre as a 5 per cent
commercial dust does not appear to have any injurious effect on the
numbers of soil microorganisms as determined by plate counts. But
instead, an obvious stimulation in the number of bacteria was appar-
ent. (Figure 24, p.59). Streptomyces appeared to be indifferent to
the presence of the insecticide (Figure 24, p.59). The mold counts
graphically represented in Figure 25, p. 60, show no significant effects
of the insecticide. Table 12, p. 63, is a comparison of the ammonia,
nitrite, and nitrate analyses obtained at the time the soil counts
were made.

Ammonification and Nitrification

The results from the nitrification studies obtained by using
the field-treated soil verify in many respects the results obtained
from the laboratory-treated soil. Ammonification in the presence
of 1,000 p.p.m. peptone nitrogen was stimulated by the addition of
aldrin after 10 days incubation. In the presence of this added
peptone nitrogen, ammonification was stimulated   by 24.6 per cent

FIGURE 24: THE INFLUENCE OF SOIL INSECT TOXICANTS ON THE NUMBERS OF SOIL MICROORGANISMS IN SCHOTH GARDEN SOIL (AVERAGE OF FOUR REPLICATIONS)
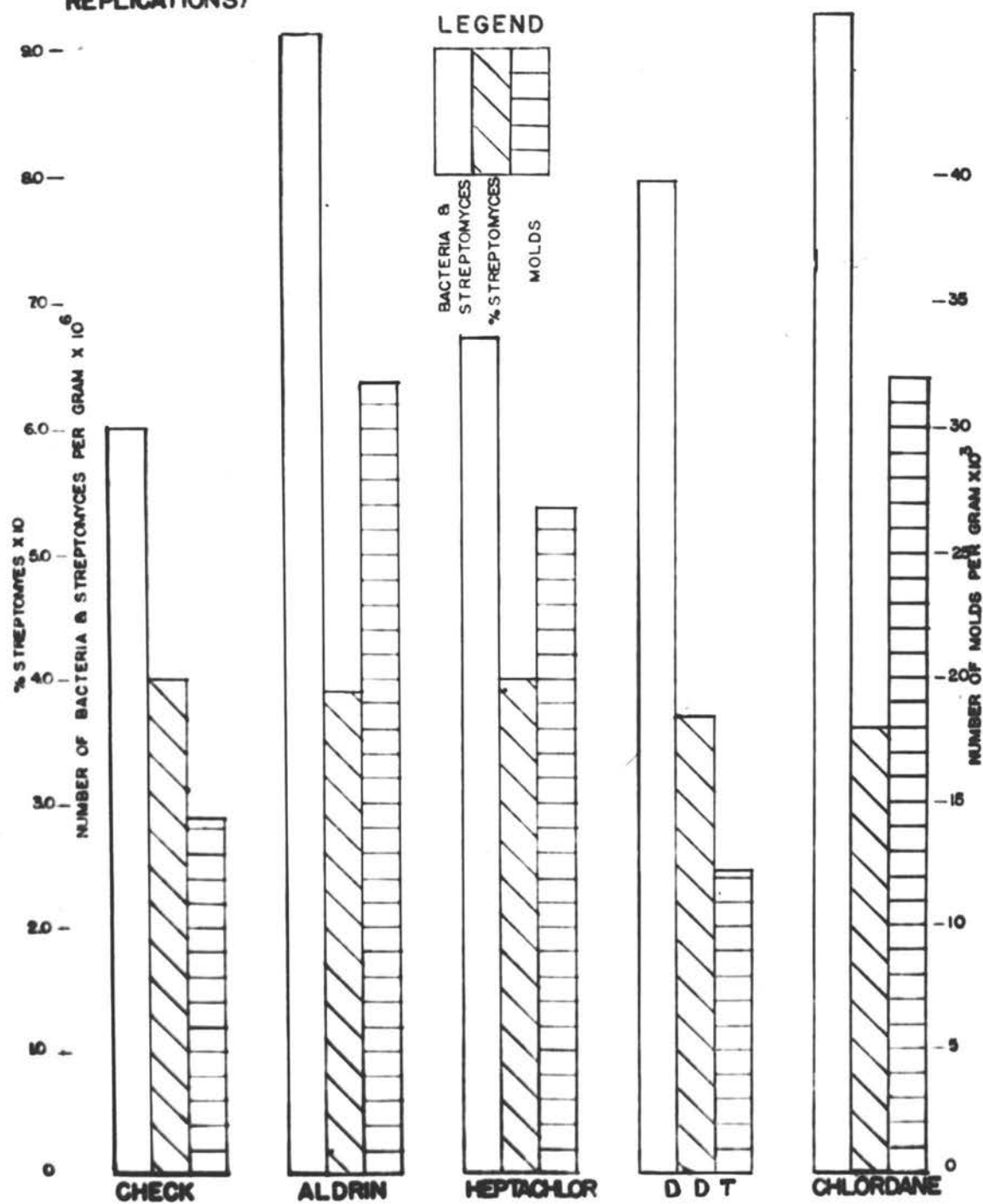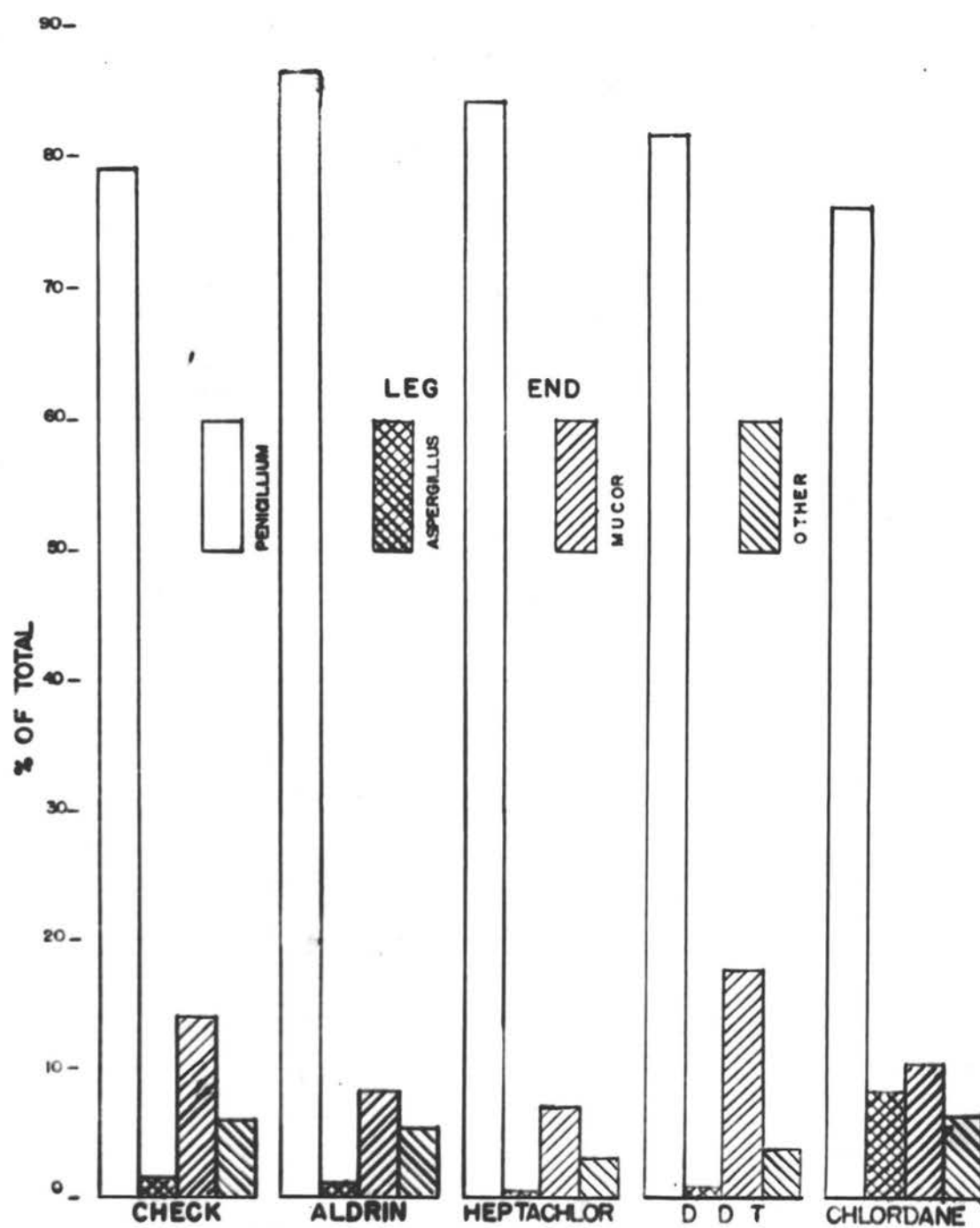
FIGURE 25: DIFFERENTIAL MOLD COUNT FROM SCHOTH GARDEN SOIL
(FIELD TREATED)
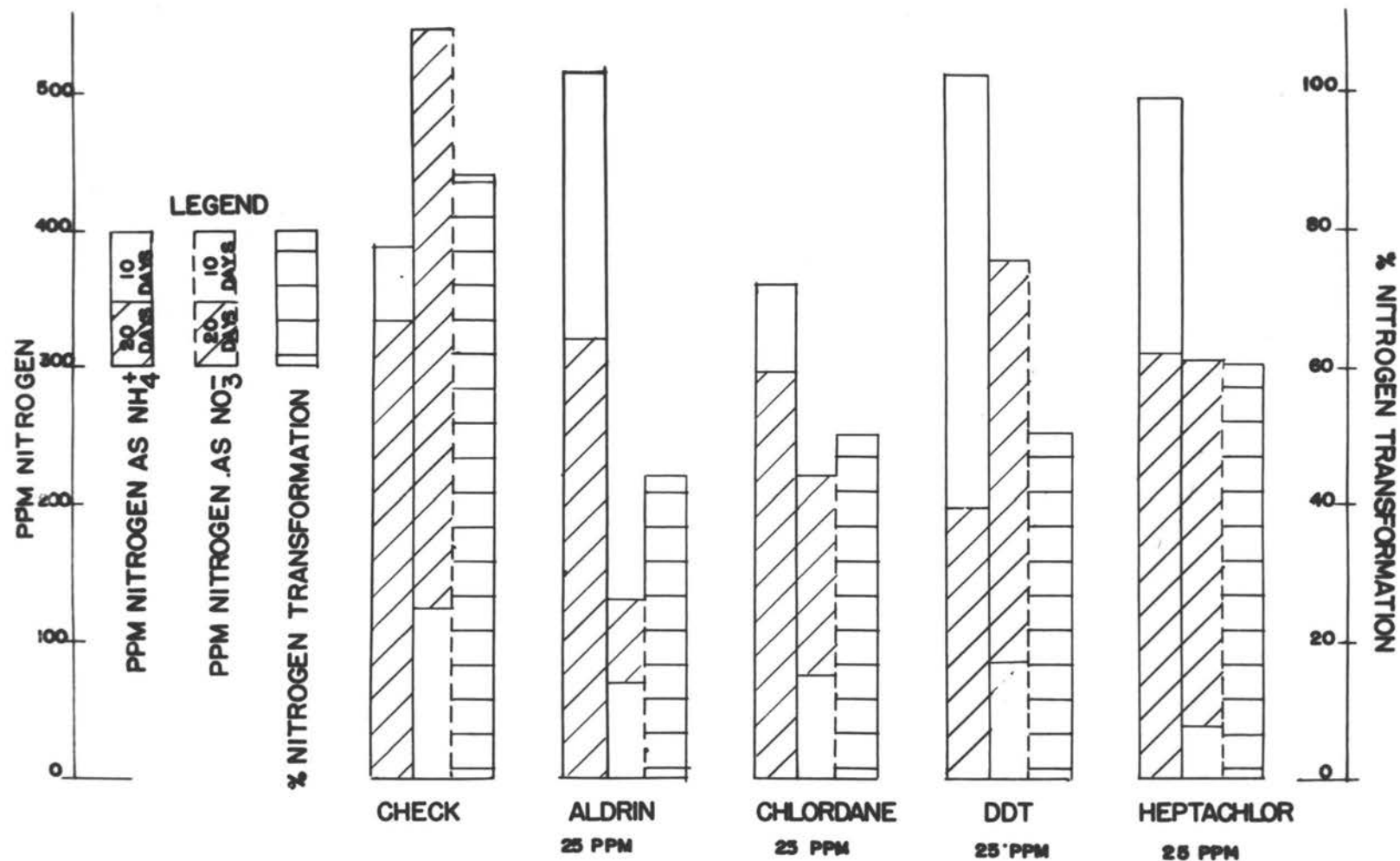
FIGURE 26: THE INFLUENCE OF SOIL INSECT TOXICANTS ON AMMONIFICATION AND NITRIFICATION IN FIELD TREATED SCHOTH GARDEN SOIL (VALUES FOR SOIL ONLY SUBTRACTED)

61.

over the control. After 20 days incubation, ammonification values

for the treated soil were back to normal. However, when ammonium

chloride was added at 500 p.p.m., the results indicate no conver-

sion of ammonia to nitrite and consequently, there was no nitrate

formation. This effect was apparent at 10 and 20 days incubation.

Nitrate formation was slight in the treated soil in the presence

of 500 p.p.m. sodium nitrite after 10 days incubation. After 20

days, nitrate formation was inhibited. Nitrate formation was

greatly inhibited at 10 and 20 days incubation where peptone had

been added to the treated soil. This inhibition was manifested

as a 41.7 per cent reduction in nitrite conversion, as based on

the control soil, which was arbitrarily set at 100 per cent.

After 20 days incubation, nitrate formation was more severely

effected in that a 76.6 per cent inhibition was apparent.

Aldrin Analysis

Residual aldrin as chloride was determined as 22.5 p.p.m.

TABLE 12

Nitrogen Analyses of Insecticide-Treated Field Soils
Obtained Simultaneously with Plate Counts
Average of Four Random Plot Samples

Sample	Treatment	pH	p.p.m. N as NH_4	p.p.m. N as NO_2	p.p.m. N as NO_3
A	Check	5.9	44	T*	0.97
B	Aldrin	6.2	23	T	0.76
C	Heptachlor	6.0	68	T	0.54
D	DDT	6.1	15	T	1.0
E	Chlordane	6.1	1	T	0.19
Composite Samples					
21	Check	5.9	20	T	1.42
22	Aldrin	6.2	10	T	0.48
23	Heptachlor	6.1	20	T	1.28
24	DDT	6.0	5	T	0.50
25	Chlordane	6.1	8	T	T

* T denotes trace.

III   SOIL EXTRACT STUDIES

A.   INTRODUCTION

In an effort to expedite the action of aldrin under condi-
tions of excess nitrogen, a shake flask technique, using a soil
extract as an inoculum, was adapted to the study of the effects
of aldrin on the soil microflora. Thus it was hoped that this
method would show more obvious differences than were obtained
from the conventional nitrogen transformation studies in the
soil.

B.   EXPERIMENTAL METHODS

Preparation of the Extract and the Nutrient Solution

In all of the soil extract studies, the medium and the ex-
tract were prepared in essentially the same manner. Two liters
of Difco peptone solution containing 1,000 p.p.m. nitrogen per
100 ml. of solution were admixed with 20 grams (water-free) of
the soil. This suspension was vigorously agitated for 30 minutes.
It was then removed and filtered through Whatman #2 filter paper.
The resulting filtrate was relatively clear and suitable for tur-
bidimetric analyses for the estimation of microbial numbers.

Apparatus

A reciprocating incubator with a 3.5 inch stroke and 96
excursions per minute, held at approximately 28° C., was used
throughout the study.

General Procedure

One hundred ml. of the extract filtrate were dispensed into 500 ml. Erlenmeyer flasks, which contained 0, 20, 200, and 1,000 p.p.m. recrystallized aldrin. These flasks were then fitted with carbon dioxide adsorption units identical with those used in the soil respiration studies. The flasks were placed on the reciprocating incubator and connected to a supply of carbon dioxide-free air. Carbon dioxide evolved was collected in N/1 NaOH. The carbon dioxide evolved was collected in two of the experiments in an effort to determine a possible utilization of aldrin as a carbon source. At intervals of 24, 48, and 120 hours, one flask of each treatment was removed. The turbidity, ammonia, and pH were determined. In the carbon dioxide respiration study, the carbon dioxide evolved was determined after 4, 8, 12, 16, 20, 24, 48, and after each 24-hour period to the conclusion of the experiment at the 120-hour interval.

Plate Counts

In some cases, plate counts were made at selected time intervals. The plates were poured with sodium albuminate agar for the determination of bacteria and Streptomyces. Peptone glucose acid agar at pH 4.0 was used to estimate the molds present.

Soils

Willamette clay loam soil from the Schoth Garden was used
for the soil extract inoculum. These samples represent composite
portions of four field plots. The composite soils sample designated
"aldrin treated" (Table 11, p.57) had been sampled at random from
four plots which had been previously treated with 25 p.p.m. of
commercial aldrin added to the soil as a 5 per cent dust. Analyses
of this soil by the method of Koblitsky and Chisholm (23, p.781)
were made at approximately 12 months after the initial treatment.
The aldrin concentration was determined as 22.5 p.p.m. The composite
soil sample designated at "check" (Table 10, p.56) was randomly
sampled from the four check plots containing no insecticide. All
samples were obtained by using the recommended procedure as des-
cribed by the Association of Official Agricultural Chemists (4,p.1).

C. RESULTS

Results of the ammonification and nitrification studies in-
dicated that aldrin did have a pronounced effect upon ammonification.
Figures 30, 31, and 32, page 69, and 27, 28, and 29, page 68, show
the results of these experiments. The experiment was set up pri-
marily to determine whether there had been any adaptation of the
general soil microflora to aldrin. Such an adaptation is evident
upon examination of Figures 29, page 68, and 30, page 69, showing
the rate and the quantity of ammonia produced in the aldrin composite
and the check soils. After 24 hours incubation, the check soil

extract shows severe inhibition to the ammonification process at all concentrations of the insecticide. Twenty p.p.m. at this incubation interval shows a 20.5 per cent inhibition in ammonification; while at concentrations of 200 and 1,000 p.p.m., the inhibition is doubled and essentially the same at approximately 45 per cent inhibition. Thirty-seven per cent inhibition in ammonification was observed at concentrations of 20 and 200 p.p.m. after 48 hours incubation. At 1,000 p.p.m., the inhibition had reached a maximum of 52.2 per cent, and at the conclusion of the experiment (120 hours) the original inhibition noted at 20 p.p.m. disappeared completely and was now back to the control levels. At 200 p.p.m., an actual stimulation of 36 per cent in the ammonification processes was evident. This stimulation was even more strikingly evident at 1,000 p.p.m. and had reached a value of 42.5 per cent.

Thus we do show that soil extract-inoculated peptone solution in the presence of aldrin recovers sufficiently from an initial inhibition in ammonification after 120 hours incubation in shake flask cultures to yield an actual stimulation in these processes (Figure 30 p. 69).

The extract of the aldrin-treated composite showed much less susceptibility to the presence of aldrin; however, the curves were essentially the same, although there was a lesser effect. At 24 hours, all three concentrations produced a 28 per cent inhibition in the liberation of free ammonia. After 48 hours, 20 p.p.m. had no effect, 200 p.p.m. produced an 8 per cent inhibition, while 1,000 p.p.m. yielded a 22 per cent inhibition in these processes. Further

FIGURE 27: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN
.100 ML PEPTONE SOL'N SHAKE CULTURES INOCULATED WITH
1.0 GM SCHOTH GARDEN "ALDRIN" COMPOSITE SOIL

LEGEND
⊖ CONTROL
◓ + 20.0 PPM ALDRIN
◔ + 200.0 PPM ALDRIN
● + 1,000.0 PPM ALDRIN
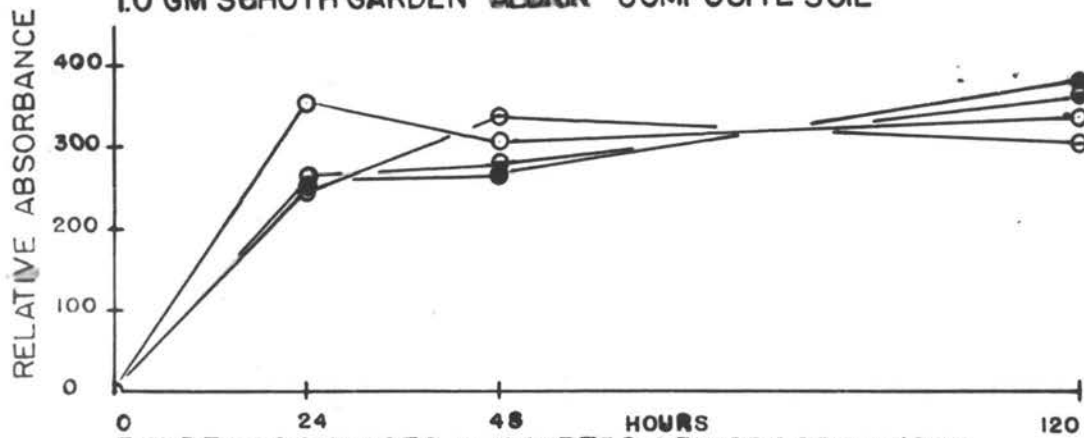
FIGURE 28: CHANGES IN NUMBERS OF MICROORGANISMS
DURING AMMONIFICATION (SEE FIG. 27)
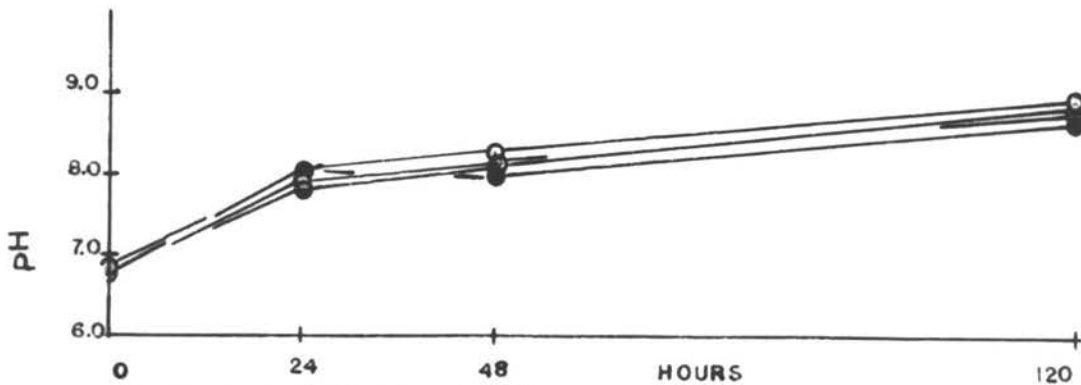
FIGURE 29: THE INFLUENCE OF ALDRIN ON THE pH OF
PEPTONE SOL'N SHAKE CULTURES (SEE FIG. 27)
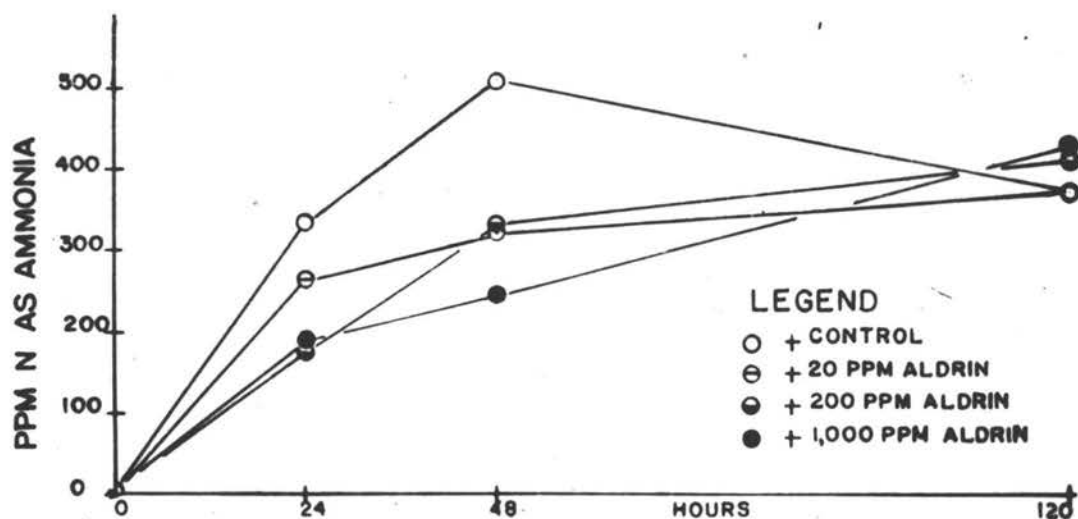
FIGURE 30: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN 100 ML PEPTONE SOL'N SHAKE CULTURES INOCULATED WITH 1.0 GM SCHOTH GARDEN "CHECK" COMPOSITE SOIL.

LEGEND
○ + CONTROL
⊖ + 20 PPM ALDRIN
◐ + 200 PPM ALDRIN
● + 1,000 PPM ALDRIN
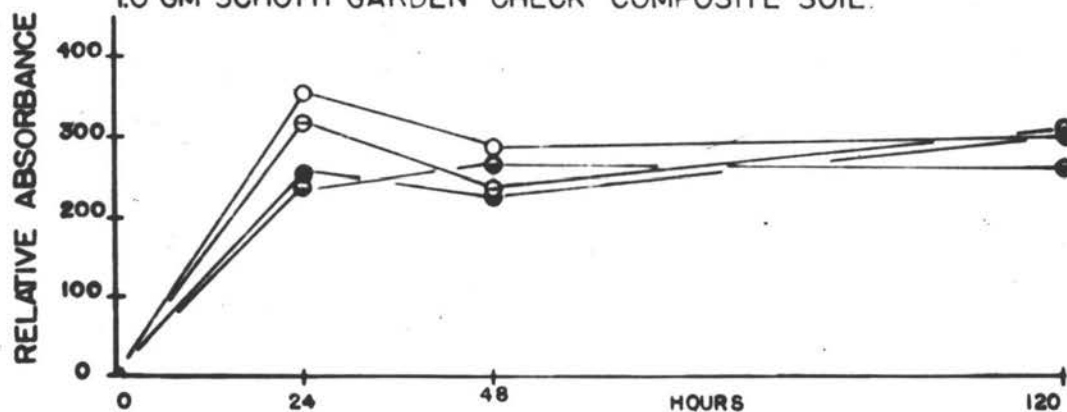
FIGURE 31: CHANGES IN NUMBERS OF MICROORGANISMS DURING AMMONIFICATION (SEE FIG. 30)
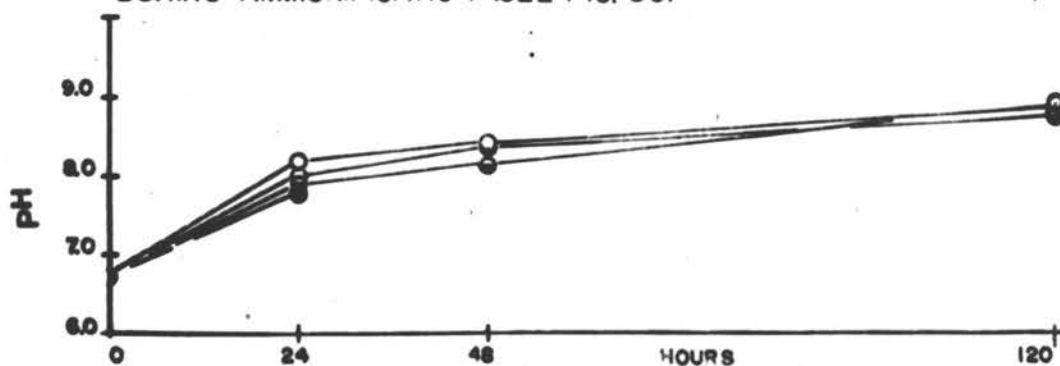
FIGURE 32: THE INFLUENCE OF ALDRIN ON THE pH OF PEPTONE SOL'N SHAKE CULTURES (SEE FIG. 30)

incubation produced essentially the same results as was indicated
in the preceding experiment; at 120 hours, 1,000 p.p.m. produced a
10 per cent stimulation in ammonification. However, concentrations
of 20 and 200 p.p.m. maintained their inhibitory action at levels
of 54.0 and 29.5 per cent respectively. The significance of these
results have not yet been determined; however, there appears to be
a critical aldrin concentration between 200 and 1,000 p.p.m. The
pH during the entire experiment for all concentrations remained sig-
nificantly below the control, as illustrated in Figure 29, page 68.
The growth of the organisms as determined by turbidimetric methods
closely parallels the ammonification processes, showing an initial
depression in the early stages of incubation and subsequent stimula-
tion at the conclusion of the experiment. These results were evi-
dent in both the "check" and the "aldrin-treated" soil extracts.

Little or no significant data were obtained by determining
the nitrite and nitrate concentrations at the characteristic time
intervals, for there appeared to be a concentration of soluble
organic matter in excess to the concentration for optimum autotrophic
proliferation. Thus, the production of nitrite and nitrate was
limited to trace amounts. The results of these experiments indicate
that there is a development of gradual resistance to the inhibiting
effect of aldrin by the general soil microorganisms, for it is evi-
dent that the degree of effect in the two soil extracts is signifi-
cantly different.

To eliminate the possibility that the manifold effects il-
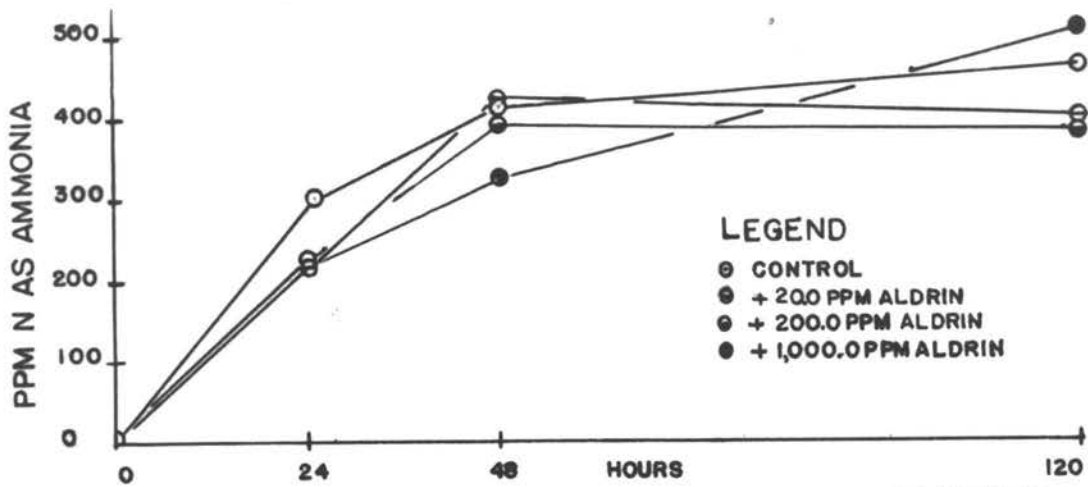lustrated in these experiments is the result of a physical phenomena,
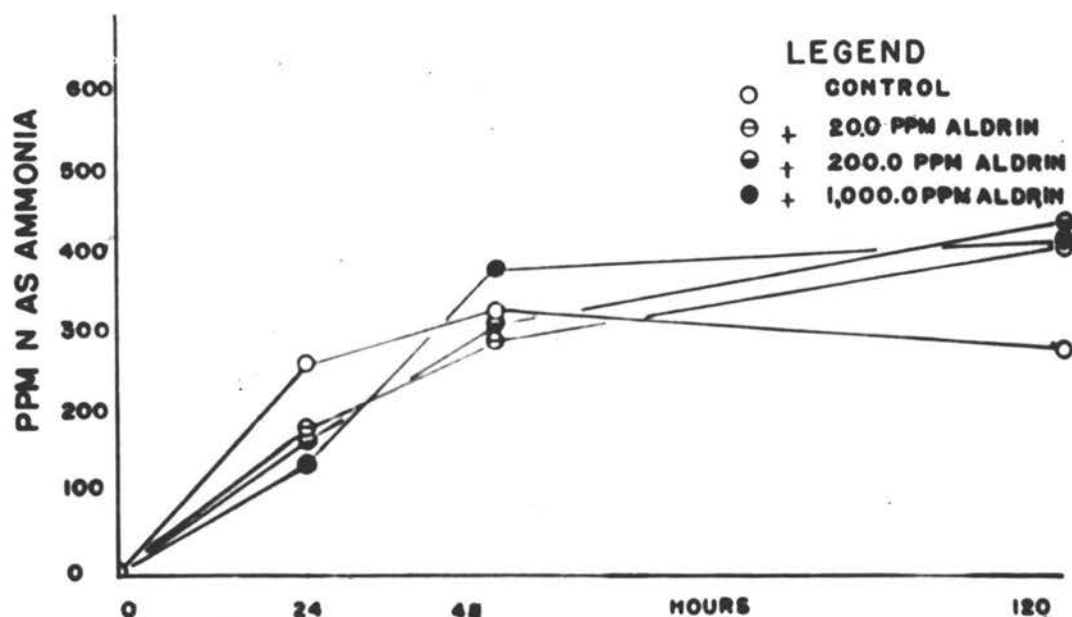
FIGURE 33: THE INFLUENCE OF ALDRIN ON AMMONIFICATION
IN 100 ML PEPTONE SOL'N SHAKE CULTURES INOCULATED
WITH 1.0 GM SCHOTH GARDEN "CHECK" COMPOSITE SOIL.

CO_2 PRODUCTION DETERMINED SIMULTANEOUSLY (SEE
FIG. 35)
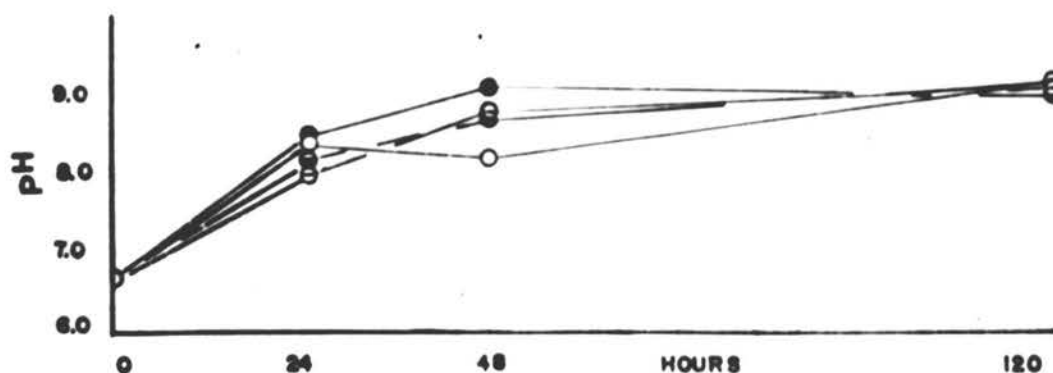
FIGURE 34: CHANGES IN pH OF ALDRIN TREATED PEP-
TONE SOL'N SHAKE CULTURES (SEE FIG. 33)

cultures similarly treated were incubated under static conditions.
The results are given in Tables 13 and 14, pages 74 and 75, and in-
dicate ciate that the effect is in all probability of a two-fold nature.

Plate Counts

In previous experiments it was shown that aldrin, although capa-
ble of inhibiting the normal nitrogen transformation in the soil, did
not have an injurious effect upon the numbers of heterotrophic micro-
organisms; but that a stimulation in the general microflora was observed.
This was evident from the results of plate counts obtained after 48
hours incubation. This appears to be in direct contrast to the infor-
mation obtained by the increased turbidity of the suspensions. There
appeared to be no obvious differences in the microflora of either of
the soil extracts or between the various extracts and all concentra-
tions of the insecticide. However, there was no growth of molds on
plates at any concentration or dilution. The molds in all probability
had been removed by the filtration of the inoculated medium prior to
incubation.

In view of this, it appeared advisable to repeat the experiment
without initial filtration of the medium. The results of this experi-
ment are shown in Figures 33, 34, and 35, pages 71 and 73. It also
appeared advisable to determine the effect of aldrin on the carbon
dioxide liberation by organisms present in the extract. The experi-
ment was set up in essentially the same manner as those preceding,
the only major change being that the coarse particles were removed by
passing the nutrient solution after inoculation through absorbent

FIGURE 35: THE INFLUENCE OF ALDRIN ON THE CUMULATIVE CARBON DIOXIDE PRODUCTION FROM SCHOTH GARDEN SOIL EXTRACTS IN PEPTONE SOLUTION.

FIGURE 35A: PLOT OF THE CUMULATIVE CO_2 PRODUCTION FOR THE FIRST 24 HOURS EXPANDED

LEGEND
O   PEPTONE ONLY
⊖ + 20 PPM ALDRIN
◐ + 200 PPM ALDRIN
● + 1000 PPM ALDRIN

MG CARBON AS CARBON DIOXIDE

MG CARBON AS CO_2

HOURS

73
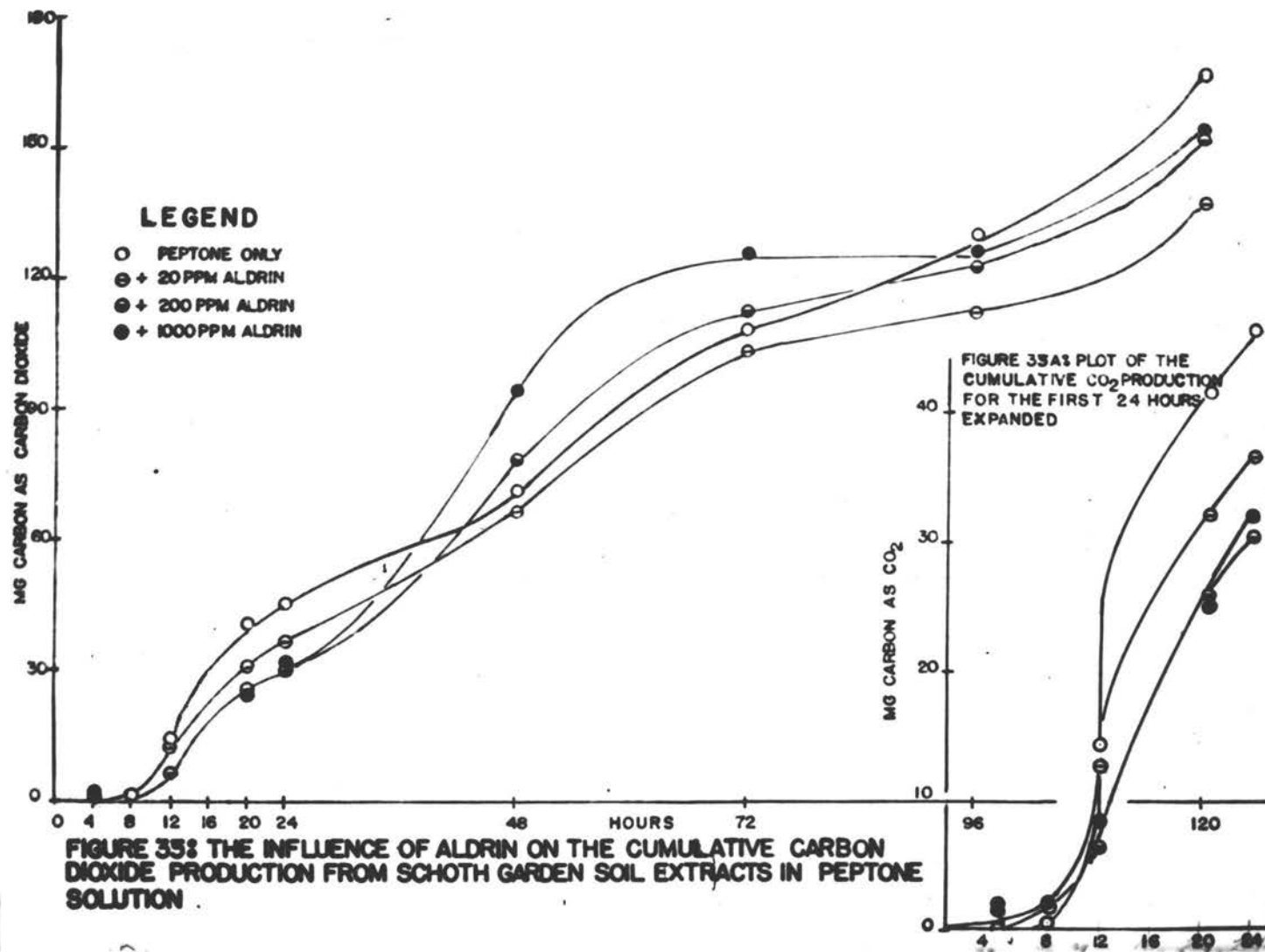
TABLE 13

The Influence of Aldrin on Ammonification in Soil Extract Inoculated Peptone Solution Incubated in a Static Condition, at 28°C., for Twenty Days

Soil Sample	Treatment p.p.m. Aldrin	Ammonia N Produced p.p.m.
Aldrin Composite	0	400.5
Check Composite	0	253.0
Aldrin Composite	20	218.0
Check Composite	20	100.3
Aldrin Composite	200	222.4
Check Composite	200	231.1
Aldrin Composite	1,000	313.9
Check Composite	1,000	114.0

TABLE 14

Plate Counts Obtained From Soil Extract Shake Flask Studies After 120
Hours Incubation

Soil Sample	Treatment ppm Aldrin	Number of Bacteria and Streptomyces per ml. x 10^6	Per cent Streptomyces
Aldrin Composite	0	27.6	31
Check Composite	0	18.9	43
Aldrin Composite	20	456.0	33
Check Composite	20	424.0	30
Aldrin Composite	200	930.0	40
Check Composite	200	857.0	38
Aldrin Composite	1,000	1,350.0	27
Check Composite	1,000	1,280.0	26

cotton, instead of filtering the medium through filter paper. As a
result, the suspension remained quite turbid, eliminating the possi-
bility of following the numbers by increased turbidity. It appears
from the results that the filtration process did remove a large num-
ber of the microorganisms present, although plate counts again revealed
that there was no mold growth.

The ammonification process showed the characteristic inhibition
after 24 hours. The 48-hour samples revealed a stimulation normally
observed after 120 hours incubation. This early effect is probably due
to the greater number of microorganisms present, or due to a species of
microorganism foreign to the subsequent experiments. Thus, it is shown
(Figure 33, p.71), that 1,000 p.p.m. aldrin exhibits a 20 per cent stimu-
lation in ammonification, with this stimulation becoming more and more
evident after prolonged incubation. Twenty and 200 p.p.m. aldrin sig-
nificantly stimulated the ammonification process after 120 hours. The
pH of the treated samples illustrated in Figure 34, page 71, reflects
the ammonification process. Static cultures incubated at 28°C. for 120
hours revealed no effect upon ammonification, growth, or pH of the me-
dium. The significance and the validity of this finding remains in
question.

A plot of the cumulative carbon dioxide production (Figure 35,
p.73) from the shake flask cultures indicates that aldrin at all three
concentrations prolongs the initial adjustment period of the microor-
ganisms to the medium. This inhibition is evident after 24 hours
incubation. Carbon dioxide production was significantly stimulated
after 48 hours incubation and maintained the stimulation through 90
hours, at which time carbon dioxide production dropped to control levels.

IV PURE CULTURE STUDIES

A. INTRODUCTION

In order to determine the effect of recrystallized aldrin on pure cultures of microorganisms, ten commonly encountered soil microbes were used in this study. These included Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Streptomyces griseus, Streptomyces coelicolor, Aspergillus niger, Penicillium sp. Mucor sp., and Trichoderma sp., all of which were obtained from the laboratory stock cultures or fresh soil isolates. It was the purpose of this study to determine the effect of aldrin on several of the organisms which are represented by the three well defined taxonomic groups: bacteria, fungi, and Streptomyces. Where the test organism was a bacterial species, a 1.0 ml. suspension of the 24-hour nutrient broth culture was used as an inoculum. In applicable cases a spore suspension was used as an inoculum in the study of the fungi and Streptomyces. The spores were harvested in 25 ml. of sterile water from slant cultures grown on nutrient agar and then thoroughly mixed. One ml. of this suspension was pipetted into the nutrient medium as the inoculum.

B. EXPERIMENTAL METHODS

Culture Medium

A simple medium consisting of 0.63 grams (water-free) Difco peptone in 100 ml. of water which contained an equivalent of 1,000 p.p.m. nitrogen, was used throughout the study.

Apparatus

A reciprocal type shaker equipped with an incubator was used to insure adequate aeration in all the pure culture studies. The temperature was maintained at approximately $28^{\circ}C$. although the temperature was not considered critical. Cell density was determined turbidimetrically by determining the logarithm of the absorbance with a Klett-Summerson photocolorimeter, Model 900.3, equipped with a deep red filter No. 66. Inoculated peptone solution at zero time was used as a reference point and the colorimeter scale set at 0. All subsequent readings were made in this manner with a standard 1/2-inch by 4-inch cuvette. Ammonia was determined by the Micro-Kjeldahl method (27, pp. 280-282). The pH was determined by the use of the Coleman glass electrode pH meter. Microscopic examinations of wet mounts were made with the American Optical Company phase attachment using the dark contrast objective.

Procedure

Recrystallized aldrin was weighed directly into 500 ml. Erlenmeyer flasks at concentrations of 2.0, 20.0, and 100.0 mg., which represent 20, 200, and 1,000 p.p.m., respectively. One hundred ml. of the standard peptone solution was dispensed into these flasks to the nearest 0.1 ml. These flasks were plugged with cotton and autoclaved at 10 pounds pressure and $103^{\circ}C$., for 20 minutes. This lower temperature was used to reduce the possibilities of a loss due to the volatilization of aldrin which has a melting point of approximately $104^{\circ}C$. (24, p.176). The experiment

was set up so that a sample at each concentration in its entirety could be removed from the shaker at 24, 48, and 120 hours, thus eliminating the possibilities of contamination while sampling and also avoiding cumbersome calculations due to changes in volume. One flask containing each of the concentrations characteristic of the experiment plus a control were removed at these times and their contents placed into chemically cleaned 100-ml. graduated cylinders and made back up to the original volume of 100 ml., with the addition of distilled water. These suspensions were then thoroughly mixed and approximately 10 ml. removed and placed into calibrated Klett colorimetric cuvettes, the turbidity and the pH were determined on the same samples. Appropriate aliquots were then taken from the suspension for the Micro-Kjeldahl determination of ammonia. In some cases the numbers of microorganisms were determined at these times.

C. RESULTS

Bacillus subtilis

Each concentration of the insecticide after 24 hours incubation showed severe inhibition to ammonification, and subsequently to growth. The same effect was noted after 48 hours. However, concentrations of 20 and 200 p.p.m. were less severe, the cultures showing a gradual recovery to the initial effect. After 120 hours there was very little difference in the effect of concentration, thus indicating a rapid recovery to the initial response. In the case of 1,000 p.p.m., the slope of the curve indicates that the rate of ammonification exceeds that of the control. In this one experiment, 100 minus

the per cent light transmittance, obtained from the Leitz Colori-
meter, was used as a measure of cell density. Ammonification also
reflects the growth of the organisms as is shown in Figure 36,
page 81. The severe inhibition to growth as determined by turbidi-
metric analyses is manifest at each concentration, although at 120
hours the 1,000 p.p.m. treatment shows a somewhat greater turbidity
than the control. A predominant portion of the turbidity noted may
be the result of the aldrin particles.

The influence of aldrin on the pH is a suppression of the ex-
pected rise normal to early stages of growth and ammonia production.
A gradual recovery occurs after 48 hours, and at 120 hours is almost
complete. In all cases, 1,000 p.p.m. had the most severe effect.
There is a definite metabolic and growth lag due to the presence of
the insecticide at each concentration.

A microscopic examination of the control and the treated
cultures was made after 24, 48, and 120 hours, in an attempt to show
an intimate contact of the cells and the aldrin particles, thus pre-
senting a logical explanation of the observed effects.

Considering the lack of growth or the complete inhibition of
growth in the treated samples after 24 hours, these early observa-
tions yielded little or no useful information. However, after 48
hours, the following significant changes were noted:

(1) Control: A great increase in the number of microorgan-
isms per field were observed. The cells were approximately 0.7 microns
by 3 microns long, and always appeared singly.
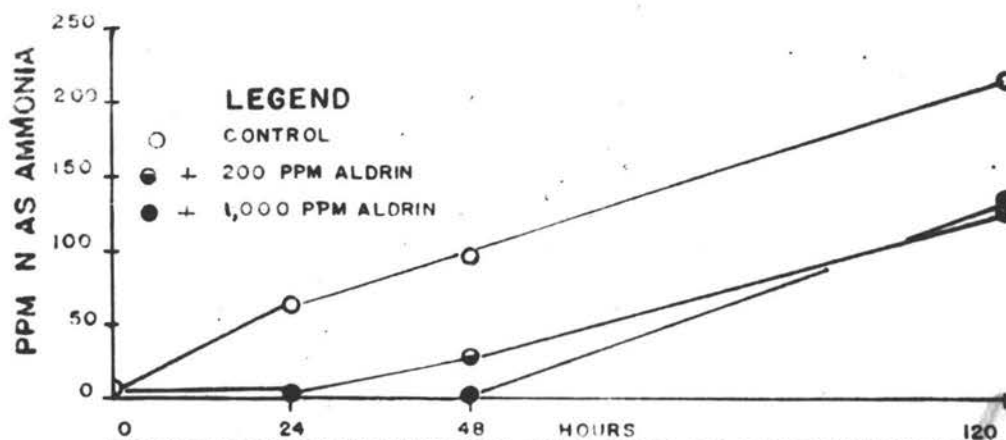
FIGURE 36: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF <u>BACILLUS</u> <u>SUBTILIS</u>

FIGURE 37: THE INFLUENCE OF ALDRIN ON THE GROWTH OF <u>B. SUBTILIS</u> IN PEPTONE SOL'N SHAKE CULTURES
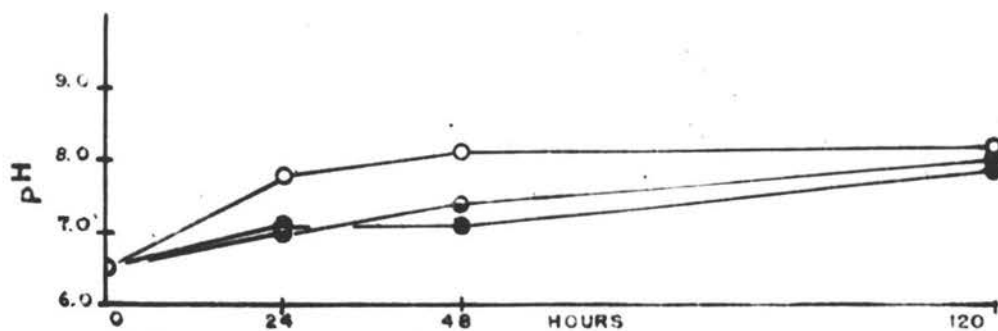
FIGURE 38: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 36)

(2) 200 p.p.m.: The field was much less densely populated with some long chains of organisms present; however, most of the cells appeared in short chains and pairs. Cell division appeared complete with the formation of cross walls, but they remained intimately connected. An average cell had the following dimensions: 0.7 microns by 4.5 microns. Aldrin particles were numerous with an average size of 1 micron by 1 micron, and in this case there was no visible contact between the aldrin particles and the cells.

(3) 1,000 p.p.m.: There were many more aldrin particles present with comparatively few microorganisms. The aldrin particles had no specific shape. The outstanding characteristic observed was that huge cells were abundant with approximate dimensions of 0.7 microns by 10--12 microns. There were many almost coccoid cells in chains. The morphology definitely appeared distorted. These observations at 970 X magnification yielded no indication that there was any contact between the cells and the aldrin particles.

Due to the artifacts inherent in all staining procedures, attempts to stain cell suspensions failed. Aldrin particles immediately occluded about the bacteria cells and gave a complete reversal of the picture obtained by phase microscopy using the wet mount technique. Attempts to photograph the observed and described fields generally failed due to the inability of maintaining a stable focus on the highly motile cells and particles.

Bacillus cereus

The effect of the insecticide upon Bacillus cereus was mani-
fest in a prolonging of the initial lag phase of growth. Twenty
p.p.m. had little effect upon ammonification; however, 200 p.p.m.
slightly inhibited the process after 24 hours incubation. While this
result is apparent, it could not be considered of practical impor-
tance. The initial inhibition was maintained after 48 hours incu-
bation in the presence of 200 p.p.m. of the insecticide. And after
prolonged incubation, 120 hours, at both 20 and 200 p.p.m., ammoni-
fication was inhibited, with 200 p.p.m. exerting the most pronounced
effect (Figure 39, p.84). The effect was also evident in the case
of B. subtilis. It is interesting to note this characteristic reac-
tion of the organism to a concentration of the insecticide at 200
p.p.m., as compared with the insecticide at 1,000 p.p.m. For at
1,000 p.p.m., ammonification is completely inhibited after 24 hours.
After 48 hours the culture recovered from the initial inhibition and
although total ammonia production lagged behind the control, the slope
of the curve indicates that ammonification is being carried out at
a more rapid rate. This general trend in the rate of ammonification
is even more strikingly illustrated after 120 hours, when it approaches
control levels, thus showing a general adaptation and a consequent
stimulation in ammonification.

Figure 39, page 84, a plot of the pH versus time, shows the
depressing effect of aldrin upon the hydrogen ion concentration of
the variously treated samples. This depression in pH parallels the
inhibition of ammonification and can in all probability be attributed
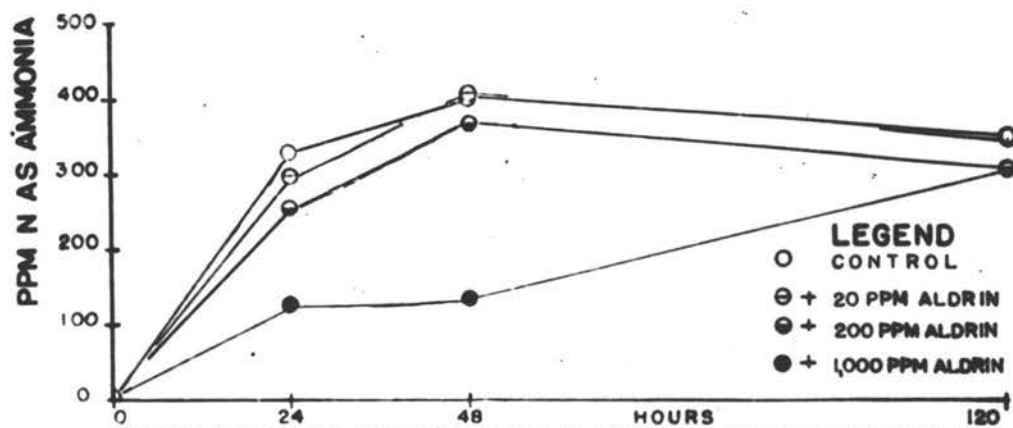
FIGURE 39: THE INFUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF **BACILLUS** **CEREUS**
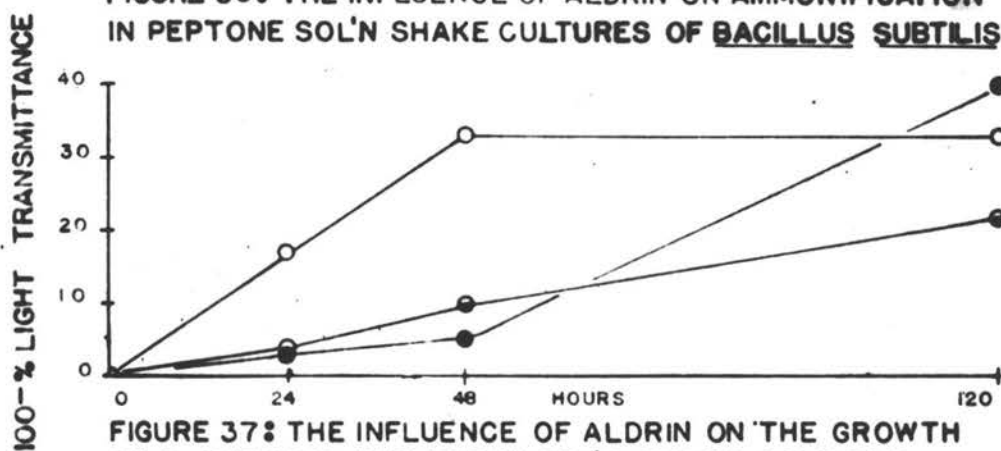
FIGURE 40: THE INFLUENCE OF ALDRIN ON THE GROWTH OF **B**. **CEREUS** IN PEPTONE SOL'N SHAKE CULTURES
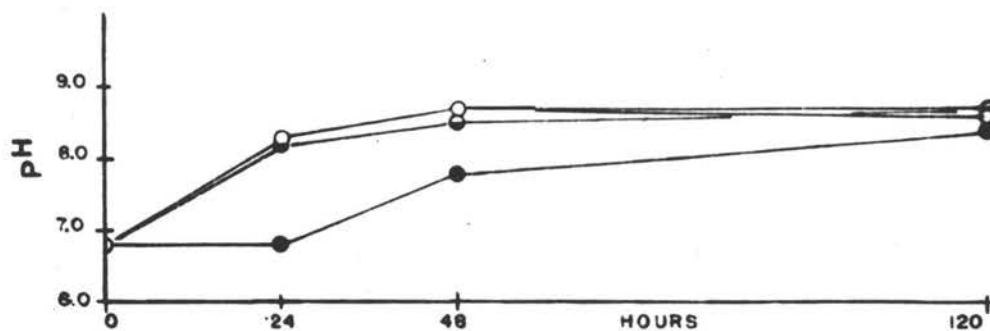
FIGURE 41: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 39)

LEGEND
O   CONTROL
⊖ + 20 PPM ALDRIN
◉ + 200 PPM ALDRIN
● + 1,000 PPM ALDRIN

to the difference in the relative ammonium ion concentrations. Although 20 and 200 p.p.m. tends to inhibit ammonification, the pH of the medium at 120 hours is 0.2 pH units above the control, which is considered significantly higher; however, the pH of the medium containing 1,000 p.p.m. lies below the control and would indicate that aldrin effects the efficiency of the carbon metabolism of the organism, or that aldrin itself is being chemically altered to yield an acid reaction. In any case this organism obviously reacts differently to various concentrations of the insecticide, and there appears to be a critical concentration between 200 and 1,000 p.p.m.

Following the growth by turbidimetric methods further emphasizes the effect of aldrin. It can be seen by comparing Figures 39 and 40, page 84, a graphic representation of the values obtained by a Micro-Kjeldahl determination of ammonia and the trubidity, that the curves are similar. However, the inhibition due to the presence of 200 p.p.m. aldrin in the medium is not manifest in growth at 48 hours. It is clearly illustrated (Figures 39 and 40, p.84), that at this period during the incubation, aldrin at this concentration does not inhibit ammonification.

Photomicrographs obtained after 120 hours incubation revealed little or no effect of the insecticide upon the gross morphology of the organism. However, several cells showed upon their exterior surfaces minute adhering particles, presumably of the insecticide. The mode of action of the insecticide is not clear, although its effect is definitely manifested in growth, pH, ammonification, and presumably in the general metabolism of the cell.

TABLE 15

Escherichia coli Pure Culture Study
Experimental Set-up

| Flask Contents	Number of Replication	Interval from treatment and sampling (hours)	Total No. of flasks
Peptone Solution and Inoculum	2	0,24,48,120	8
Peptone Solution, Inoculum, and 20 p.p.m. Aldrin	2	0,24,48,120	8
Peptone Solution, Inoculum, and 200 p.p.m. Aldrin	2	0,24,48-120	8
Peptone Solution, Inoculum, and 1,000 p.p.m. Aldrin	2	0,24,48,120	8
Peptone Solution and 20 p.p.m. Aldrin (No Inoculum)	2	0,24,48,120	8
Peptone Solution and 200 p.p.m. Aldrin (No Inoculum)	2	0,24,48,120	8
Peptone Solution and 1,000 p.p.m. Aldrin (no Inoculum)	2	0,24,48,120	8
500 p.p.m. NH_4Cl	2	0,24,48,120	8
500 p.p.m. NH_4Cl and 20 p.p.m. Aldrin	2	0,24,48,120	8
500 p.p.m. NH_4Cl and 200 p.p.m. Aldrin	2	0,24,48,120	8
500 p.p.m. NH_4Cl and 1,000 p.p.m. Aldrin	2	0,24,48,120	8
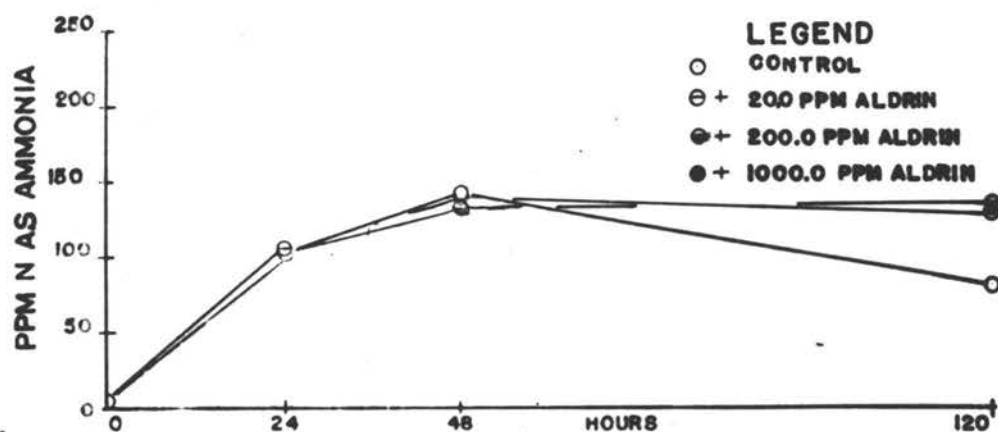
FIGURE 42: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF ESCHERICHIA COLI
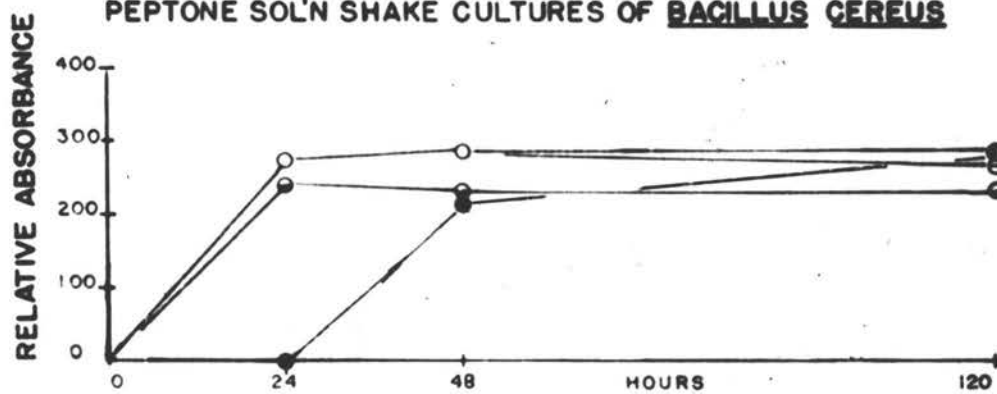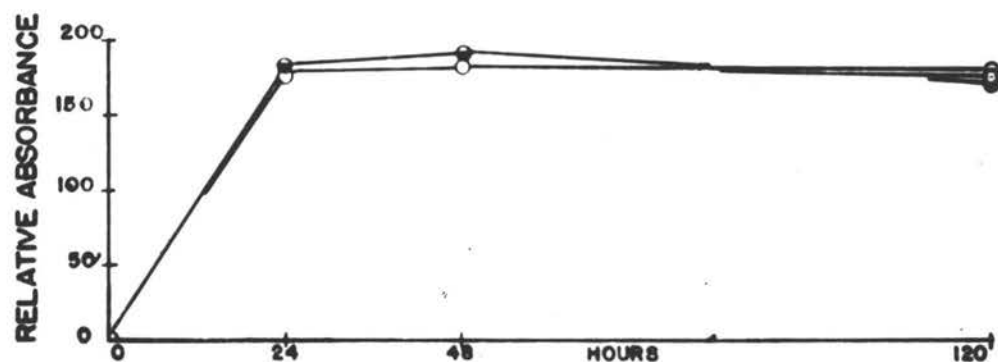

FIGURE 43: THE INFLUENCE OF ALDRIN ON THE GROWTH OF ESCH. COLI IN PEPTONE SOL'N SHAKE CULTURES
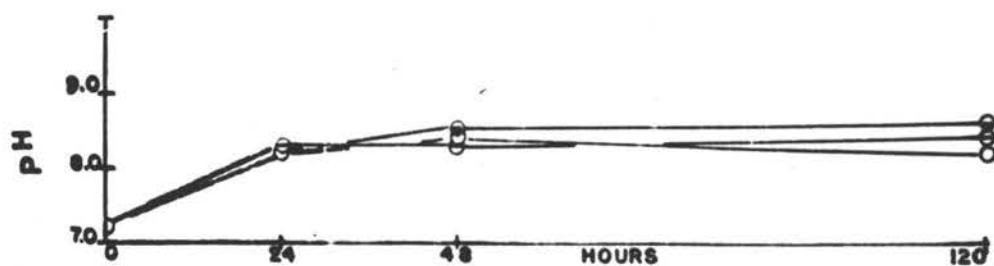
FIGURE 44: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 42)

Whether or not this effect is caused by the direct adsorption of the insecticide to the cell is dubious; however, the results of these observations would indicate that the presence of particles within the medium may play at least a minor role in the inhibition. Considering the massive concentration of the insecticide at 1,000 p.p.m., it would seem highly possible that some inhibition in growth could come from a mechanical disruption of the cell by bombardment with these particles in shake cultures. Although this action is possible, it seems doubtful that it plays a major role in the effect.

Escherichia coli

Recrystallized aldrin was used at concentrations previously noted. However, the experimental controls were run in an effort to rule out the possibility of an aldrin-ammonium complex, or a reaction between aldrin and peptone. The experimental set up is diagrammed in Table 15, page 86.

A definite but slight stimulation, though not considered serious, was noted after 24 hours incubation (Figure 42, p.87). The 200 and 1,000 p.p.m. treatments indicate a more marked stimulation after 48 hours incubation. These differences were maintained throughout the experiment.

Aldrin effected a general rise in the pH of the culture after 48 hours. After 120 hours, all treatments were 0.5 pH units above the control. Aldrin plus sterile peptone solution shaken for 120 hours at 30°C. shows a similar rise in the pH, possibly due to mechanical degradation of larger particles, increasing the solubility.
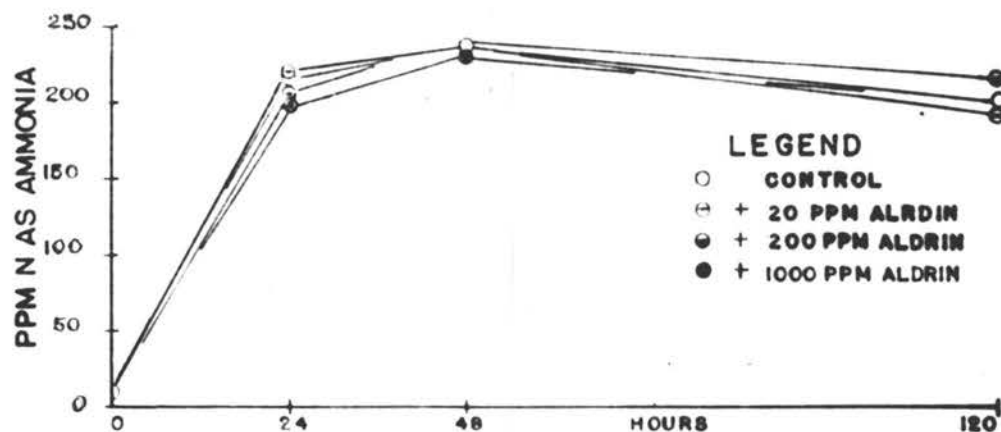
FIGURE 45: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF <u>PSEUDOMONAS AERUGINOSA</u>

LEGEND
○ CONTROL
⊖ + 20 PPM ALRDIN
◐ + 200 PPM ALDRIN
● + 1000 PPM ALDRIN
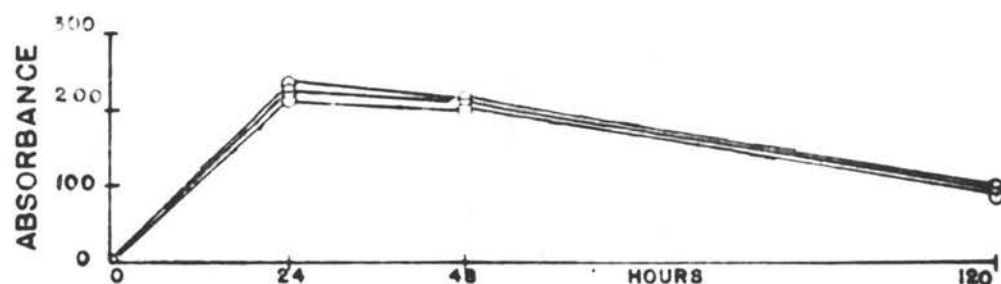
FIGURE 46: THE INFLUENCE OF ALDRIN ON THE GROWTH OF <u>PS. AERUGINOSA</u>
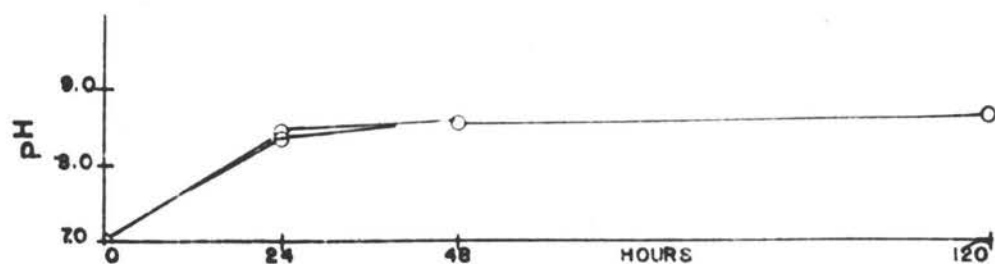
FIGURE 47: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 45)

The turbidimetric method showed gradual stimulation of growth by aldrin at each of the three concentrations (Figure 43, p. 87). The significance of this finding has as yet not been assessed.

The results of this experiment show that aldrin has no reaction with peptone or the ammonium ion nor does it enter into any absorption complex. Consequently, this elaborate control was eliminated from subsequent experiments.

Pseudomonas aeruginosa

Aldrin had no apparent effect upon shake flask cultures incubated at 30°C. for 120 hours. There were no assessable differences in ammonification or growth at any period during the incubation with any concentration of insecticide used (Figures 45, 46, and 47, p.89).

Streptomyces griseus

Although ammonification was not pronounced after 24 hours incubation, obvious differences between the control and the treated values are evident (Figure 48, p. 91). This inhibition was also evident after 48 hours, 200 and 1,000 p.p.m. aldrin being more effective and essentially equal in their effect. After 120 hours incubation there were no significant differences between each of the treated and the control samples, thus again indicating a general adaptation and possible subsequent stimulation of ammonification. A definitely injurious effect of the insecticide upon growth of the microorganism is shown in Figure 50, page 92. The turbidity of

FIGURE 48: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN
PEPTONE SOL'N SHAKE CULTURES OF STREPTOMYCES GRISEUS

FIGURE 49: CHANGES IN pH DURING AMMONIFICATION (SEE FIG.48)

FIGURE 50

The Effect of Aldrin on The Cell Volume of <u>Streptomyces</u> <u>griseus</u> produced in Peptone Solution Shake Cultures After 48 Hours at Approximately 28°C.

Cylinder Number	Aldrin Treatment (p.p.m.)
1	0
2	20
3	200
4	1,000
5	Peptone Solution Only

Streptomyces and mold suspensions in shake flask cultures are not
necessarily indicative of the plate counts or the density of the
growth. Aldrin visually appears to affect the morphological charac-
teristics of the organism and consequently there is little or no
agreement between the turbidity, optical density, or per cent light
transmittance and the numbers. This is due to the rather particulate
nature of the cell aggregates which form in the liquid medium and
their tendency to settle out of suspension. Therefore, where it was
possible to photograph the cultures in graduated cylinders used to
adjust the volume of the medium before analysis and show the relative
cell density, it was so done. This, it appears, is a more striking
method of demonstrating the effect of aldrin on the growth of these
various microorganisms where it is impossible to obtain reasonably
accurate turbidimetric analyses. Cultures in the graduated cylinders
(Figure 50, p.92) were allowed to stand for approximately two hours
before they were photographed.

Figure 50, page 92, represents the 24-hour samples of the
experiment and strikingly illustrates growth differences due to the
presence of the insecticide. After 120 hours all the cultures
appeared to be lysed; no intact cells were visible. The culture had
a pungent odor of ammonia and possibly some ammonia was lost by
volatilization. Due to the lack of intact cells after this incuba-
tion period, a photograph would serve no useful purpose. Whether or
not the cells were attacked by an actinophage or whether the pheno-
menon was one of autolysis remains questionable; however, during the
experiment the incubator and shaking device used were subject to
infection by actinophage specific for S.griseus.

FIGURE 51: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN
PEPTONE SOL'N SHAKE CULTURES OF ASPERGILLUS NIGER

FIGURE 52: CHANGES IN pH DURING AMMONIFICATION (SEE FIG.51)

FIGURE 53

The Effect of Aldrin on the Cell Volume of <u>Aspergillus niger</u> Produced
in Peptone Solution Shake Cultures After 48 Hours Incubation at
Approximately 28°C.

Cylinder Number	Aldrin Treatment (p.p.m.)
1	0
2	20
3	200
4	1,000
5	Peptone Solution Only

Streptomyces coelicolor

Aldrin at concentrations used in this experiment had no effect upon the organism.

Aspergillis niger

There was little or no physiological activity of the organism after 24 hours incubation. This is evident by a comparison of the control and the treated samples with respect to their ammonia production, wherein the differences were slight. The total amount produced was slightly above the original ammonium content of the medium. A definite inhibitory action on both growth and ammonification appeared after 48 hours. It is surprizing that the response does not correspond in degree to the concentration of insecticide; 200 p.p.m. exhibited a much more pronounced depression than 1,000 p.p.m. The relative positions of the curves (Figure 51, pp. 94) remain the same after 120 hours incubation. The greatest inhibitory action was exhibited in the flask containing 20 p.p.m.

Differences in pH at 24 hours are slight; however, there appears to be a significant lowering when at 48 hours the pH of the control dropped to approximately 5.7 (Figure 52, p.94). The effect of aldrin on the pH is strikingly demonstrated at this point during the incubation, for all three concentrations of the insecticide tend to maintain the original pH, approximately 7.3. Two hundred p.p.m. shows the greatest effect on the pH. After 120 hours, pH values for all concentrations of insecticide remain above the control; however, there appears to be a general adaptation to the insecticide in that
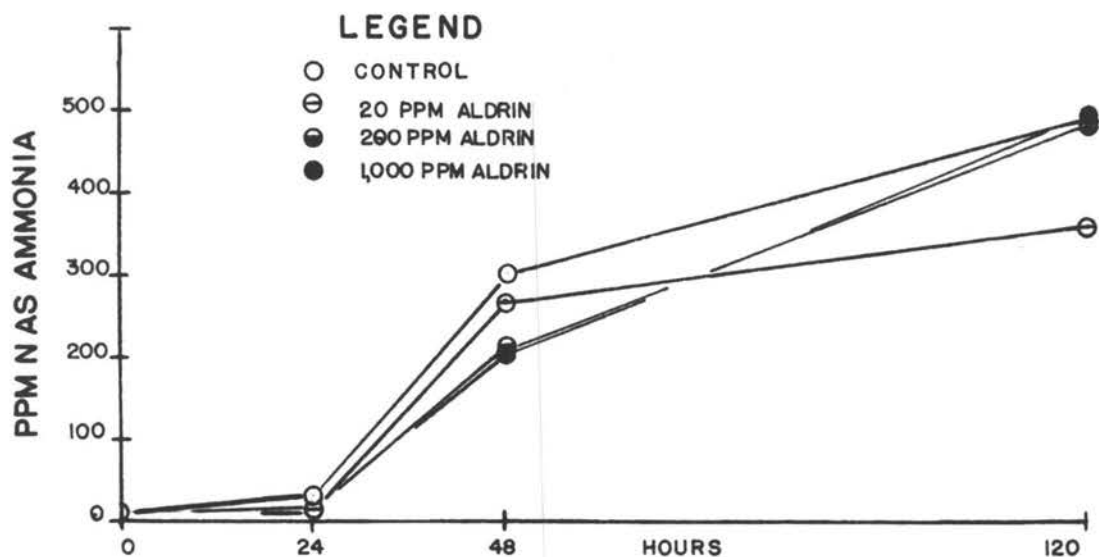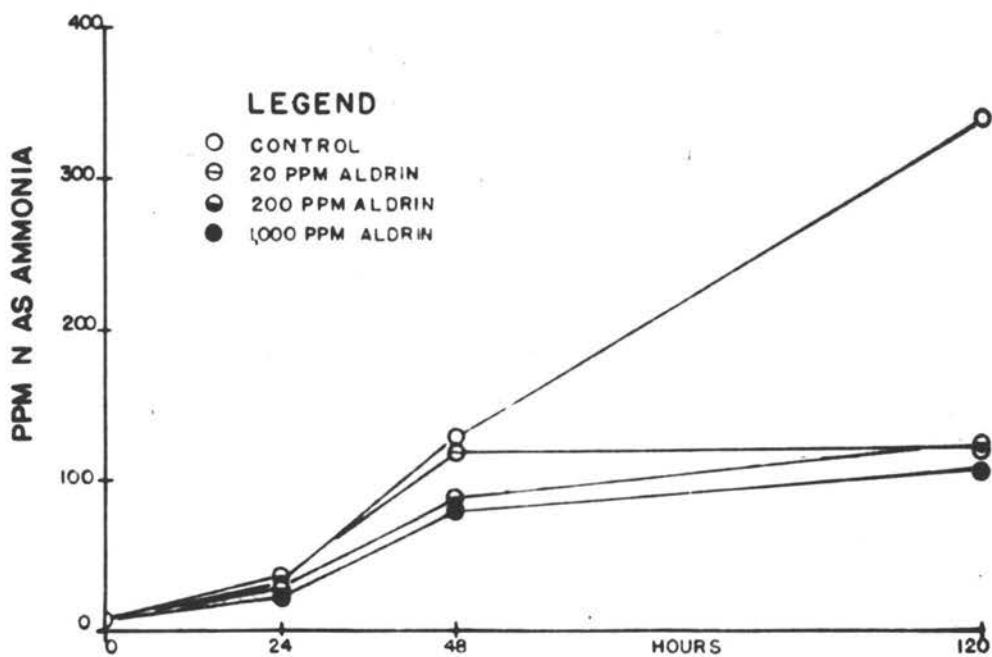
FIGURE 54: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN
PEPTONE SOL'N SHAKE CULTURES OF PENICILLIUM SP.
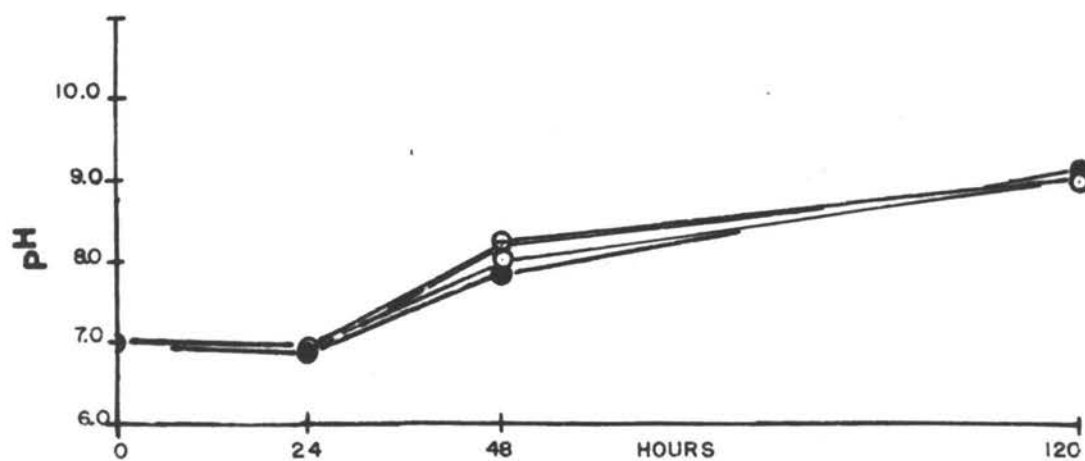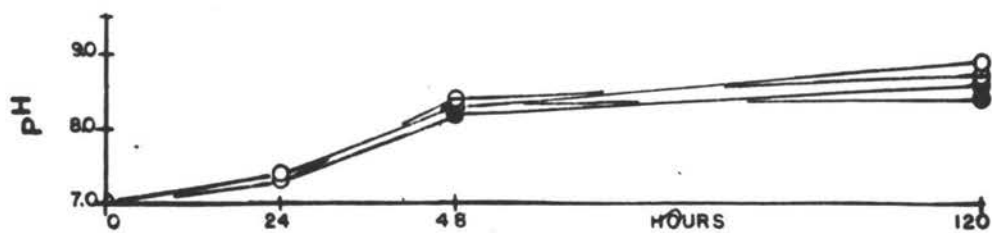

FIGURE 55: CHANGES IN pH DURING AMMONIFICATION (SEE FIG.54)

FIGURE 56

The Effect of Aldrin on the Cell Volume of <u>Penicillium</u> sp. Produced
in Peptone Solution Shake Cultures After 48 Hours Incubation at
Appr oximately 28°C.

Cylinder Number	Aldrin Treatment (p.p.m.)
1	0
2	20
3	200
4	1,000
5	Peptone Solution Only

the differential between the treated and the control samples is not nearly as wide as was noted previously in earlier samples.

Photographs were taken of the cell suspension which had been allowed to settle for approximately two hours in the graduated cylinders. The insecticide at each concentration visibly reduced the volume of cell mass produced (Figure 53, p. 95). It is also noted that there are definite morphological differences in the discrete colonies formed in these shake cultures. Growth in the control sample appeared comparatively homogenous with particles of smaller dimension, while colonies formed in the presence of aldrin were much larger and fewer in number.

Penicillium sp.

Aldrin has little or no effect upon ammonification in the early stages of incubation. The differences which are slight at 24 hours become more and more obvious after 48 hours. The degree of inhibition to ammonification is a function of the concentration of the insecticide, with 1,000 p.p.m. exerting the most pronounced effect. This is illustrated in Figure 54, page 97. Figure 56, page 98, a photograph of the graduated cylinders allowed to settle after ammonia determination, illustrates aldrin inhibited growth. PH determinations revealed no significant differences between the control and treated samples (Figure 55, p. 97).
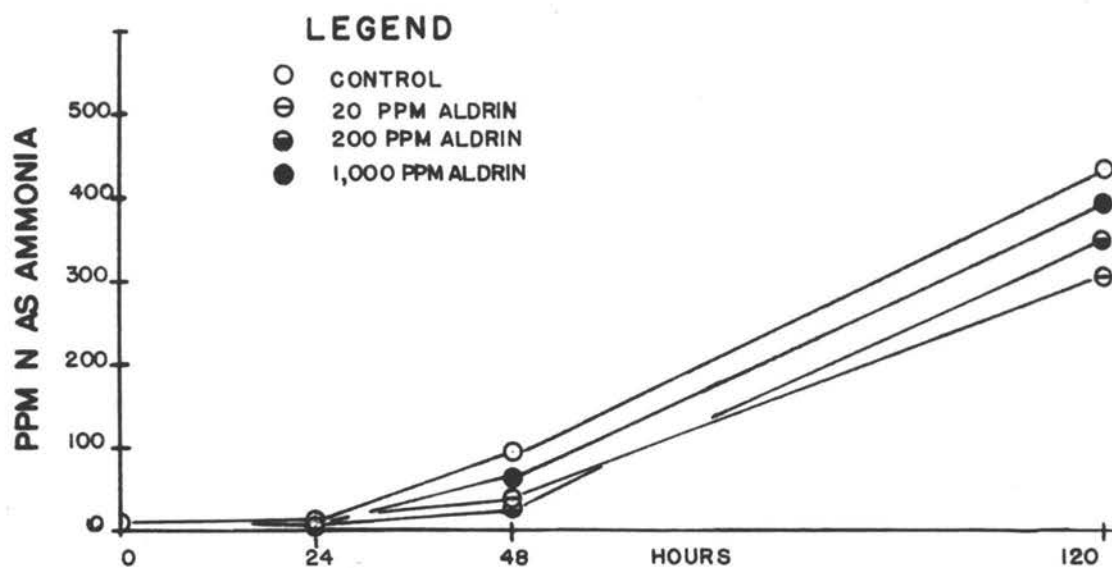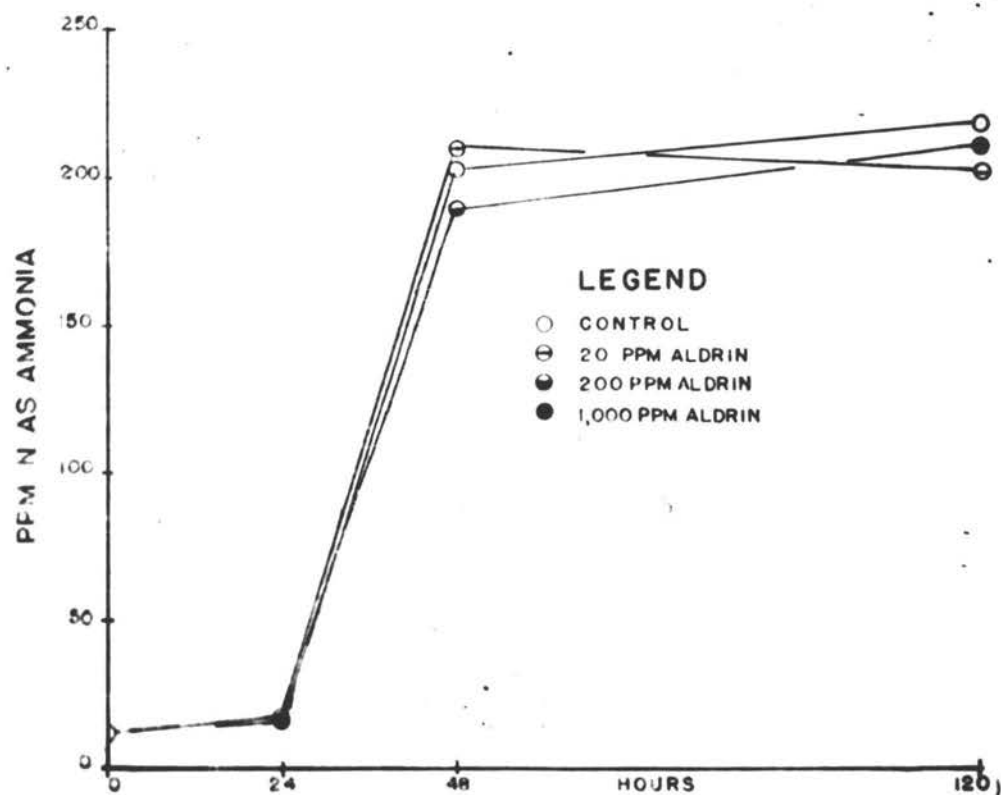
FIGURE 57: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF TRICHODERMA SP.
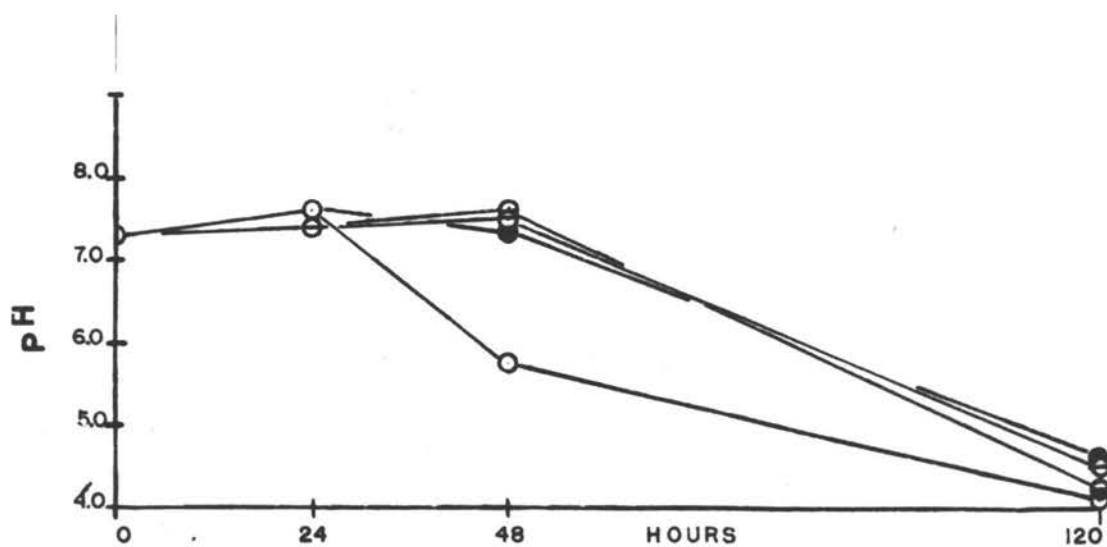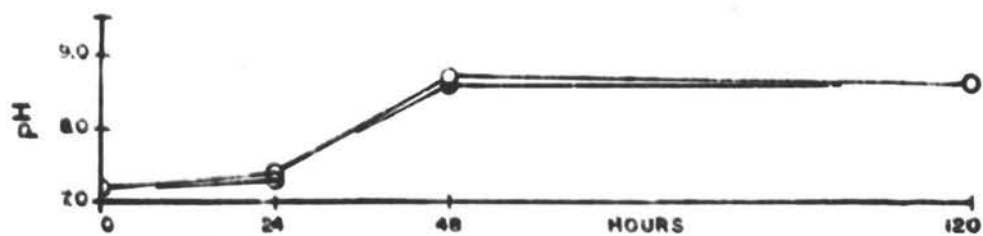

FIGURE 58: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 57)

FIGURE 59

The Effect of Aldrin on the Cell Volume Produced by <u>Trichoderma</u> sp. in Peptone Solution Shake Cultures After 48 Hours Incubation at Approximately 28°C.

Cylinder Number	Aldrin Treatment (p.p.m.)
1	0
2	20
3	200
4	1,000
5	Peptone Solution Only
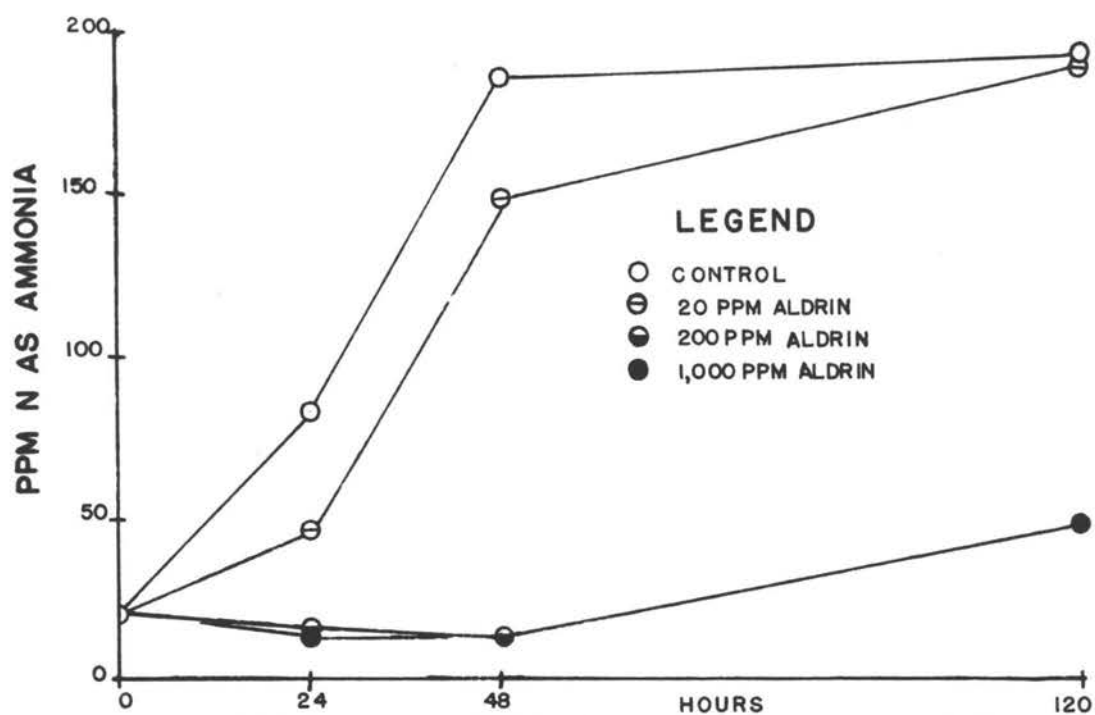
FIGURE 60: THE INFLUENCE OF ALDRIN ON AMMONIFICATION IN PEPTONE SOL'N SHAKE CULTURES OF MUCOR SP.
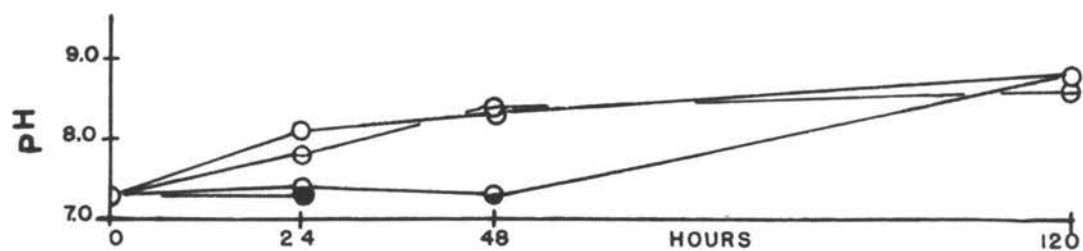
FIGURE 61: CHANGES IN pH DURING AMMONIFICATION (SEE FIG. 60)

FIGURE 62

The Effect of Aldrin on the Cell Volume Produced by <u>Mucor</u> sp. in Peptone Solution Shake Cultures after 48 Hours Incubation at Approximately 28°C.

Cylinder Number	Aldrin Treatment (p.p.m.)	Cell Mass* (Grams)
1	0	0.05
2	20	0.03
3	200	0.01
4	1,000	0.01
5	Peptone Solution Only	0.00

* Dry weight basis.

Trichoderma sp.

The Trichoderma species used in this experiment appears to
be completely indifferent to the presence of aldrin (Figures 57,
58, and 59, pp. 100-101).

Mucor sp.

Aldrin at 200 and 1,000 p.p.m. all but completely inhibited
the liberation of ammonia after 24 and 48 hours incubation. The
data indicate a slight adaptation to the presence of the insecticide
at these concentrations after 120 hours incubation. This is illus-
trated in Figures 60 and 61, page 102, the latter representing the
pH taken at the characteristic time intervals. Twenty p.p.m. of the
insecticide slightly inhibits ammonification and slightly reduces
the pH; however, after 120 hours there appears to be no difference
between the pH and the ammonia produced in the control and the treated
samples. Figure 62, page 103, a photograph taken after 120 hours
incubation, illustrates the differences in growth or cell mass in
the presence of aldrin at each of the concentrations. The data
(Figure 62, p. 103) show a close correlation with the photograph;
the observed volume and the actual cell weight as determined by
filtering the contents of the cylinders through syntered glass
medium filters under slight vacuum and drying at 105°C. for 24
hours.

DISCUSSION

I EXPERIMENTAL METHODS

A justification of methods used to determine the effect of
compounds such as aldrin on soil microorganisms and some of their
activities is necessary, especially if the purpose of the investi-
gation is designed to assess the relationship between effect and
soil fertility. Therefore, the following discussion is a brief
summary of current research methods in soil microbiology.

Ammonification as an Index of
Decomposition of Soil Organic Matter

The nitrogen in the complex organic substances is present
largely in the form of proteins. Thus many investigators have attem-
pted to differentiate fertility of different soils, using the rapidity
of decomposition of organic nitrogenous compounds, either in solu-
tion or directly in soil (26, p. 187). However, results are fre-
quently difficult to interpret. Differences in the ability of the
soil to produce ammonia resulted in many factors other than the
microbial flora of the soil. Among other lesser factors, the fol-
lowing are responsible for the greatest variance: (1) The large
numbers of microorganisms capable of ammonification; (2) The amount
of ammonia produced depends upon the nature of the carbon and nitrogen
in the organic matter added to the soil; (3) A portion of the ammonia
is converted to nitrate, depending upon certain environmental condi-
tions; (4) The amount of ammonia that a soil can hold depends upon

the pH, the nature of absorbed bases, and the buffer content (52, pp.611-612).

Although it is generally concluded that ammonification cannot be used as a direct method of determining the crop-producing power of different soils, it is possible to study the effect of substances added to the soil by comparing their effect upon ammonification. Ammonia production can be used effectively as an index of the acti- vities of pure cultures of microorganisms or of the complex soil flora upon nitrogenous organic materials (52, p. 612).

<center>The Nitrifying Capacity of the Soil

as a Function of Fertility</center>

Various investigators (53, pp.55-67) have established a definite correlation between the nitrifying power of the soil and its crop-producing power. Many others are of the opinion that while nitrification is a valuable and essential asset in fertility, it probably does not, under normal conditions, become a limiting factor in productivity. The lack of correlation between certain bacterial processes, such as nitrogen transformations, and soil fertility, may be that the latter is limited by some factor other than the nitrogen supply, such as moisture, temperature, or aeration.

<center>Numbers of Microorganisms in

the Soil as an Index of Fertility</center>

Any correlation between the crop-producing power of a soil and the number of microorganisms has met with considerable success;

however, not without a certain amount of criticism (54, pp.321-330).
Those investigators justifying the correlation have placed considera-
ble confidence in the determination of numbers of microorganisms in
soil by plate count methods. A correlation was reported (54, pp.345-
346) to exist between crop yield, oxidizing power of soil, nitrate
production, and numbers, but not between crop yield and ammonia ac-
cumulation. The relative numbers of bacteria, Streptomyces, and
molds is indicative of the chemical condition of the soil, such as
soil reaction and the degree of organic matter decomposition, and
therefore must be considered a function of the environment.

Carbon Dioxide Evolution and Soil Fertility

As a result of the assimilative activities of soil micro-
organisms, carbon dioxide content of the soil varies with the amount
of organic matter present. The food most necessary for the life of
microorganisms is carbon and is consumed in largest proportion by
the majority of the soil microbes. And since most of this carbon is
oxidized to produce energy, carbon dioxide is the outstanding product
of their activity. Although some orgaic matter may be initially
resistant to decomposition and various intermediate products may be
formed, sooner or later they are completely oxidized. Waksman
(51, p. 699) summarizes, "Since all heterotrophic aerobic micro-
biological processes are accompanied by the production of carbon
dioxide, this can be readily taken as an index of the microbiological
activities in the soil." Soil respiration studies are therefore an
important measure of decomposition process and as an index of soil
fertility (6, p.353).

There is a correlation between the bacterial numbers, nitrate concentration, and carbon dioxide evolution for a rise in bacterial numbers is always accompanied by a rise in the CO_2 in the soil, and somewhat later a rise in the nitrate concentration. The principle factors affecting carbon dioxide production are, in the order of their importance, temperature, moisture, dissolved oxygen, and the growing crop. (51, p.701). Stoklasa (44, pp.589-599) states that collection of the CO_2 produced from a given quantity of soil gives an exact picture of soil under certain environmental conditions. Many later investigators fail to share the enthusiasm of Stoklasa, although most of them agree CO_2 production in soil fertility is a factor of importance.

Thus ammonification, nitrification, carbon dioxide production, and microbial numbers were established as criteria in an effort to ascertain the effect of aldrin on soil microorganisms and some of their activities in representative Oregon soils.

II EXPERIMENTAL RESULTS

The stimulatory effect upon the total numbers of microorganisms in the soil and the reason for the inability to show a concommittant increase in nitrogen transformation remains unsolved. There is, however, evidence which supports the increase in numbers of bacteria and molds in that an initial stimulation in ammonification and carbon dioxide evolution is the result when aldrin is added. The organisms responsible for ammonification and carbon dioxide evolution are heterotrophic, and the plate counts of these experiments

which show a stimulatory response to added aldrin are limited to heterotrophic microorganisms. There has been no attempt to determine the effect of aldrin on the numbers of autotrophic microorganisms by plate count methods.

It is logical to assume, considering the extremely limited solubility and the great chemical stability of aldrin, that any effect due to its presence would be of an indirect nature. Since aldrin is so highly resistant to decomposition by physical and chemical procedures, it is likely that it would resist decomposition due to microbial activity.

The residual nature of aldrin in soil is as yet not well understood. Residue studies by Fleming, et.al. (11, p.5) have established that aldrin losses in soil, after twelve months under field conditions, have been as high as 67 per cent. Lidov, et.al. (24, p.187) states that aldrin, like chlordane, exhibits residual effectiveness under field conditions for somewhat less than three weeks. It therefore falls into a class of materials which exhibit pronounced initial insect toxicity but relatively short residual action. However, Terriere (46) and others, have determined the residual effectiveness of aldrin and found it to lose little or none of its initial toxicity after twelve months under field conditions.

Chlordane, a structurally related compound which is very similar to aldrin, was shown in residue studies, to yield a sigmoid curve when results of the chemical and biological determinations were averaged for each time-concentration and expressed as percentage

remaining of the insecticide originally applied. Chlordane concen-
trations decreased very rapidly and after one and one-half years, only
about 30 per cent remained in the turf (11, p.4). Below this point
further decreases could not be determined definitely, since the
amounts were outside of limits of the chemical and biological me-
thods. It was expected, however, that trace concentrations would
persist in treated plots for several years. The existance of the
sigmoid curve would suggest that the loss of chlordane was the re-
sult of biological factors rather than a physical phenomenon, since
the sigmoid curve represents the trend of the growth and death, and
also the trend of the vital biochemical activities of a biological
population.

The ambiguity of results indicate a wide variance of opinion
as to the residual nature of aldrin. However, a close examination
of the various methods of analyses will yield a clearer picture of
aldrin's residual effectiveness. Many investigators have relied
solely on biological assay methods while others are divided between
two common chemical analyses. Those finding no loss in effective-
ness have, in general, based their results on biological assay
methods. While others having determined an actual loss of the com-
pound, have obtained results by chemical analyses specific for
aldrin. And to further confuse the problem, the tendency of authors
to use the terms "residual effectiveness" or "residual toxicity"
and "residue" or "residual concentrations" interchangeably, have
contributed to the ambiguity of the literature.

The outstanding and most widely used chemical analyses are the methods of (1) Koblitsky and Chisholm (23, p. 781) and (2) Danish and Lidov (8, pp.190-197). The method of Koblitsky is based on the analysis of chloride ion after sodium decomposition of the compound in the soil extract; it is therefore not specific for aldrin. The Danish method is a specific colorimetric analysis for aldrin and has a sensitivity of 0.1 p.p.m.

Apparent losses in aldrin concentration (Table 9, p.52) indicate volatilization of the compound during the incubation and air-drying periods. Although aldrin exhibits relatively high vapor pressures (18, p.1), it is illogical to assume a loss of 100 p.p.m. (10 mg.) as a result of this characteristic. A predominate proportion of the undetermined aldrin was probably tenaciously held in adsorption at the base exchange and resisted solvency in the hexane extractive. If losses in aldrin were a physiological consequence of assimilative activities of soil microorganisms, there should be greater decreases in aldrin concentrations in soil samples incubated with added peptone. Since added peptone nitrogen does result in an extreme elevation in microbial numbers, it is not likely that organisms responsible for such processes would fail to appear in these soil counts (Figure 2, p. 28).

Results of these experiments (Table 9, p. 52), therefore, indicate a loss of the insecticide, a loss which is concluded to be the result of factors other than microbial activities. Further speculation as to the nature of these factors is beyond the scope of this investigation.

Likening aldrin to a similar compound, DDT, Smith and
Wenzel (40, pp.228-232), and Jones (20, p.59), expressed the stimu-
latory response in numbers of soil microorganisms to this compound
as due either to the utilization of impurities present as food or
to direct stimulation of the cell to better utilization of nutrient
materials already present in the soil. There exists the possibility
that the response due to aldrin may be of a similar nature.

An obvious conclusion would be that to have any effect, the
compound must come into intimate contact with the cell in such a
manner as to alter its physical or chemical properties so that the
passage of nutrients would be inhibited or enhanced. It is diffi-
cult to conceive a compound exerting toxic effects to bacteria which
is so limited in its water solubility. However, there is little
question as to the effect observed, and not to preclude any possi-
bility which might help explain the method of the insecticides
action, it is desirable to discuss the bacterial cell wall and some
of its functions as related to the problem.

The cell wall of bacteria is not a discrete chemical or
physical entity. The results of microchemical and macrochemical
investigations (22, pp. 119-125) have brought out two facts which
are reasonably sure: The first is that the chemical composition
of the cell wall in a variety of bacteria is different; the second
is that one of the principal components of the cell wall, in prac-
tically all bacteria investigated, consists of complex carbohydrate-
containing molecules. In many bacteria the principal cell wall
constituent appears to be a complex protein conjugated with

carbohydrate, lipid, or nucleic acid. Evidence of the presence of lipid in the intact cell wall of certain bacteria has also been observed (10, p.17).

The cell wall, slime layer, and the cytoplasmic membrane all play an important role in the control of material exchange between the internal and external environments of the cell, and consequently influences metabolic activity. It is apparent that the insolubility of aldrin in water will limit its bacteriostatic properties and we must assume that it cannot be externally digested to simpler materials of greater solubility. How then is it capable of influencing such important cell functions as carbon dioxide production, protein hydrolysis, and ammonia or nitrite oxidation? Speculation allows for the following analogy: Sulfur is so insoluble in the medium in which Thiobacillus thiooxidans grows that the solubility has never been determined. Nor is there any hydrolysis to simpler materials. Vogler and Umbreit (49, pp.331-337) found that in order to oxidize sulfur, a direct contact between the organism and the sulfur particle is necessary. These same authors (48, pp.141-148) discovered that the cell of T. thiooxidans contained a "fat globule" which is placed in contact with the sulfur particle. This "fat globule" is capable of dissolving sulfur and is thus able to bring the insoluble sulfur inside of the cell.

Therefore in instances where an aldrin manifested response is observed, the microorganism doubtlessly displays rather unusual reasons for susceptibility, i.e., by carrying its own solvent.

Undoubtedly the solubility of aldrin is greatly enhanced in contact with a bacterial cell of high cell-wall lipid content.

Observations of Bacillus cereus in pure culture, revealed what appeared to be an adsorption of aldrin particles on the cell wall. However, similar observations of Bacillus subtilis gave no indication that aldrin had been adsorbed. The fact that both organisms are severely effected by the compound only clouds any explanation of its action. Doubtlessly the particles observed adhering to B. cereus cells were aldrin, although there is no direct proof. In any case the compound may alter the interfacial tension relationships about the cell to the point that it would become incapable of carrying out its normal metabolic activities.

Hummer and Kenaga (16, pp.653-655) find an interesting relationship of chemical structure to toxicity between rotenone, methoxychlor, and DDT. These authors believe that the toxicity of these compounds can be evaluated and compared from the standpoint of the molecule as a whole, taking into consideration properties such as molecular weight, solubility, polar groups, hydrogen bond acceptors (or donors) and molecular shape and dimensions.

Considering the structure, molecular weight, and a marked similarity in toxicity to the same species of insects, and comparing these properties with those of DDT, methoxychlor, and heptachlor, there exists a striking similarity to aldrin. To the knowledge of this writer, there has been no attempt to compare the toxicity of these compounds with respect to microorganisms, other than qualitative estimates of their effect on the gross metabolic activities

of soil microroganisms. Although many investigators have observed
effects on the activities of soil microorganisms, there has been
little effort or interest in determining the mode of action. As
a consequence, the only working premise resolves itself in a trans-
position and extrapolation of information gleaned from studies of
insect physiology to microbial physiology in an effort to interpret
the effect of insecticides upon soil microorganisms, and their ac-
tivities.

It is well known that a growth-stimulating or growth-inhibiting
substance must be converted into a part of the living structure,
that is, it must be adsorbed by molecules of the living substance.
It is reasonable to postulate that in the molecular system of the
living substance there are spaces that can be occupied by mole-
cules of the growth regulator. This means that the growth regulator
has a certain affinity for a certain "space". This hypothesis pos-
tulates two kinds of spaces; the first filled in by growth substances
effects a growth promotion, the second type also shows a certain
affinity, but in this case growth inhibition results (25, pp.69-70).

Any organic molecule (aldrin) brought from outside into the
cell will not ordinarily be able to fill the "spaces" of the growth
substance with the necessary active compound for the growth effect;
consequently, the molecules accumulate somewhere on the living
"structures," where they disturb their functions and exert an
inhibiting influence to normal growth and metabolic activity.

Speculation allows for the possibility that aldrin may act
catabolically on extracellular enzyme systems; however, there is no
experimental evidence to support this view.

To give a mechanistic view of the mode of action, aldrin may be visualized to "fit into a lock-and-key relationship" with some essential region of the polypeptide chain of an enzyme. The importance of such molecular shape factors in relation to biologically active molecules and proteins such as enzymes has been widely emphasized by Pauling (30, pp.2643). In this case the toxicant would be held in position by short-ranged forces such as van der Waals, dipole-dipole, or hydrogen bonding forces. This seems to provide a logical basis for the bacteriostatic action of aldrin. Thus aldrin may fall into a class of enzyme inhibitors referred to as competative, since its action appears to rest in its affinity for the site on the enzyme protein molecule which is normally reserved for the substrate or coenzyme molecules. As a result the effect of aldrin on certain microorganisms may be a competative phenomenon between itself and compounds considered as essential respiratory intermediates. Shibata (38, pp.6-10) has shown a competative inhibition of phenolic substances and structurally unrelated alanine in Escherichia coli.

Srivastava (41, pp.403-404) has described an interesting metabolic relationship between meso-inositol and lindane (the gamma isomer of benzene hexachloride). Using the German-cockroach as a test organism, he found an inhibition of the insecticidal properties of lindane where insects had been reared on meso-inositol. However, he found no level of the vitamin which would completely neutralize the toxic action of the insecticide. It is suggested that there may be several metabolites affected in the cell by the gamma-isomer, and meso-inositol may perhaps be one of these, although there does

not appear to be a direct metabolic relationship between the two
compounds, and the inhibition shown in the test insects may have
been due to resistance acquired or developed by them after feeding
the vitamin. It should be noted, although these compounds are simi-
lar they are not isomorphous. Some evidence indicates that the gamma
isomer of hexachlorohexane (lindane) is an antagonist of inositol
in certain microorganisms. Kirkwood and Phillips (21, p.251), found
that the inhibition of growth of certain fungi, which is caused by
lindane, can be reversed in some cases with inositol. Schopfer,
Posternak, and Boss (37, p.443) as well as Doisy and Bocklage (9,
p.490) found that there are many species, however, in which this is
not true. Possibly one reason why difficulty is experienced in
demonstrating the antagonism in all species is that inositol is
water soluble and not at all taken up by fat solvents, whereas
the chloro analog is fat soluble and only very slightly so in
water (61, p.43).

The noted responses of microorganisms to aldrin may in rea-
lity be the result of factors other than chemical in nature. For
doubtlessly a portion of the aldrin used in all of these experiments
was in colloidal form. And a predominate portion of the material
used as carriers for the insecticide, when it is applied in the field,
is in a colloidal state. Conn and Conn (7, pp.99-100) have demon-
strated a distinct value of colloids in the nutrition of some bac-
teria. Bacterial numbers have been stimulated by colloids of bentonite,
kaolenite, beidelite, and illite types. These compounds appear to
act as carriers of nutrient bases, particularly calcium, and tend

to provide increase surface for the absorption of harmful products
of growth.

ZoBell (62, pp.57-58) has shown that the growth of certain
bacteria is stimulated by the addition of solid surfaces to aqueous
media. Therefore, in these experiments the stimulation in ammoni-
fication and nitrosofication may be the result of added surface
provided by the aldrin or carrier particles.

Quastel (33, pp.1-10) has shown that nitrifying microorganisms
proliferate at the expense of ammonium and nitrite ions adsorbed
at the base exchange complex of the soil. If this be true, the in-
secticide added to the soil may react with or inactivate these
exchange complexes or enter into competative adsorption with soil
nutrients. Thus serving as a logical explanation of the severe
inhibition to nitrification in soil treated with massive concentra-
tions of the insecticide.

This same investigator (33, pp.1-53) has recently shown that
a soil can be saturated with respect to a physiological type of
microorganism, and it then may be considered as an isolated enzyme
system. He has conclusively shown that nitrification within soil
takes place at the base exchange complex; therefore, if a dilute
solution of ammonium chloride is perfused through a soil in such a
manner as to prevent water logging and thus insure adequate aeration,
ammonium ions will become adsorbed at the base exchange and become
the site of Nitrosomonas* proliferation. There are few if any viable

* A physiological genus of bacteria autotrophically oxidizing
NH_4^+ to NO_2^-.

nitrifying organisms present in the perfusate. Thus, further elaborating his hypothesis that the site of nitrification is at the base exchange complex.

If enough ammonium ion is passed through the soil to saturate the base exchange, then the numbers of <u>Nitrosomonas</u> species will proliferate at a constant rate, shown by Quastel to be a linear function. Hence, the oxygen uptake of equal quantities of soil should be the same. This was found to be the case. However, when aldrin is added to the soil at a concentration of 1,000 p.p.m., perfused with 0.01 M NH_4Cl for approximately six days, the oxygen uptake determined by Warburg techniques, and compared with the control, an obvious stimulation is noted. This response in increased oxygen uptake may either be manifested by a greater number of nitrifying organisms present or in a greater metabolic acticity per cell.

Plate counts made on the perfused soil showed that the molds were completely inhibited in the treated soil; however, very little mold count was noted on the control plates. The significance of this finding is yet to be assessed. An even more pronounced effect was noted in the bacterial counts. Ethyl alcohol used as the aldrin solvent, drastically reduced the numbers of heterotrophic organisms developing on sodium albuminate agar. However, soil treated with solvent plus aldrin exhibited counts similar to the control. This indicates either a direct stimulation due to aldrin on the numbers of microorganisms or an indirect effect due to the presence of aldrin, which acts to nullify the inhibitory response due to the alcohol solvent.

One might speculate as to the reason for the noted stimulation of nitrite production on the basis of inaccuracies inherent in the nitrite analysis. It is obvious from the results that the quantity of nitrite determined is far below theoretical values; however, nitrite concentrations do not normally build up under the conditions of the experiment. It is indeed questionable whether the differences in nitrite production are significant; but in view of the results obtained from the perfusion studies, there is an apparent stimulation due to the presence of the insecticide. In view of the decrease in nitrate formation, it could be assumed that the increased nitrite accumulation resulted from retarded nitrite oxidation. However, in view of the experimental evidence, the build up of nitrite appears to be a stimulation in nitrosofication.

A logical assumption yet remaining in the realm of speculation is the possibility of a general inhibition of the bacterial-feeding protozoa as a result of the insecticide. This would help to explain the stimulation in the total number of microorganisms. Much more experimentation is necessary before valid conclusions along these lines may be drawn. However, this indirect effect remains a possibility.
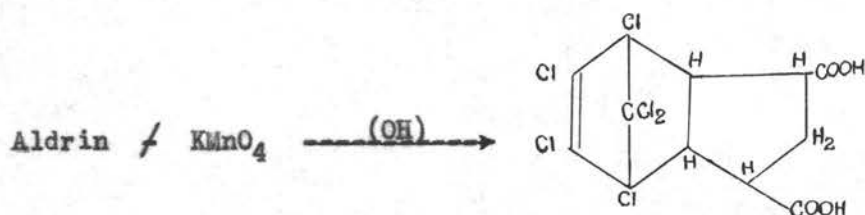
So far as determined, aldrin appears to have no effect upon Azotobacter. In four soils tested by the soil plaque method, there appeared no visible effect upon the numbers of Azotobacter colonies developing on the surface of the insecticide-treated soils.

A chance observation made on Stayton soil being incubated for the study of ammonification revealed an interesting effect of aldrin

upon fortuitously developing seedlings. Apparently the soil had been heavily seeded prior to sampling, for at approximately four days incubation a number of young sprouts were noted in all of the test samples. Striking differences in development of the young plants were apparent in the differently treated samples. There was approximately a two-fold stimulation in the growth of the plants in the samples treated with 200 p.p.m. of the insecticide. There were no differences in the height or the development of plants between controls and the samples treated with 1,000 p.p.m. No attempt was made to determine the actual number or the kinds of plants developing, for all samples were used in the ammonification study analyses.

It has recently been established that massive applications of aldrin will result in enhanced growth and early germination of many field crops (14, p.713; 12, pp.37-39; 13; 15, p.36; 24, p.187; 45, p.811; 47,p.45). This effect would indicate that aldrin acts similarly to a plant growth regulator. In view of the chance observation that aldrin appeared to cause an elongation of Bacillus subtilis cells without subsequent cell division, it is necessary to recognize the possibility of aldrin, or an oxidation product of aldrin, acting as a bacterial growth regulator.

The double bond in the unhalogenated ring of aldrin is readily attacked by mild oxidizing agents. Chromic acid in acetic acid, and potassium permanganate in alkaline solution oxidize the compound to a dicarboxylic acid, 4,5,7,7,8,8a-hexachloro-3a,4,7,7a-tetra-hydro-1,3-dicarboxy-4,7-methanoindane (24,pp.177-178).

Aldrin $\neq$ KMnO$_4$ $\xrightarrow{\text{(OH)}}$ (dicarboxylic acid structure: Cl, Cl, CCl$_2$, Cl, Cl ring system bearing H, COOH, H$_2$, COOH groups)

The dicarboxylic acid resulting from such oxidations bears structural and group relationships to several potent plant hormones. If this reaction were established as normal to aldrin in cultivated soil, it would be a simple matter to explain the increasing degree of inhibition to nitrification due to the presence of correspondingly greater aldrin concentrations. For it would appear that this compound is much more soluble than aldrin due to the carboxyl groups within its structure. This too would rule out the possibility that the effects were caused by the one per cent tolerated impurities common to recrystallized aldrin. As of this writing there is no available information concerning the chemical or physical properties of these impurities.

Aldrin tends to slightly raise the pH of soil to which it has been added. In some cases this rise in pH may be of a magnitude of 0.2 to 0.5 pH units; generally, the increase is 0.1 to 0.2 units. The changes observed are not considered sufficient to reflect any significant change in the nutritional functions of the soil microorganisms. Aldrin at 200 and 1,000 p.p.m. in distilled water has no effect upon the pH.

The gross effect of the insecticide as determined for the ten soils studied is one of slight inhibition (Table 17, p.   ). In but one case did the overall nitrogen transformation appear to be stimulated in the presence of the insecticide. This response was demonstrated in the Deaver School soil which is Willamette silty clay loam.

A correspondingly treated sample designated as Stayton, also Willa-
mette silty clay loam, indicated no effect upon the microbial ac-
tivities of the soil. These samples were unique in this respect
in that all others showed the characteristic slight inhibition.

It was noted that these two soils were the only represen-
tatives of Willamette silty clay loam present in the ten composite
samples. Further correlation of soil type with the degree of effect
is impossible, with the possible exception of a relationship between
a peat and a fine-sand soil. In these two soils, trends in the re-
sults show a more marked effect of the insecticide at both concen-
trations (Table 4, p.26).

These results may be justified, to some degree, on the grounds
that the surface of soil particles determines to some extent the
degree of adsorption, a fine-grained soil may be expected to adsorb
more than a coarse-grained soil. Since adsorption in soils is largely
a function of the silt, clay, and humus, the type of soil does appear
to have an important effect upon the insecticidal as well as the
microbial response to the insecticide.

Results of the field-treated soil studies (Figure 26, p.61)
indicate that aldrin exhibits its effect over a period of several
months. For when these soil samples were incubated with 1,000
p.p.m. peptone nitrogen, ammonification was characteristically stimu-
lated and nitrification, as previously discussed, was severely in-
hibited. It is somewhat paradoxical to note that aldrin applied at
a rate of 25 p.p.m. twelve months prior to sampling maintains the

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characteristic effects to the activities of soil microorganisms. It has been previously shown (24, p.182), and there is little doubt as to the validity of the results, that aldrin does not maintain residual insecticidal characteristics for a period of more than three weeks, and also, considering the results of these experiments (Table 9, p.52), which indicate rather large decreases in aldrin concentration over a period of 20 days. An aldrin analysis by the Koblitsky method shows that residual concentrations of aldrin are apparently the same as application rates. The validity of this analysis could be questioned on the grounds that it is not specific for aldrin. These results indicate that a compound other than aldrin, but resulting from a chemical change in aldrin, is responsible for the noted affects on soil microorganisms.

It was established by the results of the soil extract shake cultures that there was a general acclimitizing of the organisms to the insecticide. The results (Figures 27, 28, and 29, p.68; 30,31,32, p.69) of the ammonification, pH, and turbidimetric analyses of the "treated soil"* extract are essentially the same as its analogue, the "check soil"[X] extract, although the degree of effect in all cases appears to be lessened. There is an apparent adaptation to the insecticide at 1,000 p.p.m., for in each case after 120 hours incubation, the ability of the culture to produce ammonia is considerably enhanced. It is surprizing that concentrations other than 1,000 p.p.m. do not yield this stimulation. Here

* Soil treated with 25 p.p.m. aldrin twelve months prior to sampling (Table 11, p.57).

[X] Soil containing no aldrin (Table 10, p.56).

again it is important not to preclude the possibility that a compound other than aldrin is exerting the effect. This compound may be the result of a decomposition of the aldrin molecule or may be a result of impurities, the reason being that 1,000 p.p.m. would offer greater possibilities for molecular rearrangement and would subsequently yield larger quantities of the impurities. In any case, it is apparently safe to conclude that certain soil microorganisms will adapt to the presence of even large concentrations of the insecticide. From these results it would appear that the organisms were enzymatically adapting to aldrin as the cells enter the stationary phase of growth. This assumption is based on (1) the inhibition or reduction in growth rate is for the most part temporary and (2) upon further incubation the initial inhibitory response reverses to yield an increase in microbial numbers, and subsequently a stimulation in culture activity. Pinsky and Stokes (31, pp. 345-346), have recently shown that the age of microbial cultures greatly influences enzymatic adaptation, and that in certain enzymatic processes of Escherichia coli and Pseudomonas fluorescens adaptability steadily decreases during the period of active growth and is restored as the cells enter the stationary growth phase. Thuse these authors conclude that adaptation is favored by aging rather than physiological youth.

It must be recognized that cultures containing aldrin, although showing a marked delay in the onset of growth, nevertheless eventually obtain control levels. This is apparent for particular concentrations of aldrin; the threshold concentration in these

experiments appears to be between 200 and 1,000 p.p.m. In a re-
view of the patterns of growth inhibitors by Moore and Boylen
(28, pp. 319-320), these authors demonstrate a similar response of
Escherichia coli to pyridine-3-sulfonic acid and attribute it to
either the existence of a striking "inoculum effect" or as being due
to selection of resistant organisms or to adaptation to resistance.

The effect of aldrin upon ten commonly encountered soil micro-
organisms in pure culture shows no consistancy, although it was
noted that Gram positive bacteria appeared to be more "susceptible"
to the temporary bacteriostatic action of aldrin; while Gram
negative bacteria appear immune to the effects of the compound. This
difference might serve as a premise for future experimentation and
further elaboration as to the modus operandi. The response of the
two Gram positive bacteria, Bacillus subtilis and Bacillus cereus,
was essentially the same, an early initial inhibition to ammoni-
fication, but here again showing the apparent adaptation of the
organisms to 1,000 p.p.m. upon further incubation. Gram negative
Escherichia coli did show some "susceptibility" to the toxic action
of aldrin, but when compared with the affects noted upon the Gram
positive organisms, its toxicity appeared almost non-existant.

After noting the obvious effects of the compound upon re-
production and ammonification in treated Streptomyces griseus cul-
tures, it was assumed that the response to Streptomyces coelicolor
would be of a similar nature; however, this was not found to be the
case. While aldrin exhibits an initial, and rather severe, inhibition
to multiplication of S. griseus, S. coelicolor appears to be immune

to any concentration of the compound. This is surprizing in that
there is very little difference in these two organisms, metabolically
or morphologically, which might help explain their widely varied
response. The initial reaction to the three concentrations of the
insecticide by S. griseus is manifested by an obvious inhibition
to ammonification and/or reproduction; however, after 120 hours
the organism apparently has become completely readjusted to its new
environment and exhibits none of its initial inhibitory response.
It is indeed questionable whether the effect of the compound is a
result of an inhibition of extracellular enzyme systems responsible
for ammonification or whether it is the result of an actual inhi-
bition, of the vital processes necessary for cellular reproduction.
The latter appears more logical because of the obvious differences
in numbers of cells early in the incubation period, the treated
cultures showing far fewer cells than the control cultures.

The effect of aldrin on four commonly encountered molds shows
no consistancy in their response. Multiplication by Aspergillus
niger, Penicillium Sp., and Mucor Sp. is inhibited by the compound,
while Trichoderma Sp. is completely indifferent. It would be in-
teresting to determine the lipid content of microorganisms suscep-
tible to aldrin and compare them with those microorganisms showing
no response to the compound. In all cases when aldrin is shown to
exert an affect it appears to be the result of a prolongation of the
period of adjustment and the lag phase of growth. This is shown in
the results of carbon dioxide evolution from soil extracts in peptone
solution treated with aldrin. Although the period of adjustment,

the lag phase, is prolonged, the cultures show a stimulation in the logarithmic phase of growth and then returns to control levels in the stationary and death phases.

III CONCLUSIONS

At rates of as high as 1,000 p.p.m., excessively high from the standpoint of recommended field applications, aldrin at most was only slightly inhibitory to the over-all development of micro-organisms in the soil. Different organisms were found to respond differently to aldrin. The degree and duration of the response, whether stimulation or inhibition, varied with the specific organ-ism, the concentration of aldrin, and environmental factors. Even at rates of 200 p.p.m., 400 pounds per acre, which is approximately 200 times normal field application, it is thus apparent that while certain bacteria may be stimulated and others retarded there is little evidence that aldrin will so effect microorganisms as to cause any significant change in soil fertility. Therefore, it is doubt-ful that recommended concentrations for insect control would ad-versely influence the soil microorganisms to adegree that would result in a detectable change in soil fertility. It is doubt-ful whether residual concentrations would accumulate even after successive reapplication to manifest any significant change in their over-all development.

TABLE 17

Total Nitrogen Transformation from Ten Laboratory-Treated Soils

Treatment	Total N	ppm $NH_4(N)$	ppm NO_2 (N)	ppm NO_3 (N)
Control (Soil ≠ 1,000 ppm pep N--soil only)	15,140.5	13,510.8	259.1	1,488.5
≠ 200 ppm Aldrin (Soil ≠ 1,000 ppm pep N ≠ 200 ppm aldrin--soil ≠ 200 ppm Aldrin)	14,469.2	12,923.0	295.2	1,299.5
≠ 1,000 ppm Aldrin (Soil ≠ 1,000 ppm pep N ≠ 1,000 ppm aldrin-- soil ≠ 1,000 ppm aldrin)	13,878.6	12,629.0	308.0	1,068.4

Differences from Control

200 ppm Aldrin	- 671.3	- 587.8	≠ 36.1	-189.0
1,000 ppm Aldrin	-1,261.9	- 881.8	≠ 48.9	-420.1

SUMMARY

. Aldrin at concentrations of 200 p.p.m. (0.02 per cent) and 1,000 p.p.m. (0.1 per cent) in 12 different soils had a stimulatory influence upon the total numbers of microorganisms as determined by plate counts. This influence was apparent after 5 days and continued over observations periods of 10 to 20 days in laboratory studies. The effect was observed also after 12 months on field soils. No injurious effect on the general heterotrophic microflora was observed for either concentration of the insecticide.

Aldrin has a slight inhibitory effect upon the overall nitrogen transformation by soil microorganisms at each concentration. However, this inhibition is not great and appears to be of a short duration. Ammonification is enhanced at an early stage when aldrin is present in the soil. This stimulation reverses with a slight inhibition upon further incubation, and then returns to control levels. Nitrosofying microorganisms are apparently slightly stimulated at each concentration, while the nitrifying organisms are moderately inhibited. No injurious effects to development of Azotobacter on soil plaques were noted. Carbon dioxide production was slightly stimulated at each concentration.

Although the residual insecticidal effectiveness of aldrin appears to be short lived, there is no apparent microbial breakdown of aldrin in soil. Soil microorganisms adapt to even massive concentrations in the soil and in pure culture.

The response of ten commonly encountered soil microorganisms in pure culture demonstrate no degree of consistancy. The effect was an initial bacteriostasis on Bacillus subtilis, Bacillus cereus, Streptomyces griseus, Aspergillus niger, Penicillium sp., and Mucor sp. Gram negative bacteria appeared to be immune to the compound at each of the three test concentrations, 20, 200, and 1,000 p.p.m.

Carbon dioxide production from similarly treated soil extracts was initially inhibited, then characteristically stimulated after further incubation.

It is suggested that the response of soil microorganisms to aldrin, whether inhibitory or stimulatory, is a function of the degree of intimacy of contact between the cell wall and the aldrin particle, the age of the culture, the rate of diffusion into the cell, the size of inoculum, and the concentration of insecticide. Several theories designed to explain the response are presented and discussed.

Although aldrin applied at the massive concentrations characteristic of these experiments does show an inhibition to the overall nitrogen transformations in soil and appears to exert an inhibitory response by certain specific microorganisms of the soil in pure culture, it appears safe to conclude that when applied at recommended rates, aldrin will not adversely affect the general microflora of the soil nor will it affect any assessable change in soil fertility.

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