EFFECT OF CERTAIN FUMIGANTS ON NITRIFICATION AND OTHER SOIL MICROBIAL ACTIVITIES

by

PEDRO DANTE ROA

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1959

APPROVED:

[redacted]

Professor of Bacteriology

In Charge of Major

[redacted]

Chairman of Bacteriology Department

[redacted]

Chairman of School Graduate Committee

[redacted]

Dean of Graduate School

Date thesis is presented Feb. 23, 1959

Typed by Clistie F. Stoddard

ACKNOWLEDGEMENTS

To Dr. Walter B. Bollen, who as a professor made this work possible, and who as a friend gave me the counsel, encouragement and unlimited help.

To Dr. Thomas Aspitarte, Dr. K. C. Lu, and Dr. C. M. Gilmour for their helpful advice.

To Mr. William Wu, Mrs. Martha Porter, Mrs. Laura Leach, and Mrs. Vera Evenson for their assistance.

To my parents and friends who had confidence in me.

TABLE OF CONTENTS

TABLE OF CONTENTS (contd)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (contd)

EFFECT OF CERTAIN FUMIGANTS ON NITRIFICATION AND OTHER SOIL MICROBIAL ACTIVITIES

INTRODUCTION

Farmers and scientists have for centuries been concerned in the improvement of soil fertility. It was not until the eighteenth century that they became aware that microorganisms in the soil played one of the most important roles in plant growth. It was later learned that the soil is a living and dynamic system teeming with the activity of enormous numbers of organisms which include bacteria, actinomycetes, fungi, yeasts, algae, protozoa, nematodes, myxomycetes, bacteriophage, viruses, mites, insects, and worms. The microorganisms aside from being the most numerous are probably the most important component of the soil population. Crop production is dependent upon the activities of soil microorganisms in decomposing organic matter and releasing its elements in forms available as plant food. A gram of fertile soil contains from 100 million to several billion microorganisms. These vary greatly in form, food requirements and resistance to adverse conditions. Each kind produces chemical changes which influence the development of all other organisms. Many of their activities are essential to the development of higher plants and animals, and they are instrumental in the production of soil fertility.

Certain organisms on the other hand are parasitic or harmful to plant growth. When these forms become established in the soil, it is necessary to plant crops which are resistant to the pathogens or other pests involved or to treat the soil with a material which

will destroy them. A method which is becoming increasingly important as a means of controlling undesirable organisms in the soil is fumigation. A soil fumigant is an organic material, either a liquid of high vapor pressure which may or may not have a low boiling point, or a liquid adsorbed on some inert powder such as bentonite clay or talc which acts as a carrier. Generally fumigant liquids when exposed soon evaporate or lose their potency unless kept in tightly closed containers. The speed of evaporization or volatilization is determined by the character of the chemical and the temperature. Dependent on the fumigant used the treated soil is left uncovered or covered with a seal that is gas tight. The soil pests are killed presumably by action of the fumigant on the protoplasm of the cells. Although fumigants are applied to destroy phytopathogenic organisms, its effects are not necessarily limited to these undesirable forms. Many non-parasitic and beneficial forms may also be affected and useful microbial activities thus inhibited.

Brief Description of Microbial Activities

The importance of beneficial microbial activities in the soil may be briefly outlined as follows:

1. Carbon dioxide evolution - The rate of release of carbon dioxide due to the decomposition of organic residues in the soil is a reliable index of active microbial physiological activity. In this case the bacteria, actinomycetes and fungi act as agents of decay; collectively they are indispensable in the mineralization of plant and animal residues. In the atmosphere above the surface of an

acre there is approximately twenty tons of carbon dioxide. The living organisms in an acre of fertile soil return much of this carbon dioxide to the atmosphere. In the soil the carbon dioxide has important solvent action on minerals, releasing soluble phosphate, potash and other plant nutrients. By this we can evaluate the importance of microbes in the carbon cycle of nature. For the soil microbiologist this phase of the study assumes prime importance because there has been observed a close correlation between rate of carbon dioxide evolution and degree of fertility.

2. Ammonification - This process is carried out by a variety of microorganisms called ammonifiers. The ammonifiers comprise most of the heterotrophic bacteria and fungi that live in the soil. They decompose soil organic matter in order to obtain energy for growth and in the process they liberate ammonia from proteinaceous materials. Some of this ammonia they themselves use but the major portion is set free as a by-product which is rapidly assimilated by plants or transformed to nitrate.

3. Nitrification - This is the microbial transformation of ammonia to nitrite and then to nitrate. Nitrification is regarded by some investigators as non-essential and perhaps a harmful stage of the nitrogen cycle because nitrates are subject to loss by leaching and denitrification. The organisms are specific autotrophic bacteria that oxidize ammonia to nitrite (nitrosofication) and nitrite to nitrate (nitrification) to obtain energy for autotrophic fixation of carbon dioxide. Ammonia and nitrates are the

main sources of nitrogen for the plant and hence do not accumulate in the presence of an actively growing crop. Losses or absence of these forms of nitrogen retards plant growth.

4. Denitrification - This is an undesirable microbial activity in the soil. Microbial reduction of nitrates is a property possessed by many facultative aerobic bacteria. Physiologically it enables them to grow in the absence of free oxygen by utilizing nitrates as an alternative agent for oxidizing carbohydrate or other substrates to obtain energy, nitrous oxide and free nitrogen being by-products. Both are easily lost to the atmosphere and become unavailable to plants. Denitrifying organisms can be responsible for economic losses to agriculture whenever soil aeration becomes poor and prevention of their activity is desirable. In very well aerated soils, nitrogen losses caused by denitrifying bacteria are minimum.

5. Nitrogen fixation - The nitrogen fixing organisms have been the center of much attention. Their function is so useful that many attempts have been made to keep them alive and increase their numbers in the soil. Except for inoculation with the symbiotic legume bacteria, most of these attempts have been unsuccessful. These nitrogen fixing organisms are divided into three main groups:

I. The aerobes
II. The anaerobes
III. The symbiotic organisms

Biological fixation of nitrogen is closely bound up with carbon metabolism and growth. Fixation of nitrogen by Azotobacter

chroococcum and possibly by some other aerobic organisms may be limited under average conditions since these organisms are more sensitive to acidity and fix little nitrogen in presence of available nitrogen compounds. The anaerobic Clostridium butyricum is less sensitive to acidity, and will fix gaseous nitrogen even in the presence of considerable available nitrogen. The third type of nitrogen fixation is carried out by a highly developed symbiotic process. This symbiosis involves higher plants, especially the legumes such as clover and peas inoculated by species of Rhizobium.

6. Sulfur oxidation - Microbial transformations involving sulfur are largely oxidations and reductions of inorganic forms and synthesis and decomposition of organic compounds containing sulfur. Autotrophes of the genus Thiobacillus oxidize elemental sulfur and sulfides to sulfates. Waksman (45, p. 538-540) stated that the acid produced from the oxidation of elementary sulfur can be utilized as:

a) Solvent for such difficultly soluble minerals as phosphorus in rock phosphate, and potassium in glauconate or greensand marl.

b) For the neutralization of excess base in alkaline soils.

c) For the control of certain plant diseases.

The plant requirements for sulfur varies. Leguminous plants, onions and cabbage have relatively high sulfur requirements. Sulfur deficiency is often the cause when crop plants show a yellowish color in their leaves, although lack of nitrogen may cause similar symptoms.

Since most of the sulfur is found in organic form, soils high in organic matter are usually high in sulfur. Sulfates are readily leached; therefore sulfur bearing fertilizers or elemental sulfur are often required on humid soils. Since sulfur depletion affects soil fertility, the importance of the sulfur bacteria cannot be underestimated.

In order to assure a maximum rendering of these functions the organisms must have an environment which will enable them to grow and to multiply; their growth depending on such soil conditions as moisture, pH, temperature, aeration and other factors which at any time may assume major importance.

LITERATURE REVIEW

For centuries a farmer's only recourse against soil pests was to refrain from planting the affected crop in the infested field either temporarily or permanently. This led to various plans for crop rotation which often proved to be no solution to the problem. The cry for a chemical which would take care of these pests was obvious. Preliminary experiments showed that partial sterilization of soil by fumigants or other chemical brought about destruction of undesirable organisms, but their effects for unknown reasons were limited. Later soil scientists found that mere application of the chemical into the soil without regard to physical and chemical factors was not enough. Christie (10, vol. 170, p. 170-189) recommended that the soil be moderately loose and reasonable free from clods, lumps and undecomposed crop residues. Stark and Lear (39, vol. 37, p. 698-711) found that four factors appeared to be of primary importance in determining the relative persistency of phytotoxic concentrations of various fumigants in the soil:

1. The vapor pressure of the fumigants.
2. The amount of fumigant applied.
3. The concentration that plants are able to tolerate.
4. The concentration that would be effective on the pathogens.

The vapor pressures of fumigants are in general negatively correlated with their boiling points, and the boiling points can be used as a good criteria for judging volatility and the rapidity with which fumigants will escape from the soil. The lower the total

dosage of the fumigant employed and the smaller the amount applied per injection the sooner a subtoxic concentration to plants is reached.

In their effort to find a fumigant or chemical which could get rid of pests and disease causing organisms an array of compounds began to appear on the market as herbicides, insecticides, and fumigants. Whatever the name, their application into the soil was reason for concern. Soil microbiologists began to study these chemicals in the light of their effects to soil microbial activities. Nelson (28, vol. 68, p. 221-237) found that the use of sodium chlorate did not interfere with the growth of common soil organisms. Dalton and Hurtwitz (11, vol. 66, p. 233-238) determined under controlled conditions that plate counts of soil treated with chloroform and hydrogen cyanide were not sterilized after seven days. Dransfield (12, vol. 40, p. 165) applied 5% tetrachlronitrobenzene at 1/2 oz./ sq.yd. against Botrytis cinerea on lettuce and found no detrimental effect on the general soil microflora.

Bactericidal effects of certain herbicides applied to arable soils may be as significant as their herbicidal effects. Teater, Mortesen and Pratt (43) showed that normal field rates of alpha-chloro-N-N-diethylacetamide, 2-chloroallyl diethyldithiocarbamate, isopropyl-N-(3-chlorophenyl) carbamate and an amine salt of 2,4-D had little effect on nitrification and carbon dioxide evolution; higher rates of these compounds inhibited nitrification but increased carbon dioxide evolution from the soil. Newman (29, vol. 6,

p. 352) concluded from his experiments that normal rates of herbicides are non-injurious to soil microorganisms. Bollen, Morrison and Crowell (6, vol. 47, p. 302-306) found that no immediate effect on soil microorganisms were caused by aldrin and certain other insecticides as determined on numbers of bacteria, streptomycetes and fungi. Eno and Everett (13, vol. 22, p. 235-238) using heptachlor, chlordane, metoxychlor, lindane, aldrin, toxaphene, tetrachlorodiphenylethane, DDT and benzene hexachloride found no significant changes in numbers of fungi; dieldrin which showed some stimulation of fungi produced no significant changes in bacterial numbers. Heptachlor, lindane and benzene hexachloride caused decrease in nitrification rates. Smith, Dawson and Wenzel (36, vol. 10, p. 197-201) in studying the effects of herbicides on microorganisms found no consistent results. Gamble, Mayhew and Chappell (15, vol. 74, p. 347-350) reported reduction in the respiration rate and plate counts on soils three months after treatment with Dow dinitro-O-secondary butyl phenol and Penn Salt N-P-128. Hauke-Pacewiczowa (18, vol. 76A, p. 640-657) and Wilson and Choudri (48, vol. 77, p. 25-32) working with benzene hexachloride found no significant effects on microbial population when used at field rates. Wilson and Choudri (47, vol. 39, p. 537-539), Ross (32, vol. 11, p. 58-61) and Jones (20, p. 58-59) found that DDT effects did not disturb microbial activities such as nitrification and ammonification. In some cases stimulatory conditions resulted. Jones showed that benzene hexachloride produced some degree of toxicity when concentrations much above the

normal field applications were used; however, this toxicity was not evidenced against nitrogen fixing organisms. Bollen, Morrison and Crowell (6, vol. 47, p. 307-312) found no appreciable changes in nitrogen transformations and soil respiration when BHC was applied at field rates. MacLeod and Howatt (24, vol. 11, p. 60-61) using strong doses of mercuric and mercurous chloride for efficient control of common potato scab and black scurf found that these chemicals did not affect the useful activities of certain nitrogen fixing organisms.

The discovery that soil could be successfully fumigated with volatile chemicals to eliminate some of the pests has increasingly aroused widespread interest both in the development of inexpensive fumigants and in the most efficient methods of their application. As the cost of treatment has been reduced the areas of usefulness have greatly enlarged. The problem, however, has been to find a fumigant that is cheap and not too specific for organisms against which it is effective. There have been numerous reports on comparative testing of methods of application of fumigants, of factors affecting their efficiency and dosages rates in relation to various soil conditions, and on limitations in respect to organisms against which they are effective. With these ideas in mind the quest for improved soil fumigants has continued.

In spite of the large number of fumigants which were put on the market, chloropicrin and carbon disulfide became the most widely used volatile chemicals for soil fumigation. Mathews in 1919 was

probably the first to report the use of chloropicrin for nematode control. After this a period of 10 to 15 years elapsed before much additional experimental work was concluded. In 1932 Johnson (19) and Godfrey (16, vol. 26, p. 246-256) reported successful use of chloropicrin for nematode control on pineapple at a rate of 163 pounds/A in holes 5 to 6 inches deep and 18 inches apart.

Ever since the work of Johnson chloropicrin has become, for the lack of a better one, a yardstick by which other fumigants are measured. This led soil investigators to a number of studies in relation to its effects on soil microbial activities. Martin and Aldrich (26, vol. 16, p. 201-203) and Klemmer (22, vol. 57, p. 12) found that chloropicrin exerted an initial depressive effect on bacterial population to be later followed by a large increase. Studies by Stark (37) revealed that chloropicrin diffusion in the soil is dependent upon the size of the clay fraction. Further work by McClellan, Christie and Horn (23, vol. 37, p. 440) and Stark (37) showed that chloropicrin diffusion in the soil proceeded more rapidly at increasing temperatures and that the chemical was retained for longer periods when the soil was wet, around 50 to 60 per cent saturation; this last point seems not to be in agreement with Godfrey (16, vol. 26, p. 246-256) who stated that chloropicrin to be effective did not require wetting of the soil. Nitrogen utilization investigations by Stark, Smith and Howard (38, vol. 48, p. 433-442) and Tam (42, vol. 59, p. 191-205) showed that increasing dosages of chloropicrin increased the interval before active nitrification is resumed.

Ammonification is not retarded, probably because many of the ammonifying bacteria are sporeformers and are thus difficult to kill.

Up to this point an attempt has been made to briefly outline and describe the effects on soil fertility of those chemicals which were early introduced in order to eliminate pests and disease-causing organisms. Many studies were undertaken to evaluate their effectiveness and usefulness. In the past few years several new fumigants have appeared on the market. They definitely were an improvement over the early ones. Their rapid acceptance by agriculturists have once more made soil microbiologists wonder as to their effect upon microbial activities.

D-D Mixture first appeared in the summer of 1942. This fumigant began to displace chloropicrin which is more costly, extremely unpleasant in its lachrymal effects and requires ground cover to render it efficient. Miller (27, vol. 11, p. 53) and Carter (7, vol. 97, p. 383-384) demonstrated the value of soil fumigation with D-D Mixture to control parasitic nematodes in strawberry plantings on heavy soil. Martin (25, vol. 16, p. 107-122), in a greenhouse study, reported D-D Mixture markedly affected the population of fungi in the soil; although initially destroyed or reduced to very low numbers they later re-established themselves. He also found little correlation between total numbers of bacteria and fungi in treated soils. Kincaid and Volk (21, p. 1-24) reported higher ammonia nitrogen yields and lower nitrate nitrogen recoveries in soils treated with D-D Mixture. The tobacco crop with which they were working appeared

much better when treated with D-D Mixture. Fenwick et al. (14, vol. 40, p. 208-214) obtained higher yields of potatoes when the fumigant was used against potato-root eelworm. Carter (8, vol. 38, p. 35-44) studied the toxicity of the fractions of D-D Mixture; the fraction with the higher boiling point, 1:3 dichloropropene, proved to be the most toxic but when in mixture with the other fraction, propylene dichloride, the toxicity increased.

Some of the most recent fumigants are Vapam, Nemagon and Telone. Farmers are using them because of their powerful nematocidal effects. Baines (4, vol. 42, p. 192) stated that citrus nematodes were effectively controlled when 274 to 500 pounds of Vapam per acre were applied in 6 to 12 surface inches of water in basins. The low doses (274 lbs/A) were effective in sandy loams and the high doses (500 lbs/A) on loam soils. The treatments also decreased the number of non-parasitic fungi in the soil. Because of the newness of these fumigants very little is known about their effects on soil microbial activities. Conscious of these facts and considering the important implications of these investigations the Bacteriology Department undertook the laboratory studies in cooperation with the Entomology Department of the Oregon Agricultural Experiment Station which conducted field experiments.

PURPOSE OF THE INVESTIGATION

The objectives of the present investigation are as follows:

1. To determine, describe and assess any effects on the soil microbial population that might occur as result of fumigation.

2. To determine fumigant effects on specific important microbial activities, such as:

   a. Carbon dioxide evolution
   b. Ammonification
   c. Nitrification
   d. Sulfur oxidation

3. To determine the direct effect of different fumigant concentrations on specific representative soil microorganisms considered important in soil fertility.

PLAN OF STUDY

1. Effects on microbial population
   Determine by plate counts total numbers of bacteria, actinomycetes and fungi in soils treated in the laboratory at 1, 2, 5, 10 and 30 days.

2. Effects on microbial activities
   a) Soil respiration
      - At 1, 2, 4, 7, 14, 30 and 60 days
      - Treatments:
        Soil alone
        Soil + organic matter at 2000 ppm C.
        Soil + fumigant at different rates*
        Soil + fumigant + organic matter at 2000 ppm C.

   b) Ammonification
      - At 3 and 7 days
      - Treatments:
        Soil alone
        Soil + peptone at 1000 ppm N
        Soil + fumigant at various rates*
        Soil + fumigant + peptone at 1000 ppm N

   c) Nitrification
      - At 14 and 28 days
      - Treatments:
        Soil alone
        Soil + $NH_4/2SO_4$ @ 300 ppm N + $CaCO_3$ @ 5 T/A**
        Soil + fumigant at different rates*
        Soil + fumigant + $NH_4/2SO_4$ + $CaCO_3$

   d) Sulfur oxidation
      - At 30 days
      - Treatments:
        Soil alone
        Soil + sulfur @ 1000 ppm
        Soil + fumigant at various rates*
        Soil + fumigant + sulfur @ 1000 ppm

3. Zones of microbial inhibition

4. Fumigants as carbon source

---

* Fumigants rates used are outlined in Table 2, page 23.

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

CHARACTERISTICS OF THE FUMIGANTS USED

Fumigants like Vapam, D-D Mixture, Nemagon and Telone are presumed to act only on animal protoplasm, specifically that of nematodes.

Vapam - This is a water soluble fumigant very effective against weed seeds, nematodes and fungi. Promising results have also been observed when applied for control of several species of soil infesting arthropods, the garden centipedes and the bulb mite. Vapam has low toxicity to man and animals but is highly toxic to plants in the soil. Vapam decomposes in damp soil giving a gas which dissipates within a few days. It is introduced into the soil through irrigation equipment, to the plow sole, or to the ground surface in connection with the use of a rototiller. Vapam, a product of the Stauffer Chemical Company, is sodium N-methyl dithiocarbamate.

D-D Mixture - This is a soil fumigant produced by the Shell Chemical Corporation. It is a black, volatile liquid consisting mainly of 1:3 dichloropropene and 1:2 dichloropropane with traces of higher chlorides. It is only slightly soluble in water, but easily emulsified. It is inflammable. D-D Mixture is highly toxic to eelworms and nematodes. This fumigant is relatively easy to handle since its fumes are less irritating and less volatile than those of chloropicrin. Another significant advantage is that it does not require a surface seal when applied to the soil.

Nemagon - This is another product of the Shell Chemical Corporation. Nemagon soil fumigant is much heavier than D-D Mixture

and has a higher boiling point. The vapor pressure of D-D Mixture is 30 times higher than that of Nemagon. For these reasons D-D Mixture diffuses much faster than Nemagon and also leaves the soil quicker, while Nemagon remains in the soil for a longer period thus increasing its killing effect. This fumigant contains 67 per cent by weight of 1, 2 dibromo 3 chloropropane and 33 per cent of other halogenated C_3 compounds. It is a nematocide. It is relatively non-toxic to humans, although proper precautions must be observed when handling it.

Telone - This fumigant is being offered by the Dow Chemical Company. Telone is a water white to amber colored liquid with a sweetish odor. Chemically it is composed of undiluted technical dichloropropenes. It is an excellent nematocide. It could cause severe bodily damage to persons who do not handle it carefully.

PART I. CHEHALIS SILTY CLAY LOAM SOIL

The soil used was Chehalis silty clay loam obtained from the Entomology fumigation control plots on the Vegetable crops farm, Oregon Agricultural Experiment Station, Corvallis, Oregon. A bulk sample was air dried, passed through a 10-mesh screen and was stored in a covered 30-gallon galvanized container for future use as required.

Methods for Chemical and Microbial Analysis

Moisture was determined as loss of weight by drying samples at 105° C. for 24 hours.

Water holding capacity was calculated from the amount of water retained by soil samples in Gooch crucibles wetted from below by immersion and then allowed to drain to constant weight in a moisture saturated atmosphere.

For pH determinations a soil water ratio of 1:5 was used. Ten grams of soil, oven dry basis, were made up to this ratio with distilled water and shaken for two minutes to obtain a uniform suspension. Readings were made with a Model N Beckman glass electrode pH meter.

The following tests were made by the Soil Testing Laboratory, using the methods indicated:

a. Lime requirement, by the Woodruff method (49, vol. 66, p. 53-64).
b. Phosphorus, by sodium bicarbonate extraction (Olsen et al., 31).

c. Exchangeable potassium, calcium and magnesium, determined by flame photometry on an ammonium acetate extract (Schollenberger and Simon, 33, vol. 59, p. 13-14).

The nitrogen determinations on the soil included Kjeldahl, ammonium nitrogen, nitrite nitrogen and nitrate nitrogen. The following procedures were used:

1. Kjeldahl, by the AOAC Gunning method (2, p. 30), using Hibbard's mixture (Na_2SO_4- 20 parts, $FeSO_4$- 2 parts and $CuSO_4$- 1 part).

2. NH_4^+, determined by the modified method of Shrikhande (35, vol. 13, p. 187-188), which is described under Ammonification on page 53.

3. Nitrites and nitrates extracted with a 1:5 soil:water dilution were determined colorimetrically with appropriate filters in a Klett-Summerson photoelectric instrument, using the alpha naphthalamine-sulfanilic acid method and the phenoldisulfonic acid method, respectively.

Sulfate sulfur was determined by a turbidimetric procedure developed by Schreiner and Failyer (34), but modified by use of the Klett-Summerson photocolorimeter with a red filter (700 mu).

Total carbon was obtained by combustion using the AOAC method (2).

The microbial analyses were made by plate counts. Peptone glucose acid agar (pH 4.0) was used for fungi, using dilutions of 1:500 and 1:5,000; sodium albuminate agar was employed with dilutions of 1:50,000 and 1:500,000 for bacteria and actinomycetes (Waksman and Fred, 46, vol. 14, p. 27-28). Triplicate plates for each dilution

were poured. Table 1, page 21, presents the chemical and microbial analyses of the original soil sample.

Table 1. Analysis of Chehalis Silty Clay Loam Soil*

Chemical analysis:	
Moisture capacity, per cent	57.1
Moisture content, per cent	8.7
pH	6.35
**Lime requirement, tons per acre	1.5
**Exchangeable cations:	
Bicarbonate soluble phosphorus, ppm	22.3
Potassium, me/100 grams	0.31
Calcium, me/100 grams	12.65
Magnesium, me/100 grams	5.59
Nitrogen:	
Kjeldahl, per cent	0.13
Ammonium	trace
Nitrite	trace
Nitrate	12.0
Sulfur as sulfate	trace
Total carbon, per cent	1.63
Carbon:nitrogen ratio	12.0
Microbial analysis:	
Fungi, per gram	28,000
Total bacteria, per gram	4,920,000
Actinomycetes, per cent	30.1

* Data expressed on oven-dry basis.

** Determined by the Soil Testing Laboratory.

PART II. MICROBIAL POPULATION STUDIES

Experimental Methods

A preliminary experiment was made to obtain a general indication of the effect of fumigants on soil microorganisms under optimum conditions in the laboratory. The fumigants tested were Vapam, D-D Mixture, Nemagon and Telone. Each compound was applied at their respective field rates, Table 2, page 23. In some cases weaker or stronger fumigant concentrations were used to determine whether these changes would make significant differences on microbial counts. Fumigants were diluted to the desired concentrations in sterile 99 ml water blanks. To 80 grams of soil, dry-basis, placed in pint milk bottles, calculated amounts of fumigant were added to amounts of distilled water required to bring the soil moisture to 60 per cent of the water holding capacity. Five bottles of each treatment and controls were prepared. The bottles were closed with paper milk caps which were punctured with a 7 mm diameter hole in the center to allow aeration, then weighed and the weight recorded for future reference. All samples were incubated at 28° C. Losses in weight due to evaporation were restored by adding water at approximately five-day intervals. At the end of 1, 2, 5, 10 and 30 days the soil in one bottle of each treatment was diluted 1:5 with sterile water. The bottles were sealed tightly with rubber stoppers and placed on an automatic shaker for approximately 10 minutes. In each case a one ml aliquot of the suspension was transferred to a 99 ml water blank to give a

Table 2. Fumigants and Rates Used

Treatment	Field rates Gallons/A*	Additional rates in laboratory Gallons/A*
Vapam	20.0	2.0 200.0
D-D Mixture	25.0 38.0 40.0 41.0	400.0
Nemagon	4.4	44.0
Telone	36.0	3.6 360.0

* Rates calculated on the basis of one acre 6-2/3 inches equivalent to 2,000,000 pounds of soil.

1/500 dilution. These were shaken for two minutes, after which a one ml aliquot was transferred to another 99 ml sterile water blank. In doing so a 1/50,000 dilution was obtained. Based on previous experiences dilutions of 1/500 and 1/5,000 proved to be adequate for fungi counts while dilutions of 1/50,000 and 1/500,000 gave satisfactory results for total colony counts of bacteria and actinomycetes. Plates were incubated at 28° C. for 2 to 5 days for fungi and 7 to 10 days for bacteria and actinomycetes.

In order to assure accurate counting, only those plates which offered numerous but clear and well separated colonies were considered. Counts were made with the aid of a Quebec colony counter.

Results and Discussion

The results are expressed in Tables 3 and 3a, pages 25 and 26 respectively, and in Figures as follows:

Vapam, Figures 1a, 1b and 1c, pages 27 and 28.

D-D Mixture, Figures 2a, 2b and 2c, pages 29 and 30.

Nemagon, Figures 3a, 3b and 3c, pages 31 and 32.

Telone, Figures 4a, 4b and 4c, pages 33 and 34.

The effect of Vapam at field rate, 20 gal/A, upon the general microbial population appeared stimulatory for the first 10 days as evidenced in bacterial and actinomycetes counts. Number of fungi were affected in the same way, the only difference being in that the stimulation effect started three or four days earlier. In both cases these increases were followed by a depression which continued from ten to thirty days. Studies beyond this time were not made. The increasing trends observed in the early days were probably due to a stimulatory effect of the gas as it diffused in the moistened soil, which at this time appeared in non-toxic concentrations; later, further vaporization and greater accumulation of the Vapam gas rendered it toxic, thereby causing a decrease in microbial numbers.

D-D Mixture results revealed that the changes occurring with different rates were not significant. Early apparent depressive effects assume no importance because similar reactions occurred with treated and untreated samples. After a 10-day period soils exposed to D-D Mixture at 41 gal/A showed a definite increase in total

Table 3. Changes in Numbers of Bacteria, Actinomycetes and Fungi in Chehalis Silty Clay Loam Soil Treated with Various Fumigants at Different Field Rates

NEMAGON

Days	Total bacterial counts 1 x 10^6/gram			Actinomycetes 1 x 10^6/gram			Fungi 1 x 10^4/gram		
	Control	4.4gal/A	44gal/A	Control %	4.4gal/A %	44gal/A %	Control	4.4gal/A	44gal/A
1	2.3	2.5	2.4	37.8	36.7	37.5	1.4	1.6	1.8
2	3.6	2.6	2.7	29.7	33.3	40.7	2.7	2.0	2.2
5	2.3	2.5	2.6	39.1	36.7	34.6	2.2	1.4	1.6
10	2.4	2.0	2.3	40.4	45.0	45.7	1.2	1.2	1.8
30	1.9	1.2	2.0	27.0	41.7	25.6	1.7	1.3	1.3

D-D MIXTURE

Days	Total bacterial counts 1 x 10^6/gram			Actinomycetes 1 x 10^6/gram			Fungi 1 x 10^4/gram		
	Control	25gal/A	41gal/A	Control %	25gal/A %	41gal/A %	Control	25gal/A	41gal/A
1	3.8	3.6	4.5	31.6	29.6	29.2	3.8	1.9	2.8
2	2.8	2.6	2.6	32.1	41.2	47.1	3.8	3.2	3.0
5	3.1	2.3	3.1	41.9	46.7	37.7	1.6	2.0	0.9
10	2.4	3.0	2.6	50.0	48.3	42.3	1.6	2.0	0.8
30	2.7	2.4	3.6	40.7	39.6	36.6	4.0	2.6	3.1

TELONE

Days	Total bacterial counts 1 x 10^6/gram				Actinomycetes 1 x 10^6/gram				Fungi 1 x 10^4/gram			
	Cont'l	3.6gal/A	36gal/A	360gal/A	Cont'l %	3.6gal/A %	36gal/A %	360gal/A %	Cont'l	3.6gal/A	36gal/A	360gal/A
1	6.4	5.8	6.0	5.6	35.9	46.7	42.9	41.4	2.5	2.4	1.7	1.9
2	6.7	6.5	6.0	5.9	41.8	38.8	34.5	39.0	2.2	2.4	2.4	1.9
5	5.1	3.8	3.3	2.4	28.4	39.9	36.4	44.7	3.1	3.1	3.3	1.8
10	4.9	5.7	5.5	6.5	52.0	41.2	48.2	43.8	2.5	2.2	2.0	1.6
30	5.1	5.5	5.4	6.1	48.0	56.4	57.0	50.0	3.8	3.8	3.7	2.9

VAPAM

Days	Total bacterial counts 1 x 10^6/gram		Actinomycetes 1 x 10^6/gram		Fungi 1 x 10^4/gram	
	Control	20gal/A	Control %	20gal/A %	Control	20gal/A
1	7.3	6.2	21.4	30.9	5.0	0.4
2	5.5	6.5	35.5	26.2	9.5	1.4
5	5.2	6.5	32.7	32.3	26.0	30.5
10	5.6	7.2	28.6	33.3	8.4	3.5
30	4.7	4.1	43.6	43.2	3.5	1.5

Table 3a. pH Changes in Chehalis Silty Clay Loam Soil Treated with Various Fumigants at Different Rates

	D-D MIXTURE			VAPAM		NEMAGON			TELONE			
Days	Cont'l	25gal/A	41gal/A	Cont'l	20gal/A	Cont'l	4.4gal/A	44gal/A	Cont'l	3.6gal/A	36gal/A	360gal/A
1	6.2	6.2	6.3	6.9	6.2	6.2	6.2	6.2	6.3	6.3	6.2	6.2
2	6.3	6.4	6.3	6.9	6.2	6.2	6.2	6.2	6.2	6.3	6.3	6.3
5	6.3	6.3	6.3	7.0	6.2	6.3	6.1	6.3	6.3	6.2	6.3	6.1
10	6.1	6.1	6.3	6.9	6.2	6.3	6.2	6.4	6.2	6.2	6.3	6.3
30	6.6	6.6	6.3	6.9	6.1	6.5	6.5	6.5	6.6	6.6	6.7	6.5

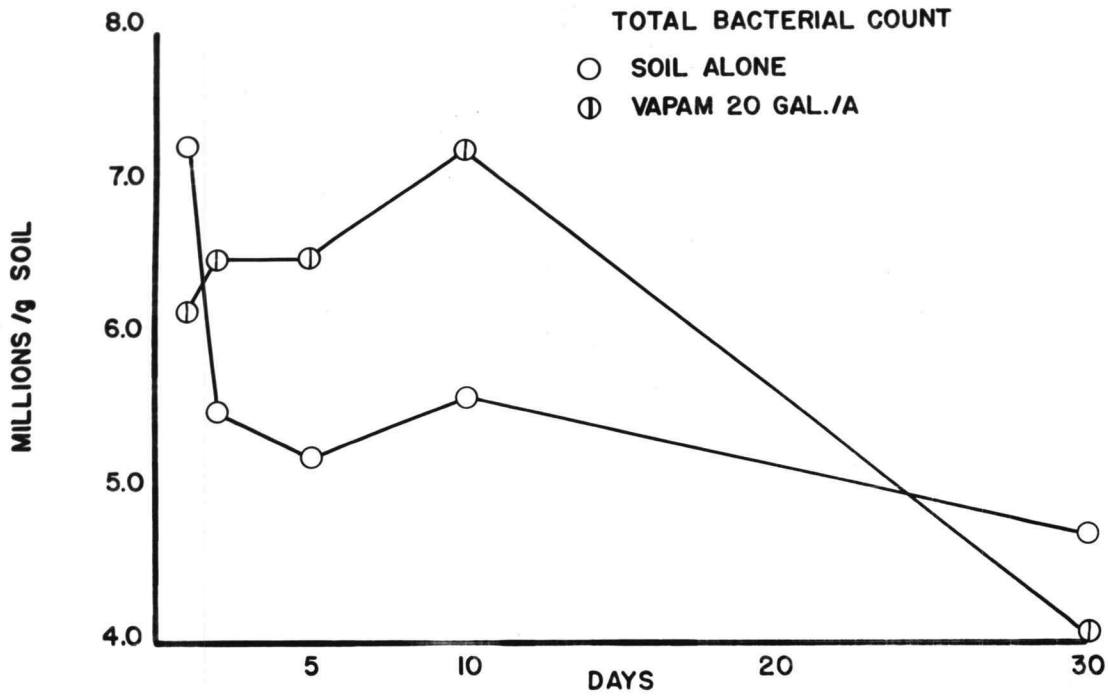

FIGURE: 1a

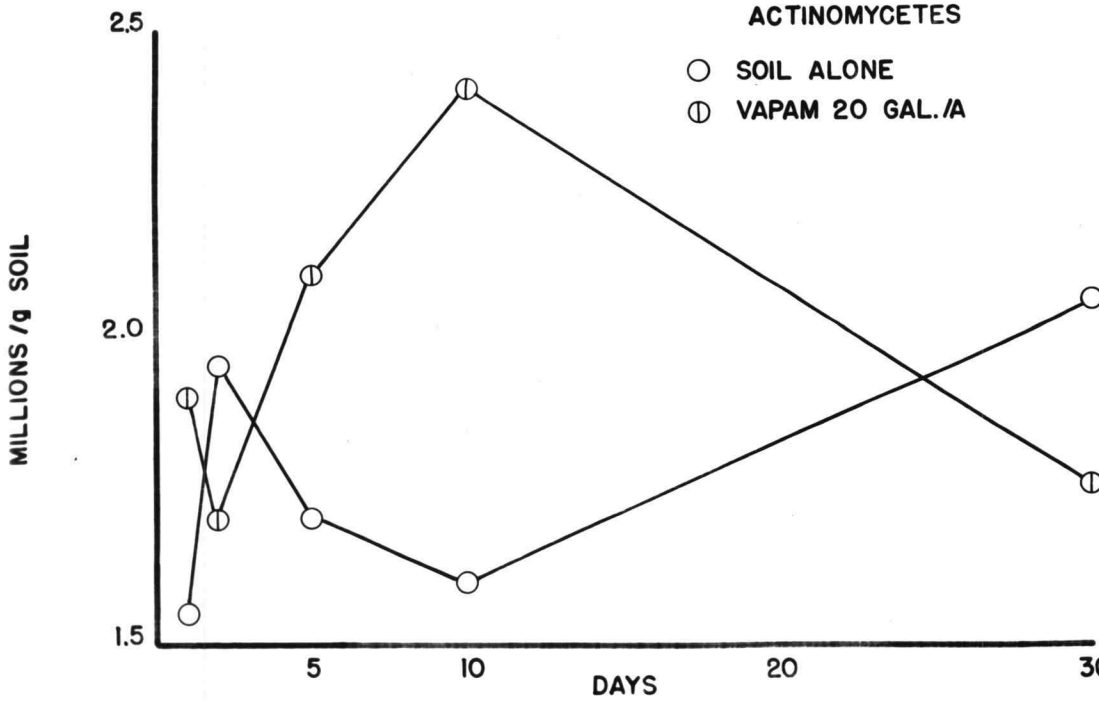

FIGURE: 1b

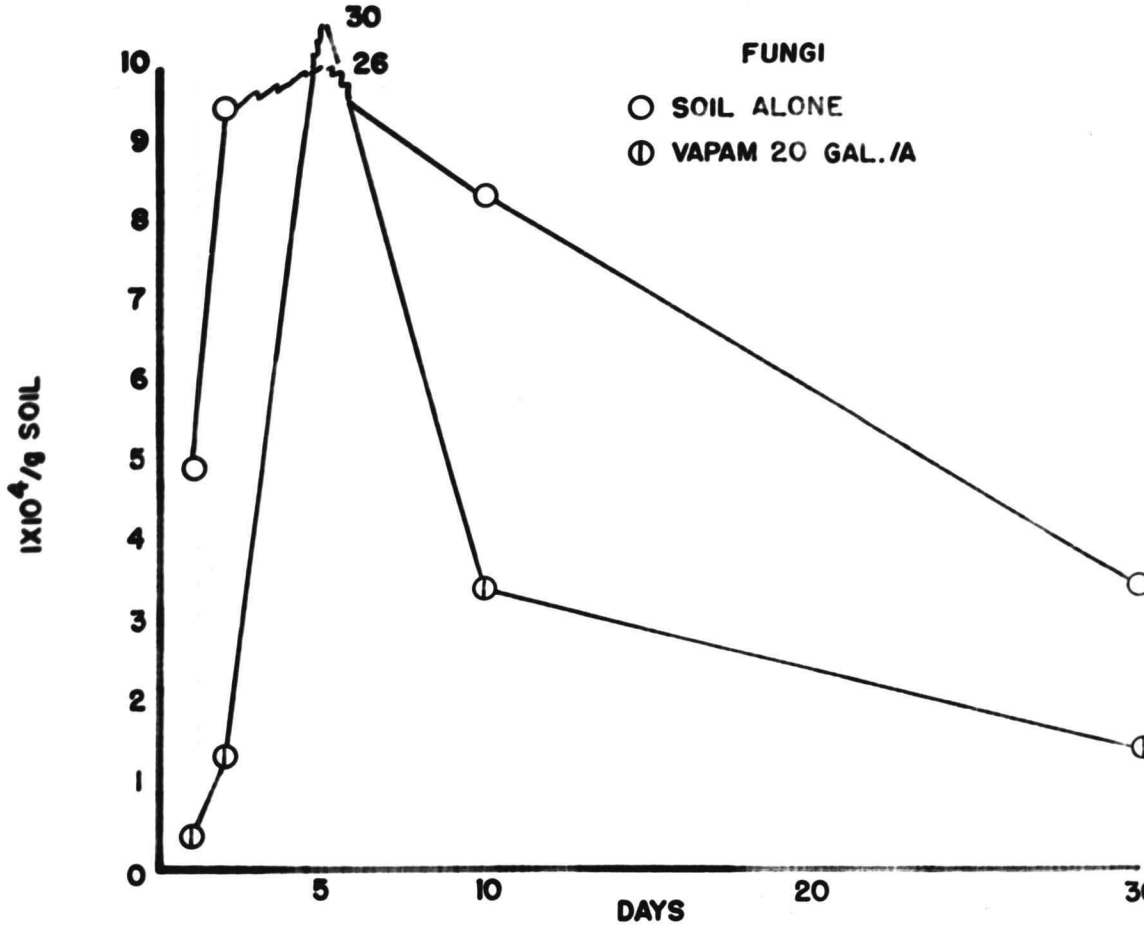

FIGURE: 1c CHANGES IN TOTAL NUMBERS OF BACTERIA, ACTINOMYCETES, AND FUNGI IN CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH VAPAM AT FIELD RATE.

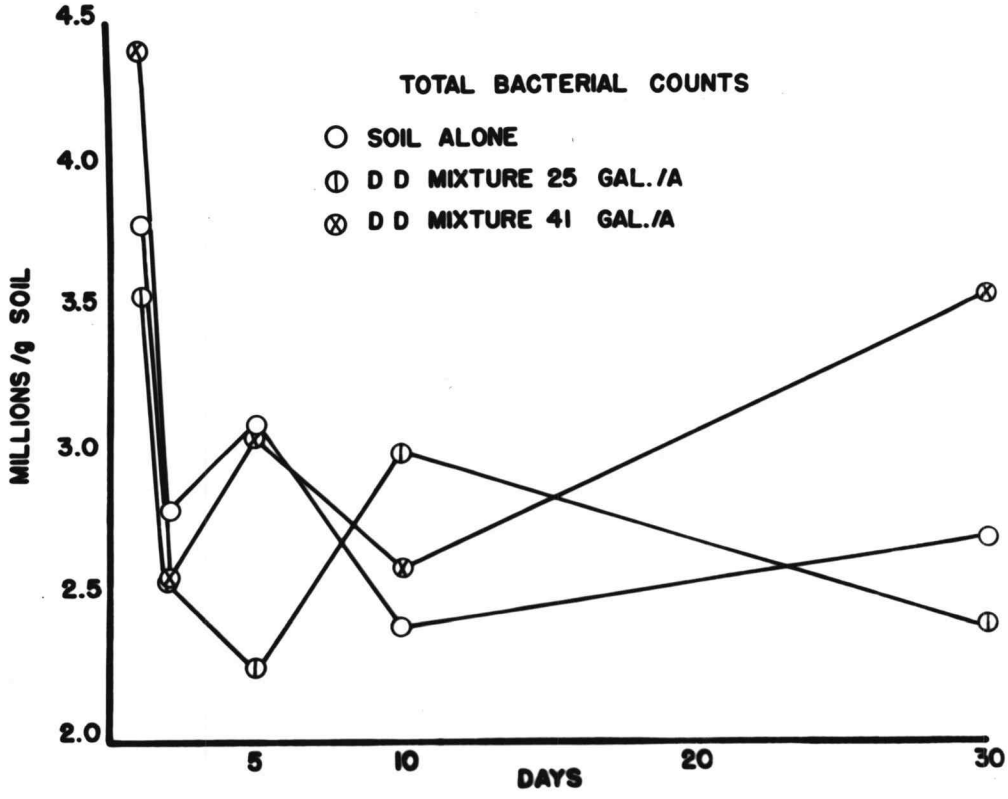

FIGURE: 2a

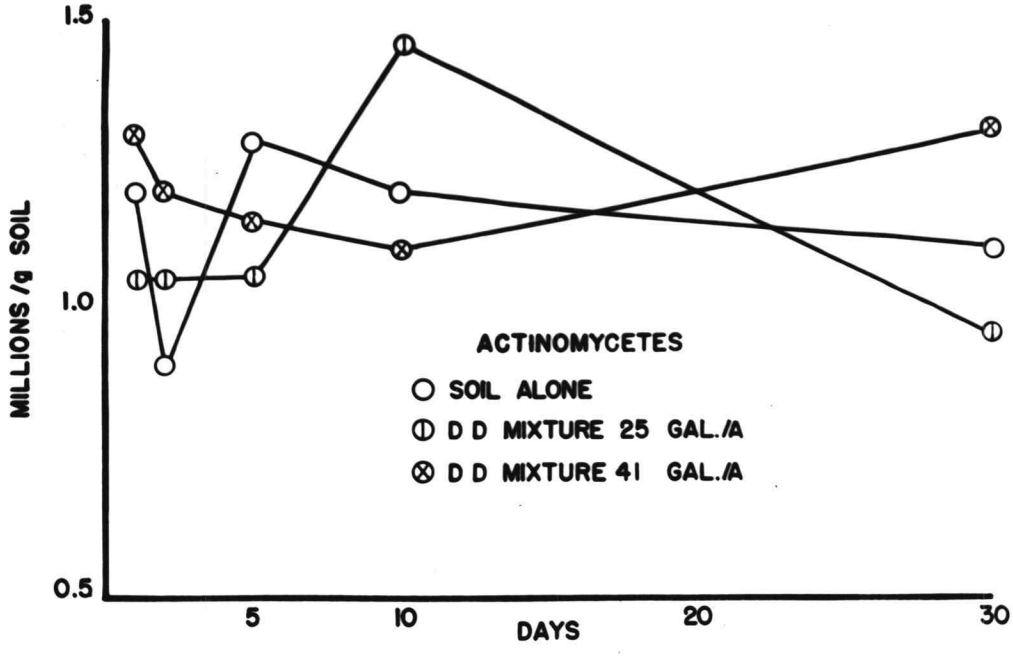

FIGURE: 2b

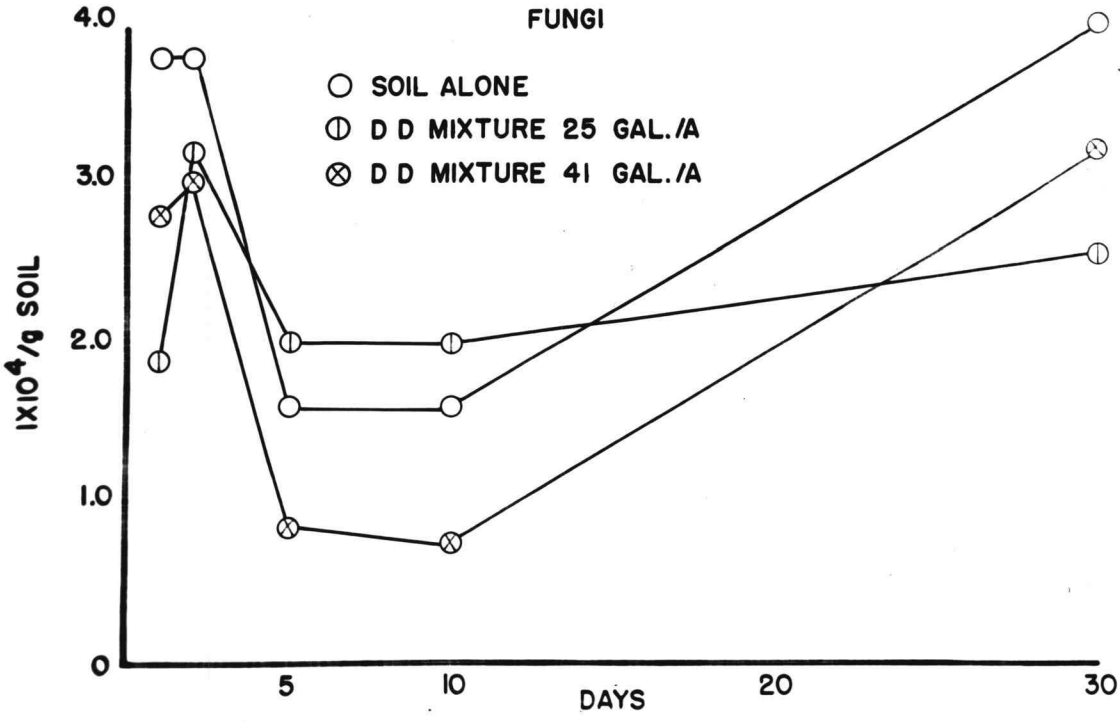

FIGURE: 2c CHANGES IN NUMBERS OF BACTERIA, ACTINOMYCETES, AND FUNGI IN CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH D D MIXTURE AT FIELD RATES.

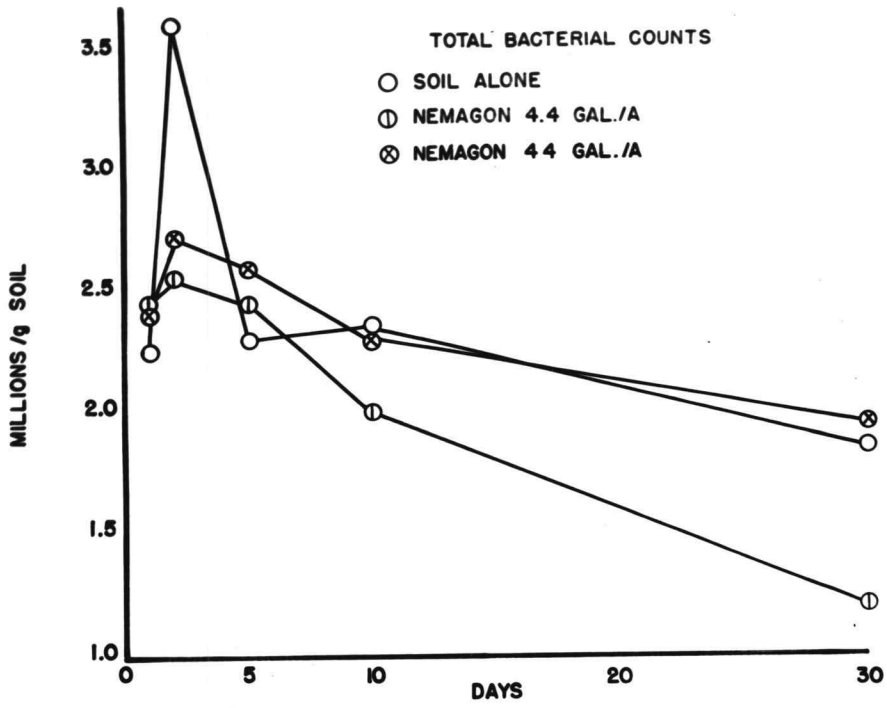

FIGURE: 3a

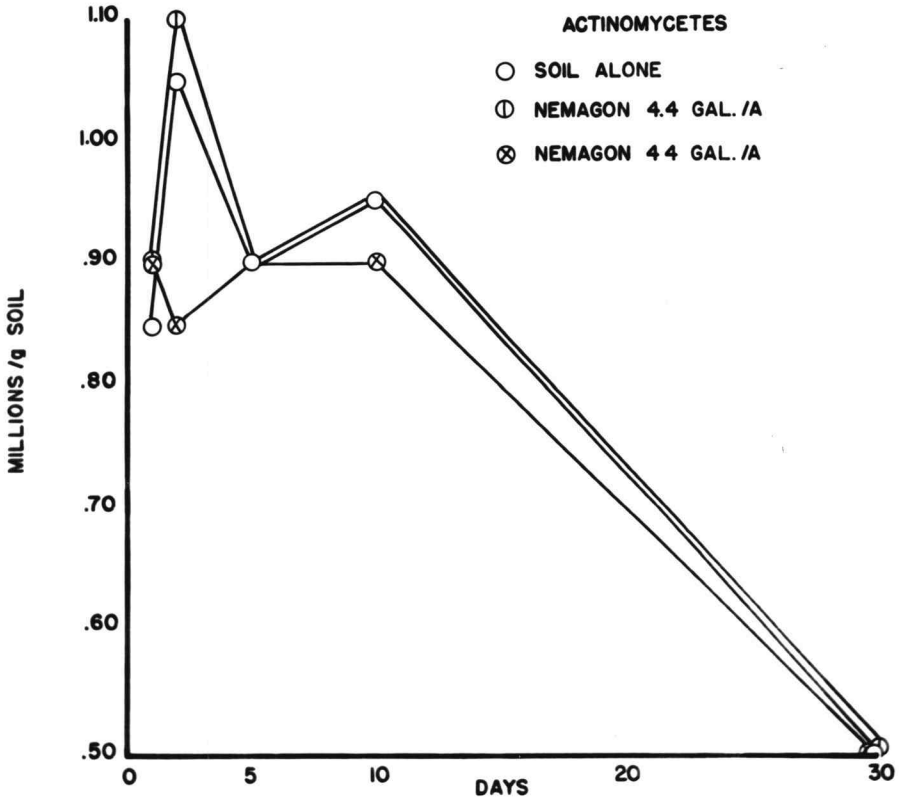

FIGURE: 3b

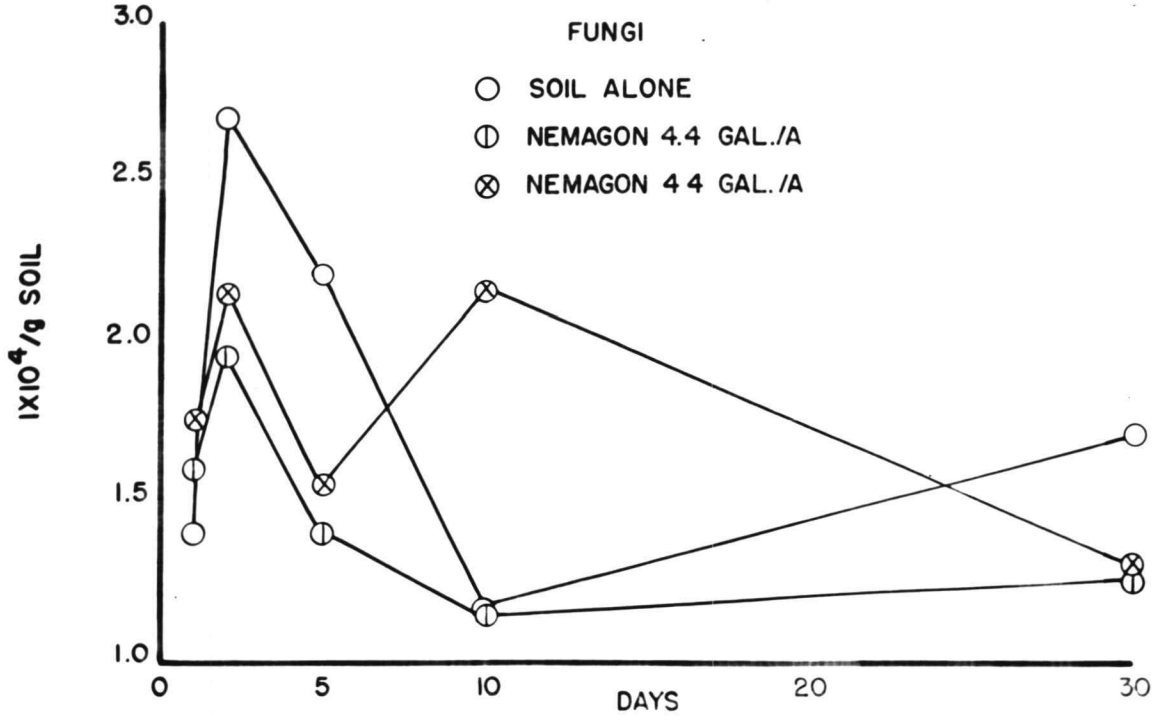

FIGURE: 3c CHANGES IN TOTAL NUMBERS OF BACTERIA, ACTINOMYCETES, AND FUNGI IN CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH NEMAGON AT DIFFERENT RATES.

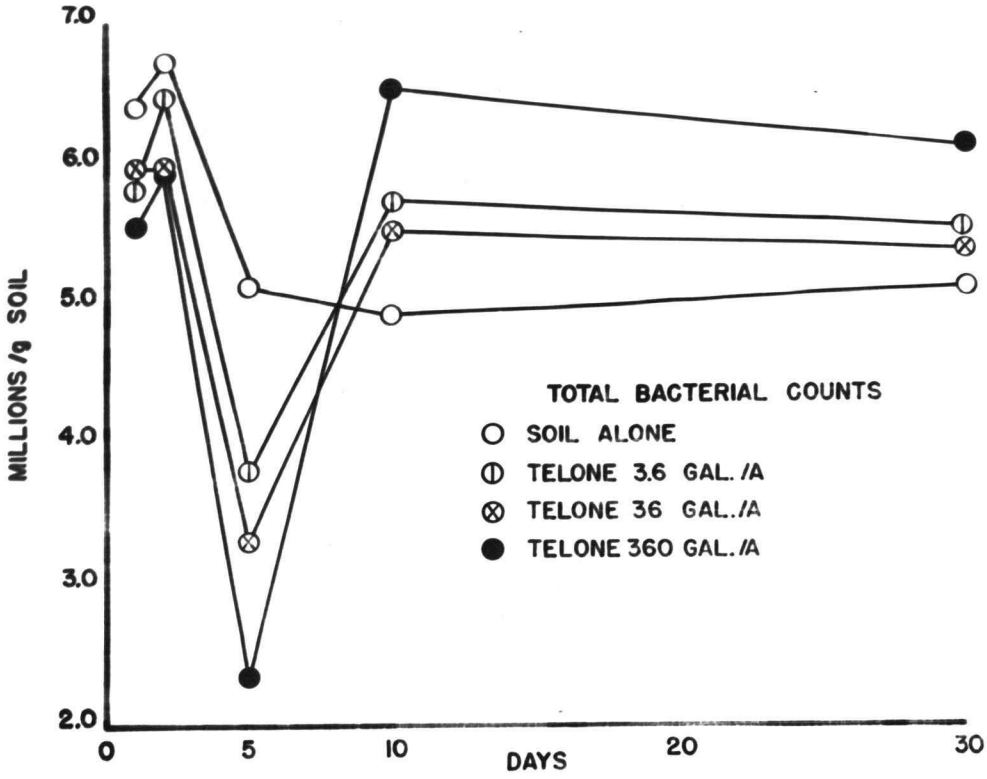

FIGURE: 4a

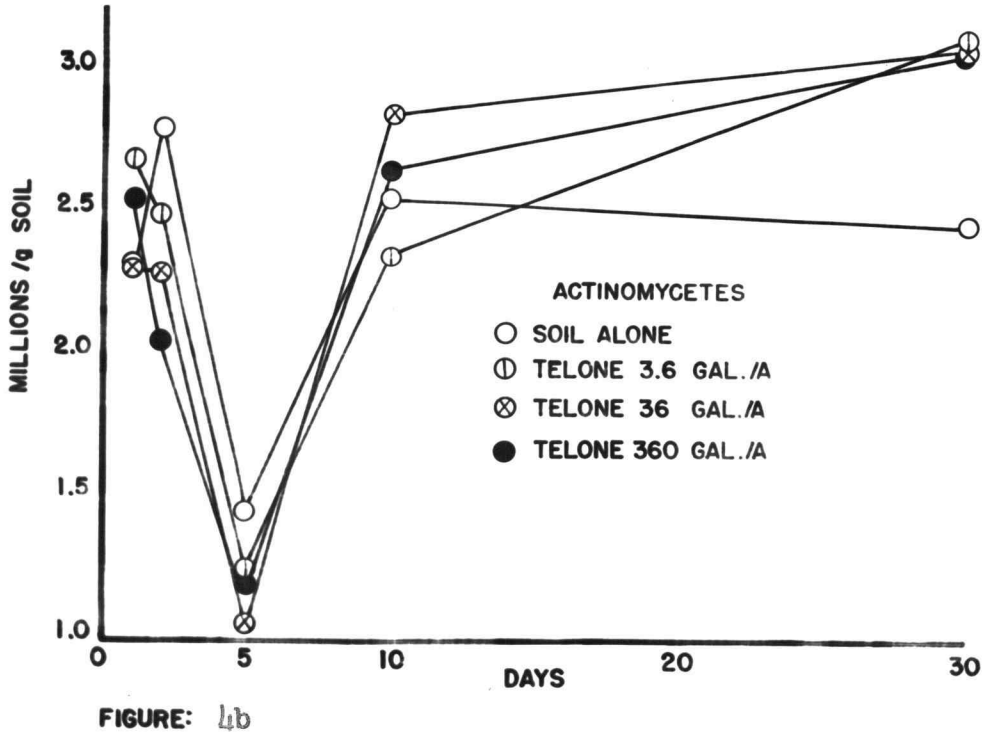

FIGURE: 4b

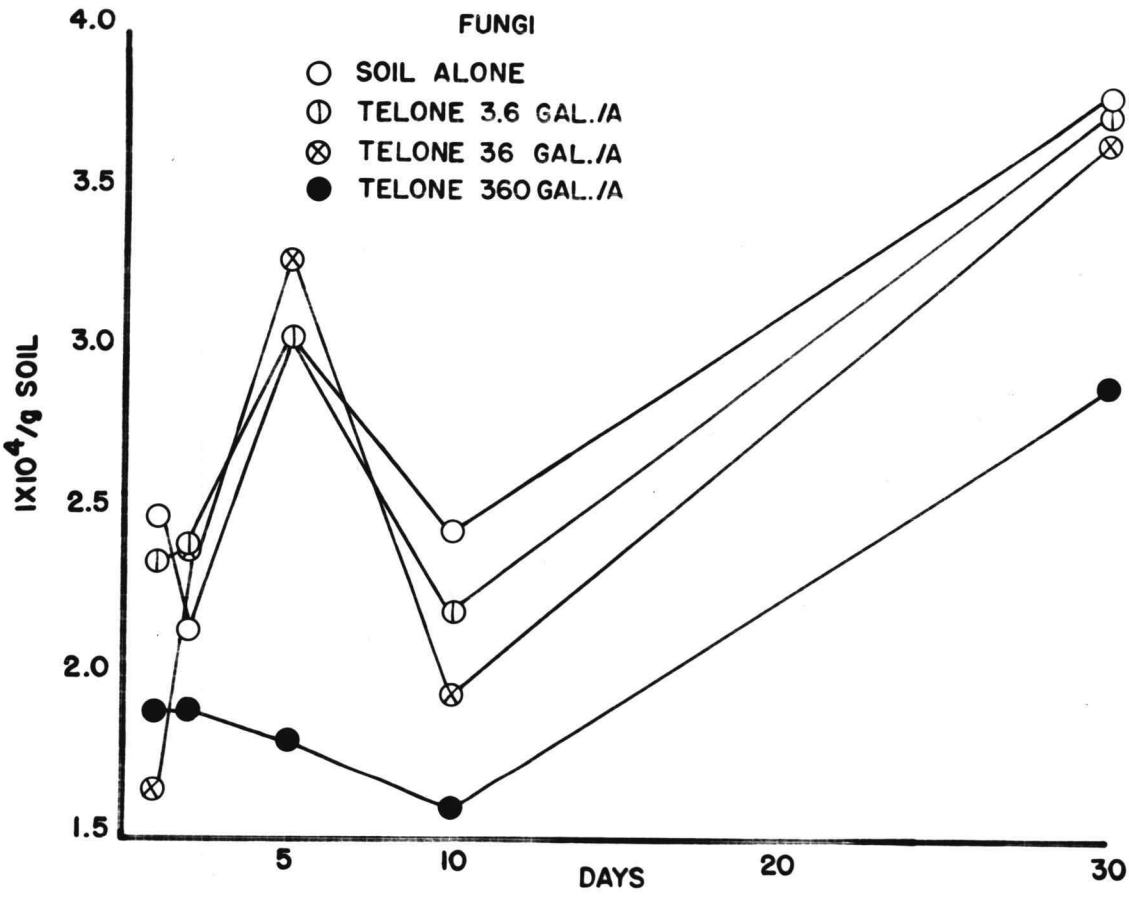

FIGURE: 4c CHANGES IN TOTAL NUMBERS OF BACTERIA, ACTINOMYCETES, AND FUNGI IN CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH TELONE AT DIFFERENT RATES.

numbers of bacteria and actinomycetes. This increase continued up to the thirty days, when the experiment was stopped. Soil treated at the rate of 25 gal/A showed irregular effects after three days, as also did the rate of 41 gal/A. At thirty days, however, the lower rate was depressive while the higher rate was eventually stimulating. Because the fumigant concentrations were not great, a closer correlation was expected; however, the 25 gal/A rate gave very low counts with a tendency to decline with time while the opposite occurred with the 41 gal/A treatment. Factors contributing to these divergencies are not known.

Martin (26, vol. 69, p. 107-122) reported that D-D Mixture initially destroyed or reduced numbers of fungi which later "re-establish" themselves in "greater numbers". This is in agreement with the results obtained here. Soils treated with this fumigant at 41 gal/A showed decreases in fungi within the first ten days, after which a progressive increase took place. Although the present experiment does not show "greater numbers" in relation to the control, due perhaps to the limited 30-day incubation period, the progressive rate observed indicated this to be possible in a longer time. Soils treated with D-D Mixture at 25 gal/A showed a more or less stationary stage in numbers of fungi, which were below the counts of the control and D-D Mixture at 41 gal/A treatments. It is of interest that the treatment of D-D Mixture at 25 gal/A showed similar slightly depressive effects, except at 10 days, on total numbers of bacteria, actinomycetes and fungi. Apparently, this rate is just about

sufficient to retard these microorganisms in the soil.

Nemagon had little effect on microbial numbers. At field rate, 4.4 gal/A, and treatments as high as ten times the field rate or 44 gal/A this fumigant had no significant effect on numbers of fungi or actinomycetes but slightly depressed the counts of bacteria at 30 days.

Total numbers of bacteria were greatly reduced in the first five days in soils treated with Telone at 3.6, 36 and 360 gal/A. This was followed by a period of repopulation, the increase being proportional to the increasing fumigant concentration. While actinomycetes were sharply reduced at five days, they were markedly increased at 10 and 30 days. Telone at field and lower rates had no marked effects on numbers of fungi. Telone at 360 gal/A depressed numbers of fungi during the first 10 days; this was followed by an increase which even at 30 days did not equal or surpass the control soil or soil with weaker fumigant treatments.

In general, all treatments produced a typical partial sterilization effect on microorganisms in the soil during the first ten days, followed by a period of re-establishment or increase. Investigations by many workers using different chemicals in the soil indicate similar trends on microbial counts. From these results it appears that the fumigants employed offer no exception as far as the general saprophytic flora is concerned.

PART III. STUDIES ON MICROBIAL ACTIVITIES

Carbon Dioxide Evolution

Since carbon dioxide evolution is a reliable index of the physiological activity of microorganisms, the measurement of this has long been used as a means to compare fertility of different soils as well as to determine response to favorable and unfavorable factors. In 1926 Stoklasa (41, vol. 4, p. 589-599) observed that the quantity of carbon dioxide produced in a given time and under specific environmental conditions offered a good picture of the correlation between carbon dioxide evolution and bacterial populations. This aspect of the present investigation serves as a supplement to the determinations of microbial counts.

Experimental Methods

The respiration apparatus and method employed were essentially as described by Bollen (5, vol. 15, p. 353-374), whose procedure is based on the fundamental investigations of Stoklasa (41, vol. 4, p. 589-599). Portions of soils weighing 200 grams on the water-free basis were placed in pint bottles. The addition of organic matter in the form of dextrose or wheat straw was made at a rate equivalent to 2000 ppm carbon. These additions were mixed with the dry soil before any further treatment. Fumigant applications were at field rates and in most cases ten times weaker and ten times strong concentrations, being added to enough distilled water to bring the soil samples up to the optimum of 60 per cent of moisture

capacity. The water or solutions were added in several small amounts to portions of soil as they were placed in the bottles. The bottles were transferred to the respiration apparatus in an incubator at 28° C. where they were connected to a source of carbon dioxide-free air. The entrance tube passed over the surface of the soil in the bottles and the air stream was then conducted into test tubes containing approximately 15 ml of N/1 sodium hydroxide, which served to absorb the carbon dioxide evolved. Slight positive pressure insured a gentle constant flow of air. The carbon dioxide absorption tubes were collected and replaced after the end of incubation period of 1, 2, 4, 7, 14, 30 and 60 days. The carbon dioxide absorbed in the sodium hydroxide was titrated with a standard N/12 H_2SO_4 in the Beckman automatic titrator. The results were expressed cumulatively as milligrams of carbon as carbon dioxide evolved per 200 grams of soil.

Results

The data obtained were statistically analyzed by the Department of Statistics. Since the values obtained varied considerably between soils treated with fumigants at different rates and soils to which organic matter was added, it was necessary to use LSD (least significant difference) calculated on two different bases. One LSD was for values below one hundred and the other was for values above one hundred. In each case the LSD between means at the 5 per cent and 1 per cent levels were calculated. Differences larger than LSD 5 per cent are referred as "significant" and those larger

than 1 per cent level as "highly significant". Significant and highly significant differences between fumigants plus organic matter and untreated checks are indicated in each table as well as in each figure. The general results of these respiration studies are shown in Tables 4, 5, 6 and 7 and Figures 5 to 10 inclusive.

Discussion

Vapam at field and below field rates had no significant effects on respiration at the end of 60 days, although at ten times the field rate, 200 gal/A, Vapam had a definite inhibitory influence upon carbon dioxide evolution. The various rates of Vapam had different effects upon the rate of decomposition of dextrose. Vapam at 20 and 200 gal/A gave highly significant and significant depressions respectively, of respiration. The greater depressive effect of Vapam at 20 gal/A is correlated with its depressive effect observed on microbial counts. The higher Vapam concentration did not proportionally increase the lethal or inhibitory effects upon microbial growth or on production of CO_2. While it is not statistically significant there was a slight microbial stimulation at the rate of 2 gal/A.

D-D Mixture is a fumigant which diffuses slowly but effectively. Perhaps this is the reason why significant microbial activities started only after the first week. The lowest field rate (25 gal/A) significantly stimulated CO_2 evolution while the higher rates (38 and 41 gal/A) showed highly significant stimulation. These results are

Table 4. Cumulative Carbon Dioxide Evolution, as mg of C, per 200 g. Chehalis Silty Clay Loam Soil Treated with Vapam at Different Rates with and without Added Organic Matter

Treatment	Incubation Period in Days							Increase over soil alone	Apparent decomposition
	1	2	4	7	14	30	60		
	mg	mg	mg	mg	mg	mg	mg	mg	%
Soil Alone	1.8	3.3	4.8	7.9	13.8	25.5	47.9	-	-
+ Vapam @ 2 gal/A	2.4	4.1	6.4	10.0	15.9	27.2	47.8	-0.1	-
+ Vapam @ 20 gal/A	1.5	2.3	3.6	6.0	11.3	25.1	46.1	-1.8	-
+ Vapam @ 200 gal/A	3.1	4.5	5.8	8.0	13.3	21.9	33.9	-14.0	-
Soil + Dextrose @ 2000 ppm C	30.2	60.0	111.3	167.6	210.2	245.1	286.0	238.1	11.9
+ Vapam @ 2 gal/A	35.7	64.4	101.2	368.4	213.1	254.8	299.4	251.5	12.6
+ Vapam @ 20 gal/A	3.5	6.6	15.4	88.3	167.5	208.9	242.5	194.6	9.7
+ Vapam @ 200 gal/A	8.5	12.0	17.0	92.5	174.2	226.0	258.6	210.7	10.5

LSD for values below 100

0.05	5.837
0.01	7.866

LSD for values above 100

0.05	21.05
0.01	28.07

Table 5. Cumulative Carbon Dioxide Evolution, as mg of C, per 200 g. Chehalis Silty Clay Loam Soil Treated with D-D Mixture at Different Field Rates with and without Added Dextrose

Treatment	Incubation Period in Days						Increase over soil alone	Apparent decomposition
	1	2	4	14	30	60		
	mg	mg	mg	mg	mg	mg	mg	%
Soil Alone	1.1	1.9	3.4	11.5	25.2	32.2	-	-
+ D-D Mixture @ 25 gal/A	1.0	3.1	4.9	14.9	26.8	42.0	9.8	-
+ D-D Mixture @ 38 gal/A	0.8	1.4	3.9	15.2	30.8	51.5	19.3	-
+ D-D Mixture @ 41 gal/A	1.6	3.2	6.1	16.9	31.0	51.4	19.2	-
Soil + Dextrose @ 2000 ppm C	35.5	60.4	122.8	230.5	291.8	352.0	319.8	16.0
+ D-D Mixture @ 25 gal/A	30.3	59.0	108.0	189.1	245.3	291.4	259.2	13.0
+ D-D Mixture @ 38 gal/A	26.0	64.3	101.2	184.9	239.1	281.1	248.9	12.5
+ D-D Mixture @ 41 gal/A	26.7	59.3	111.9	191.1	240.1	277.0	244.8	12.2

LSD for values below 100

0.05	5.837
0.01	7.866

LSD for values above 100

0.05	21.05
0.01	28.07

Table 6. Cumulative Carbon Dioxide Evolution, as mg of C, per 200 g. Chehalis Silty Clay Loam Soil Treated with Nemagon at Different Rates with and without Organic Matter

Treatment	Incubation Period in Days							Increase over soil alone	Apparent decomposition
	1	2	4	7	14	30	60		
	mg	mg	mg	mg	mg	mg	mg	mg	%
Soil Alone	2.3	2.8	5.7	6.1	14.6	30.0	55.0	-	-
+ Nemagon @ 4.4 gal/A	0.5	0.5	1.4	3.1	12.0	27.9	54.2	-0.8	-
+ Nemagon @ 44 gal/A	0.5	1.6	5.6	9.8	23.8	39.9	68.4	13.4	-
Soil + Wheat Straw @ 2000 ppm C	1.2	3.6	7.8	23.1	73.8	150.5	244.8	189.8	9.49
+ Nemagon @ 4.4 gal/A	1.2	5.6	38.7	69.4	100.7	181.9	273.5	218.5	10.9
+ Nemagon @ 44 gal/A	2.1	7.3	18.1	34.1	66.9	141.4	225.6	170.6	8.5
Soil + Dextrose @ 2000 ppm C	3.6	6.0	71.3	78.3	132.3	195.1	233.5	178.5	8.9
+ Nemagon @ 4.4 gal/A	56.8	110.8	170.4	227.4	279.0	332.4	389.2	334.2	16.7
+ Nemagon @ 44 gal/A	1.5	8.2	28.7	67.0	110.5	200.8	273.8	218.2	10.0

LSD for values below 100

0.05	5.837
0.01	7.866

LSD for values above 100

0.05	21.05
0.01	28.07

Table 7. Cumulative Carbon Dioxide Evolution, as mg of C, per 200 g. Chehalis Silty Clay Loam Soil Treated with Telone at Different Rates with and without Organic Matter

Treatment	Incubation Period in Days						Increase over soil alone	Apparent decomposition
	2	4	7	14	30	60		
	mg	mg	mg	mg	mg	mg	mg	%
Soil Alone	1.0	2.6	5.9	12.3	20.3	39.0	-	-
+ Telone @ 3.6 gal/A	4.8	8.0	12.5	17.9	33.2	52.1	13.1	-
+ Telone @ 36 gal/A	4.6	8.3	12.9	20.7	37.8	59.1	20.1	-
+ Telone @ 360 gal/A	1.5	3.9	8.0	12.5	26.1	44.0	5.0	-
Soil + Wheat Straw @ 2000 ppm C	21.4	42.3	75.6	109.5	181.6	293.0	254.0	12.7
+ Telone @ 36 gal/A	9.9	22.2	57.8	87.7	140.1	182.4	143.4	7.2
Soil + Dextrose @ 2000 ppm C	69.0	124.8	200.6	252.0	316.1	363.1	324.1	16.2
+ Telone @ 36 gal/A	75.2	116.4	219.6	264.0	314.2	365.0	326.0	16.3
Soil + Dextrose + NH_4NO_3 to make 20/1 C/N ratio	78.2	119.5	180.8	230.1	287.8	313.7	274.7	13.7
+ Telone @ 36 gal/A	81.6	186.2	281.7	319.3	371.4	405.2	366.2	18.3
Soil + Wheat Straw @ 2000 ppm C + NH_4NO_3 to give 20/1 C/N ratio	28.4	66.9	99.1	147.2	229.8	316.9	277.9	13.9
+ Telone @ 36 gal/A	17.6	27.8	72.9	138.0	221.0	289.4	250.4	12.5

LSD for values below 100

0.05	5.837
0.01	7.866

LSD for values above 100

0.05	21.05
0.01	28.07

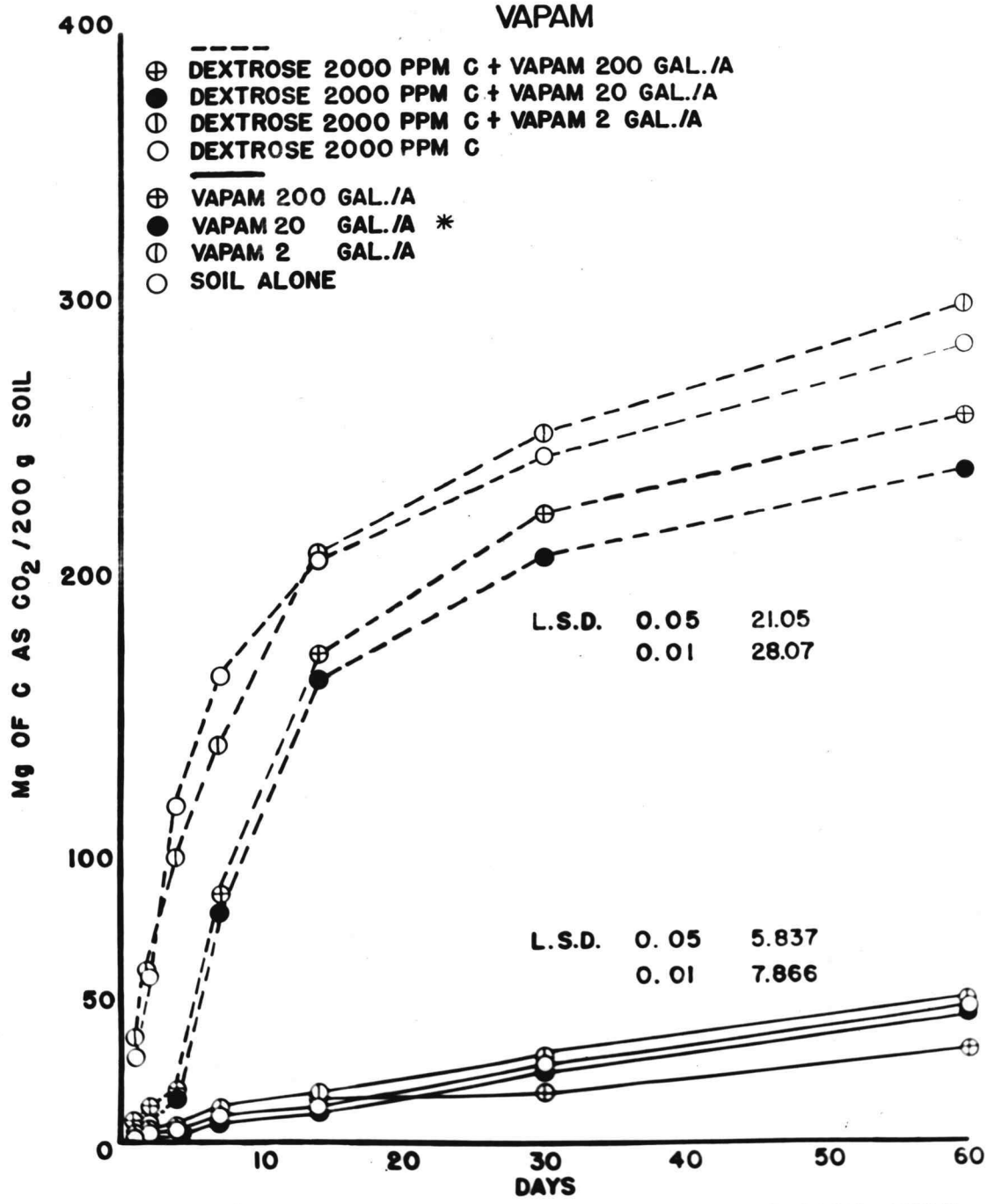

FIGURE: 5 CARBON DIOXIDE EVOLUTION, CUMULATIVE BASIS, FROM CHEHALIS SILTY CLAY LOAM SOIL TREATED AT DIFFERENT RATES WITH AND WITHOUT ADDED ORGANIC MATTER.

* FIELD RATES

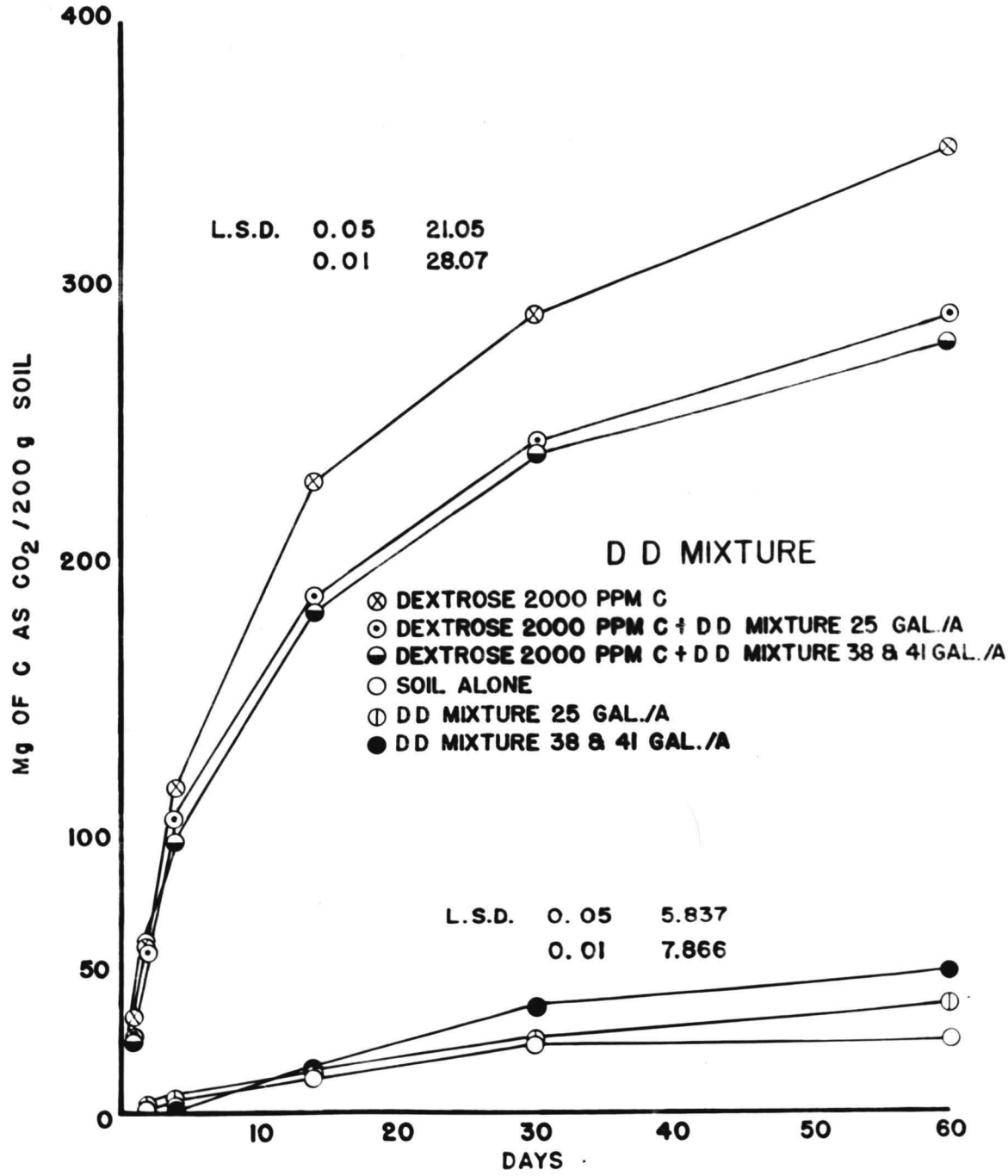

FIGURE: 6 CUMULATIVE CARBON DIOXIDE EVOLUTION FROM CHEHALIS SILTY CLAY LOAM TREATED WITH DD MIXTURE AT DIFFERENT FIELD RATES WITH AND WITHOUT ADDED DEXTROSE.

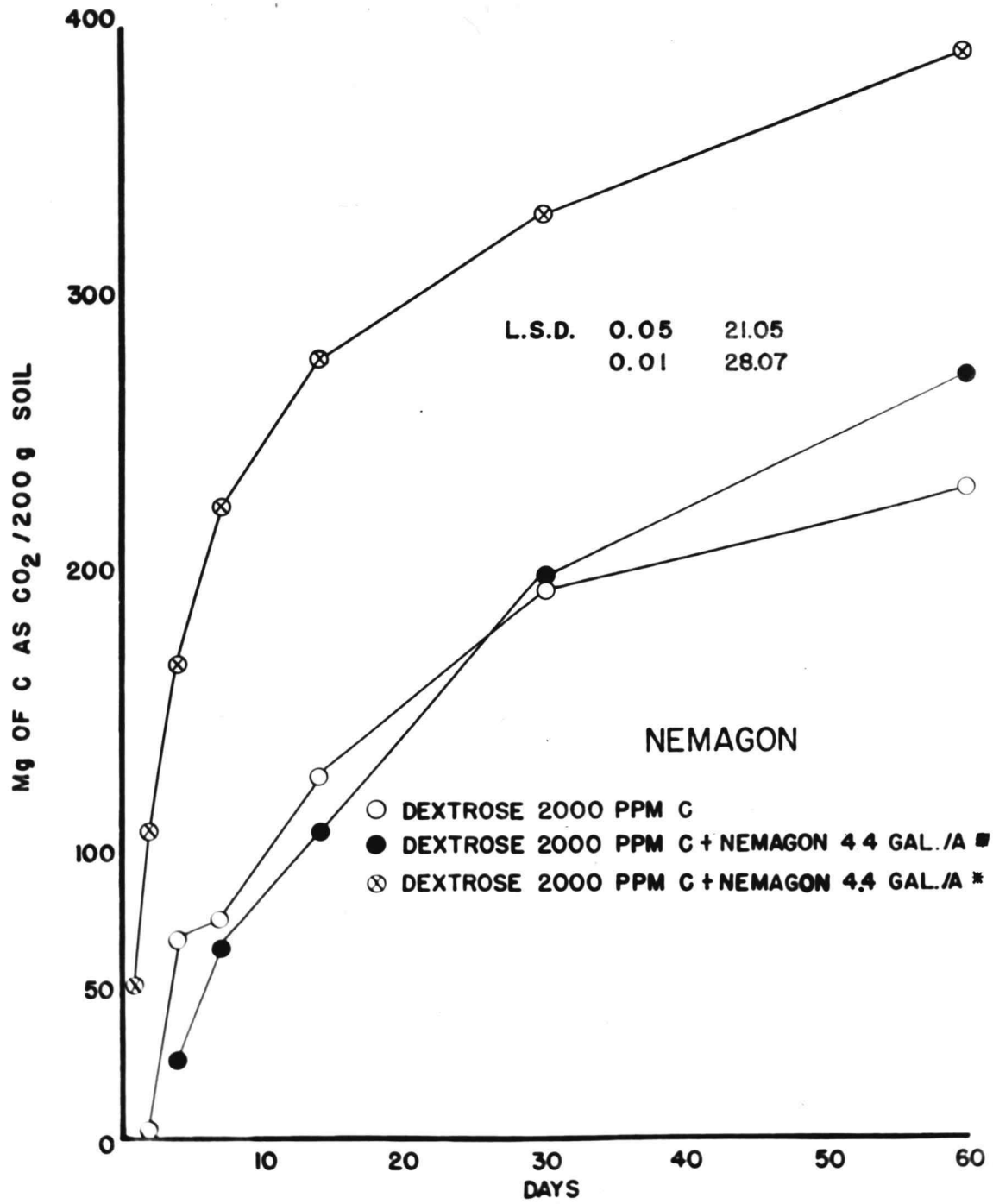

FIGURE: 7 CUMULATIVE CARBON DIOXIDE EVOLUTION FROM CHEHALIS SILTY LOAM SOIL TREATED WITH NEMAGON AT DIFFERENT RATES, WITH AND WITHOUT ADDED DEXTROSE.

* ACTUAL FIELD RATE

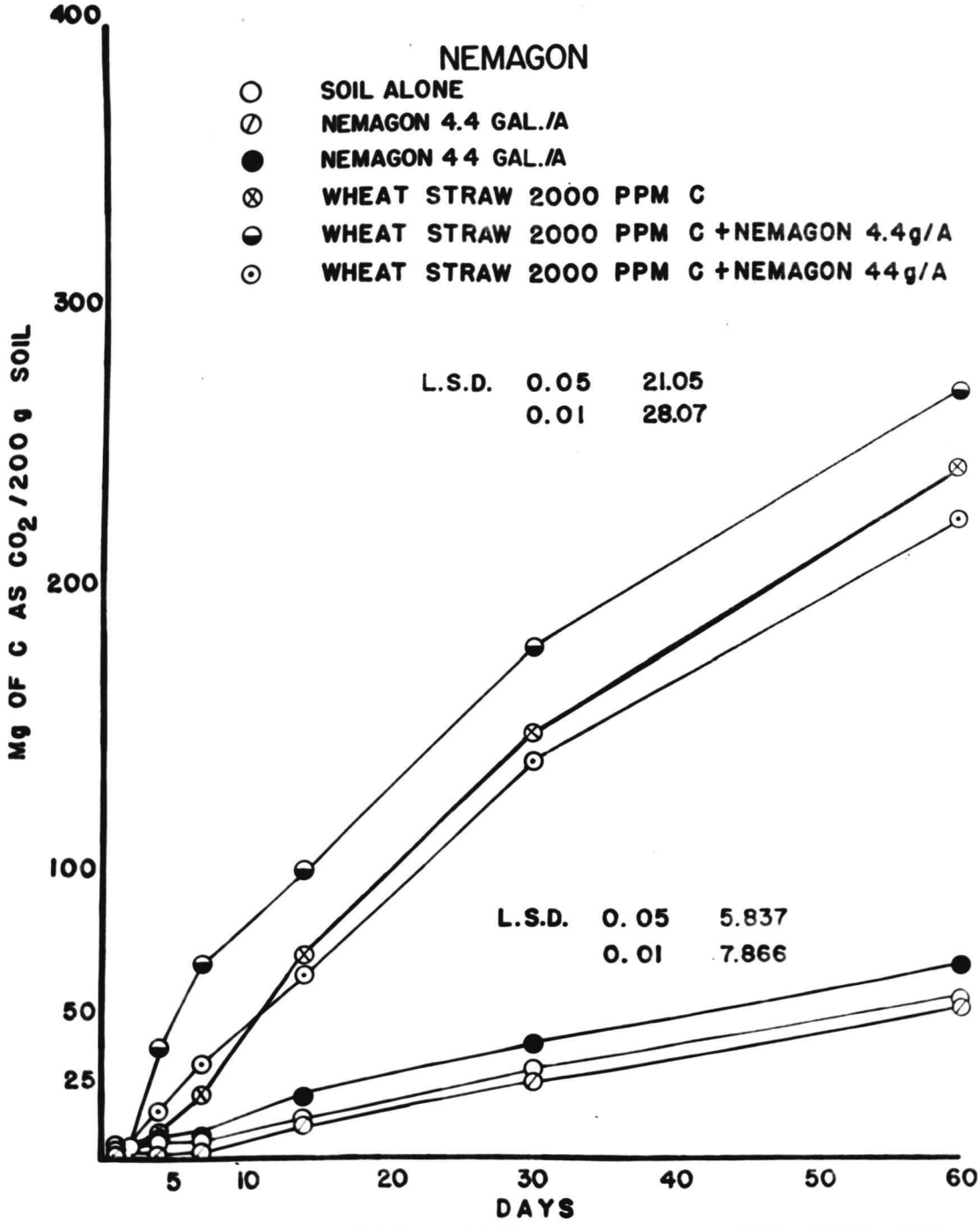

FIGURE 8 : CUMULATIVE CARBON DIOXIDE EVOLUTION FROM SOIL TREATED WITH NEMAGON AT DIFFERENT RATES WITH AND WITHOUT ADDED WHEAT STRAW.

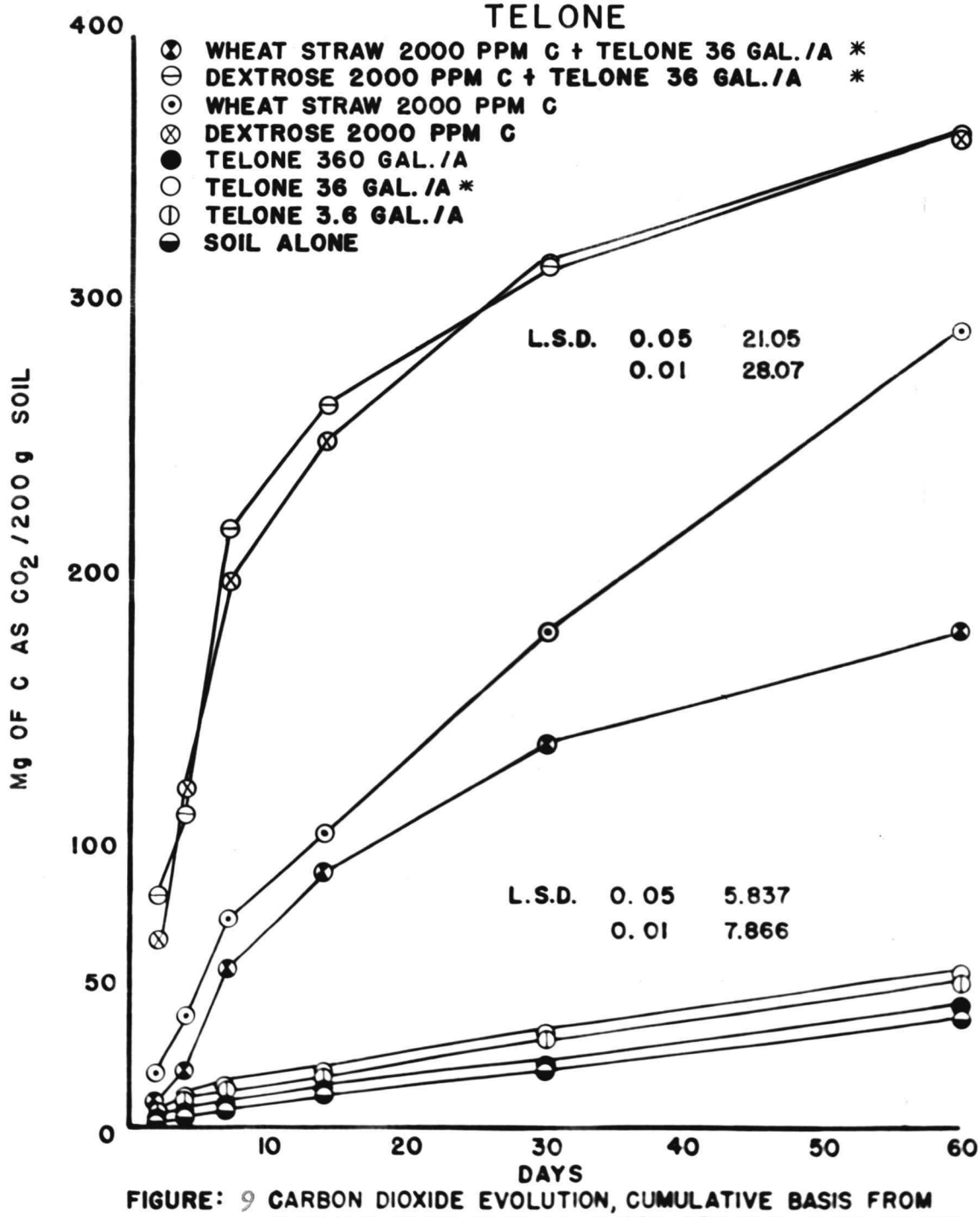

FIGURE: 9 CARBON DIOXIDE EVOLUTION, CUMULATIVE BASIS FROM CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH TELONE AT DIFFERENT RATES.

* FIELD RATE TELONE WITH AND WITHOUT ORGANIC MATTER.

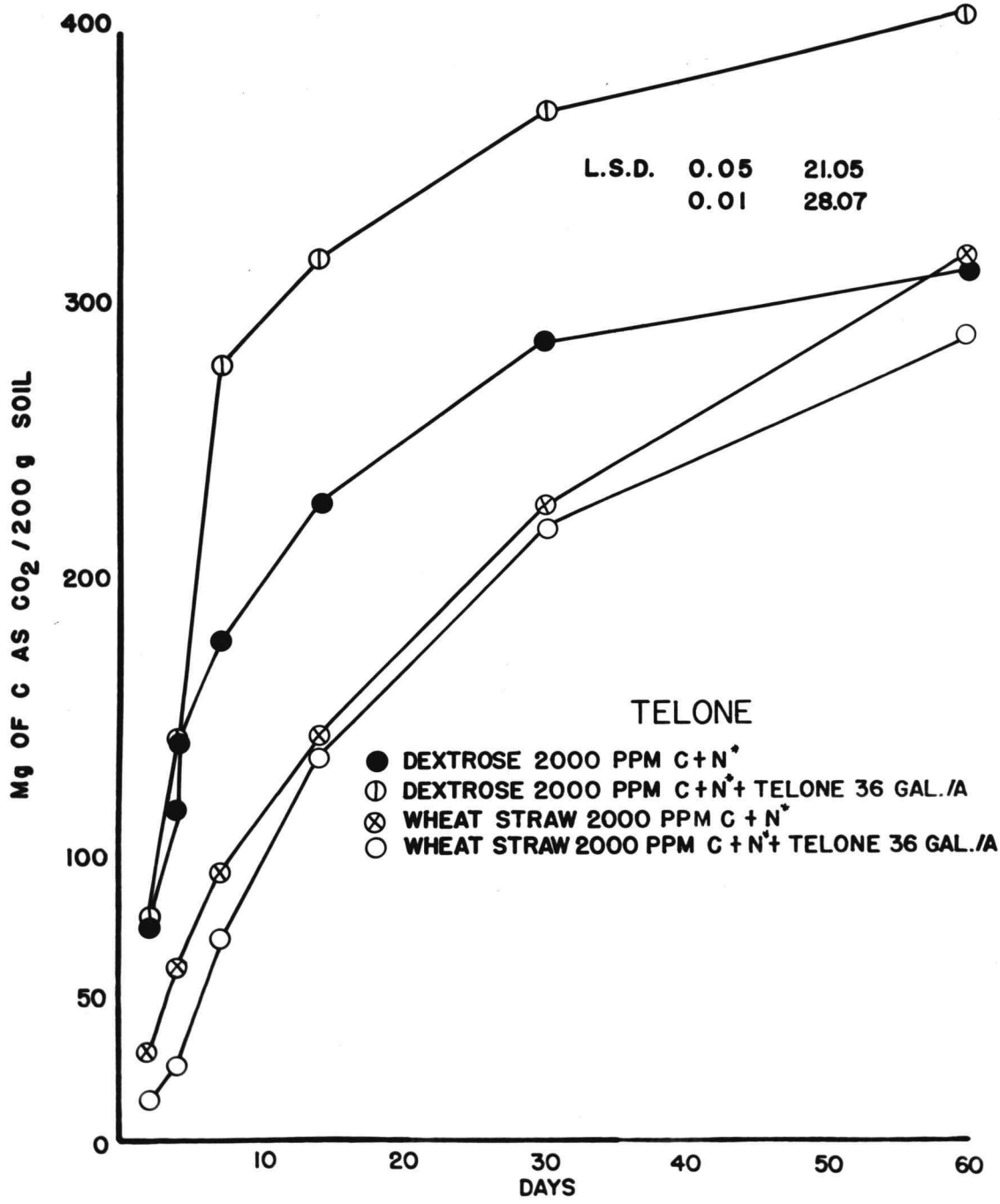

FIGURE: 10 CUMULATIVE CARBON DIOXIDE EVOLUTION FROM CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH TELONE AT FIELD RATES WITH AND WITHOUT DEXTROSE AND WHEAT STRAW.

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON:NITROGEN RATIO OF 20:1

in line with the corresponding increases previously found in microbial population numbers. Since organic matter additions are characteristic of field management a fumigant may be better evaluated if similar conditions are observed in the laboratory. D-D Mixture at field rates (25, 38 and 41 gal/A) retarded the decomposition of dextrose in Chehalis silty clay loam soil as compared with the CO_2 recoveries from control soil, Table 5, page 41.

The stimulatory effects observed with the fumigants in soil without organic matter additions and the corresponding depressive effects in the organic matter treated soil might be explained in terms of D-D Mixture gas diffusion and adsorption. In the control soil the gas diffuses freely and rather rapidly. The evenly dispersion of the chemical could have encouraged microbial activities which was expressed in the greater amounts of CO_2 evolved. Added organic matter, on the other hand, could adsorb the gas and increase its concentration sufficiently to produce local toxicity and an overall reduction in carbon dioxide evolution.

Nemagon at the field rate, 4.4 gal/A had beneficial effects on microbial activities associated with soil fertility. Respiration studies in the laboratory under optimal moisture and temperature showed significant increases in decomposition of wheat straw and dextrose when treated with Nemagon at the field rate of 4.4 gal/A. At 44 gal/A a significant stimulatory effect was observed on dextrose decomposition but its effect on wheat straw appeared slightly depressive after ten days. The greater stimulatory effect of Nemagon

at 4.4 gal/A on dextrose decomposition than on that of wheat straw can be attributed to the relative resistances of these materials.

With D-D Mixture a ten-fold increase over the field rate caused significant depressive effects on microbial decomposition of dextrose; the opposite that occurred with Nemagon can be attributed to differences in physico-chemical properties of the two fumigants. D-D Mixture diffuses much faster than Nemagon, therefore, increasing concentrations of the D-D fumigant would mean an increased rate of toxicity. On the other hand, it seems that greater concentrations of Nemagon increase its permanence rather than its toxicity in the soil, where it continues to vaporize and diffuse slowly.

More CO_2 was produced from the native organic matter in soil treated with Telone at 3.6, 36 and 360 gal/A. Telone at the field rate, 36 gal/A, caused a highly significant depression in decomposition of wheat straw but not of dextrose. Since dextrose offers rapidly available energy to organisms growing in the soil, perhaps this enables them to better resist the toxic effects of Telone. Wheat straw carbon availability is limited and apparently not rapid enough to overcome the inhibitory properties of the fumigant; consequently depressive effects resulted.

Aside from carbon, the food element required in largest amount is nitrogen. An insufficiency of available nitrogen limits microbial development and activity. Previous experiments have indicated that a carbon nitrogen ratio of 20/1 to 25/1 in soil provides the conditions for optimal microbial growth. When nitrogen as ammonium

nitrate was added to wheat straw and dextrose to make the desired C/N ratio, the results shown in Table 7, page 43 and Figure 9, page 48, were obtained.

Soils with wheat straw and dextrose at a 20/1 carbon-nitrogen ratio, aside from early depressive effects showed no significant differences at the end of a 60-day incubation period. On the other hand, with Telone at the field rate addition of 36 gal/A highly significant stimulatory results were obtained in soils with dextrose and significant depressive effects with wheat straw at the end of the same incubation time.

Soil samples to which wheat straw and ammonium nitrate were added at the carbon-nitrogen ratio of 20 to 1, gave a higher significant stimulatory effect than the one in which the C/N ratio was not altered. Addition of Telone at field rate caused significant reduction of straw decomposition. With dextrose the opposite happened. Addition of available nitrogen produced a highly significant depressive effect. This probably could be explained on the basis that in the absence of added nitrogen some soil organic nitrogen was decomposed to meet microbial requirements, thus liberating more carbon dioxide. Telone additions with nitrogen, on the other hand, greatly stimulated dextrose decomposition, and gave the greatest carbon dioxide evolution of any of the treatments.

Ammonification

Experimental Methods

Eighty grams of soil on the water-free basis were placed in pint milk bottles. Peptone at 1000 ppm N was added. To add the dry materials, the soil was spread on a sheet of paper, the peptone was sprinkled on and mixed with a spatula. The fumigant was diluted to the desired concentration; then calculated amounts were added with a syringe to successive portion of the soil as they were transferred to the bottle. Further addition of distilled water were made as necessary to obtain optimum moisture. Each of the triplicate samples were closed with milk caps, the caps being punctured with a 7 mm diameter hole to allow free air circulation. The bottles were weighed and their weight recorded. All were incubated at 28° C. for a period of 3 and 7 days.

At the end of each incubation period, the contents of each bottle were mixed and samples equivalent to 10 grams of soil, dry basis, were analyzed for NH_4^+- N. The analytical procedure was basically the one developed by Shrikhande (35, vol. 13, p. 187-188) for decomposed plant material, the adaptation for NH_4^+- N determination in soil being as follows:

The 10 gram, dry basis, soil sample was transferred to a Kjeldahl flask to which 30 ml of phosphate buffer (K_2HPO_4 14.3 grams, KH_2PO_4 91.0 grams and one liter of water) pH 7.4 was added. The flask was connected to the distilling apparatus and approximately 100 ml distillate collected in 4 per cent boric acid solution.

The adsorbed NH_4^+- N was titrated with N/14 H_2SO_4 to a grayish-red end point as obtained by the use of bromcresol green indicator (Cooper 9, vol. 13, p. 466-470). The remaining soil in the bottle was made up with distilled water to a 1/5 dilution and placed in an automatic shaker for 10 minutes. After taking a pH reading with a model N Beckman pH meter using a glass electrode, the suspension was treated with copper acetate, calcium hydroxide and ammonium carbonate to obtain a clear filtrate for NO_2^--N and NO_3^- -N analysis as is explained on page 19.

Results and Discussion

The results are shown in Table 8 and Figure 11, pages 55 and 56 respectively.

Since ammonification is a rapid process the results in 3 days are of particular interest. While little ammonium nitrogen appeared in the control soil the presence of fumigants in most cases increased ammonification of the soil organic matter. Only D-D Mixture at 25 gal/A and Nemagon at 44 gal/A had no appreciable effect; Vapam at 200 gal/A appeared depressive. Ammonification of added peptone, on the other hand, was decreased by the fumigants in all instances, especially by Vapam and very marked at the higher rate. Aside from Vapam only D-D Mixture and Telone at the higher rates gave depressions of likely significance.

Nitrites appeared only in soil with peptone and the concentrations were below significant levels. Nitrate nitrogen showed only

Table 8. Influence of Various Fumigants on Ammonification of Chehalis Silty Clay Loam Soil with and without Peptone

Treatment	pH		NO_2^- -N		NO_3^- -N		NH_4^+ -N		NH_4^+ -N increase over soil only		Ammonification of added Peptone			
											Based on NH_4^+ -N		Based on NH_4^+ + NO_3^- -N	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
			ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%	%	%	%
Soil Alone	6.7	6.3	t	t	23.0	10.0	4.0	12.0	—	—	—	—	—	—
+ D-D Mixture @ 25 gal/A	6.4	6.5	t	t	18.0	7.0	4.0	26.0	—	14.0	—	—	—	—
+ D-D Mixture @ 40 gal/A	7.3	6.7	t	t	25.0	11.0	30.0	24.0	26.0	12.0	—	—	—	—
+ D-D Mixture @ 400 gal/A	6.9	6.4	t	t	18.0	12.0	36.0	15.0	32.0	3.0	—	—	—	—
+ Telone @ 3.6 gal/A	7.6	6.4	t	t	21.0	13.0	35.0	13.0	31.0	1.0	—	—	—	—
+ Telone @ 36.0 gal/A	6.6	6.6	t	t	21.0	14.0	52.0	10.0	48.0	-2.0	—	—	—	—
+ Telone @ 360.0 gal/A	6.8	6.6	t	t	18.0	8.0	21.0	8.0	17.0	-4.0	—	—	—	—
+ Nemagon @ 4.4 gal/A	6.8	7.1	t	t	20.0	10.0	13.0	1.0	9.0	-11.0	—	—	—	—
+ Nemagon @ 44 gal/A	7.2	7.2	t	t	18.0	9.0	4.0	4.0	—	-8.0	—	—	—	—
+ Vapam @ 20 gal/A	6.8	7.1	t	t	13.0	12.0	25.0	4.0	21.0	-8.0	—	—	—	—
+ Vapam @ 200 gal/A	7.3	7.1	t	t	20.0	8.0	1.0	15.0	-3.0	3.0	—	—	—	—
Soil + Peptone @ 1000 ppm N	7.2	7.1	3.0	1.0	22.0	24.0	484.0	569.0	480.0	557.0	48.4	55.7	50.6	59.3
+ D-D Mixture @ 25 gal/A	7.3	7.3	2.0	4.0	18.0	19.0	457.0	526.0	453.0	514.0	45.5	51.5	47.5	54.5
+ D-D Mixture @ 40 gal/A	7.5	6.7	2.0	3.0	17.0	15.0	460.0	530.0	456.0	518.0	45.6	51.8	47.7	54.5
+ D-D Mixture @ 400 gal/A	7.5	6.9	2.0	2.0	16.0	14.0	423.0	521.0	419.0	509.0	41.9	50.9	43.9	53.4
+ Telone @ 3.6 gal/A	6.7	7.1	2.0	4.0	4.0	17.0	474.0	555.0	470.0	543.0	47.0	54.3	47.8	57.2
+ Telone @ 36.0 gal/A	7.1	7.2	2.0	2.0	15.0	17.0	434.0	519.0	430.0	507.0	43.0	50.6	44.9	53.6
+ Telone @ 360.0 gal/A	7.3	7.2	1.0	t	19.0	11.0	394.0	556.0	390.0	544.0	39.0	54.4	41.3	56.6
+ Nemagon @ 4.4 gal/A	7.3	7.4	3.0	2.0	17.0	15.0	469.0	531.0	465.0	519.0	46.5	51.9	48.6	54.6
+ Nemagon @ 44.0 gal/A	7.1	7.3	2.0	t	17.0	14.0	477.0	555.0	473.0	543.0	47.3	54.3	49.4	56.9
+ Vapam @ 20 gal/A	6.7	6.8	t	7.0	14.0	19.0	310.0	540.0	306.0	528.0	30.6	52.8	32.4	55.9
+ Vapam @ 200 gal/A	6.3	6.7	t	t	1.0	6.0	38.0	541.0	34.0	529.0	3.4	52.9	3.9	54.6

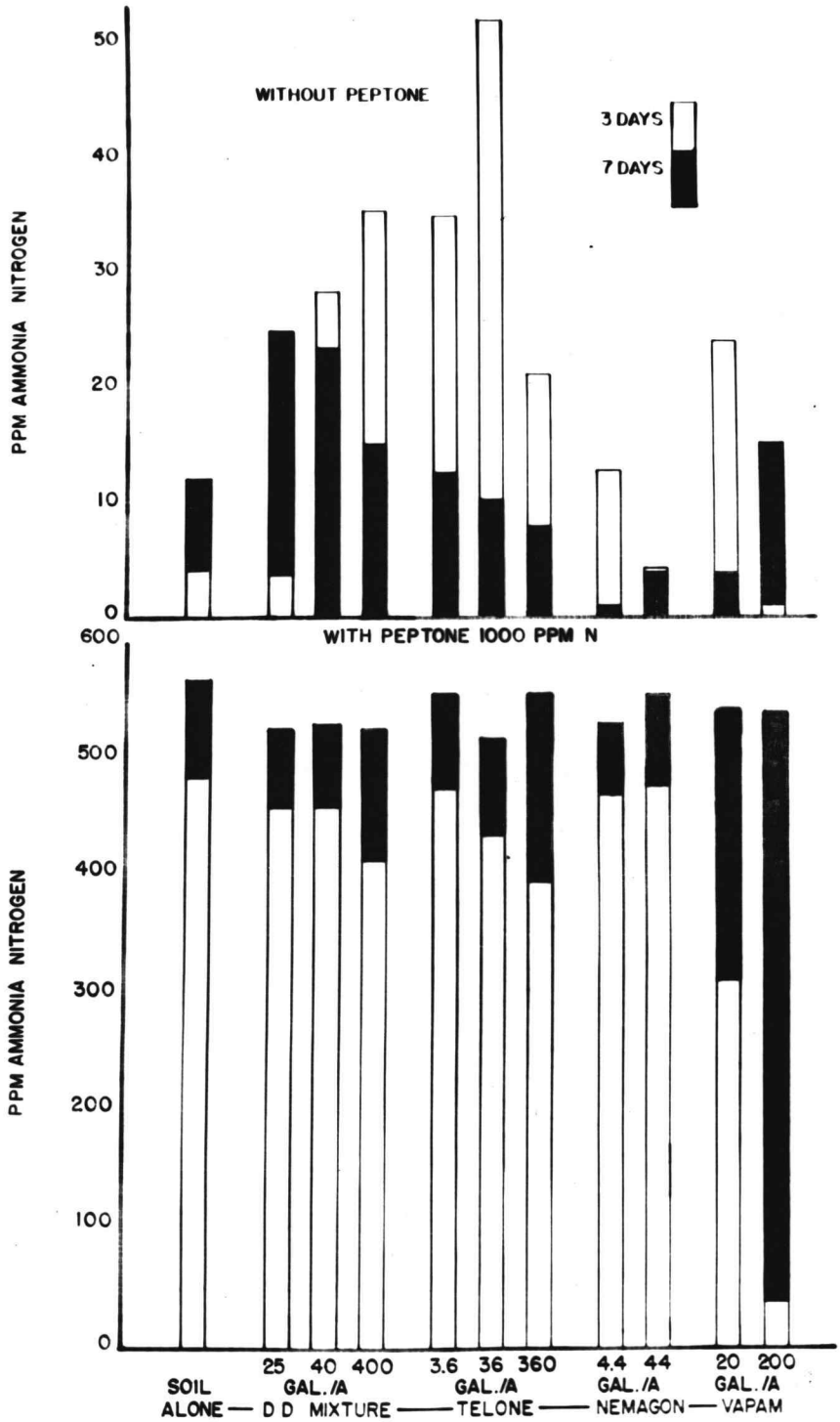

FIGURE: 11 INFLUENCE OF VARIOUS FUMIGANTS ON AMMONIFCATION OF CHEHALIS SILTY CLAY LOAM SOIL WITH AND WITHOUT PEPTONE.

minor variations, except that the concentration was distinctly depressed in the presence of Telone at 3.6 gal/A and Vapam at 200 gal/A. The effect of the lower rate of Telone appears anomalous, especially in comparison with the results at 7 days, but the influence of Vapam on nitrate formation is marked and prolonged as is shown also in the Nitrification study, Table 9, page 58 and Figure 12, page 59.

At 7 days nitrite concentrations were again insignificant in all cases. In soils without peptone, nitrate and ammonia nitrogen both generally were less than at 3 days. The relatively small differences do not correlate significantly with fumigant treatments. The ammonification of peptone at 7 days was slightly more than 50 per cent in all cases, regardless of fumigant or rate of application. Earlier depressive effects of D-D Mixture, Telone and Vapam were overcome at 7 days.

Nitrification

Experimental Methods

The preliminary procedures using ammonium sulfate instead of peptone were basically as in Ammonification. The treatments were made as outlined in Plan of Study, page 15. Two sets of each treatment in triplicate were incubated at 28° C. for periods of 14 and 28 days. At the end of each incubation time, each sample was diluted with distilled water to a 1:5 ratio and a uniform soil suspension was obtained by shaking for 10 minutes in an automatic

Table 9. Influence of Various Fumigants on Nitrification of Chehalis Silty Clay Loam Soil with and without $NH_4/_2SO_4$ @ 300 ppm N ≠ $CaCO_3$ at 5T/A

Treatment	pH		NO_2^- -N		NO_3^- -N		Total N (NO_2^- ≠ NO_3^- -N)		Increase over soil alone		Nitrification	
	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
			ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%	%
Soil Alone	6.9	6.9	t	t	12.9	13.0	12.9	13.0	-	-	-	-
≠ Vapam @ 20 gal/A	6.9	6.9	t	t	13.1	14.7	13.1	14.7	0.2	1.7	-	-
≠ D-D Mixture @ 25 gal/A	7.0	7.4	t	t	11.2	14.2	11.2	14.2	-1.7	1.2	-	-
≠ D-D Mixture @ 40 gal/A	7.4	7.4	t	t	10.2	11.3	10.2	11.3	-2.7	-1.7	-	-
≠ D-D Mixture @ 400 gal/A	7.4	7.4	t	t	8.3	13.8	8.3	13.8	-4.6	0.8	-	-
≠ Nemagon @ 4.4 gal/A	6.8	6.6	t	t	16.3	10.8	16.3	10.8	3.4	-2.2	-	-
≠ Nemagon @ 44 gal/A	6.7	6.5	t	t	16.8	12.5	16.8	12.5	3.9	-0.5	-	-
≠ Telone @ 3.6 gal/A	6.0	6.0	t	t	16.3	12.8	16.3	12.8	3.4	-0.2	-	-
≠ Telone @ 36 gal/A	5.9	6.0	t	t	13.0	10.3	13.0	10.3	0.1	-2.7	-	-
≠ Telone @ 360 gal/A	5.9	6.0	t	t	12.7	14.1	12.7	14.1	-0.2	1.1	-	-
Soil ≠ $NH_4/_2SO_4$ @ 300 ppm N ≠ $CaCO_3$ @ 5T/A	6.9	7.0	t	t	174.9	179.5	174.9	179.5	162.0	166.5	54.0	55.0
≠ Vapam @ 20 gal/A	7.1	7.0	54.3	t	16.1	189.4	70.4	189.4	57.5	176.4	19.1	58.8
≠ D-D Mixture @ 25 gal/A	7.4	7.4	t	t	119.1	157.7	119.1	157.7	106.2	144.7	35.4	48.2
≠ D-D Mixture @ 40 gal/A	7.4	7.4	17.0	t	116.2	155.0	133.2	155.0	120.3	142.0	40.1	47.3
≠ D-D Mixture @ 400 gal/A	7.4	7.4	t	t	112.7	166.7	112.7	166.7	99.8	153.7	33.2	51.2
≠ Nemagon @ 4.4 gal/A	6.3	6.3	81.0	t	4.3	161.7	85.3	161.7	72.4	148.7	24.1	49.5
≠ Nemagon @ 44 gal/A	6.3	6.5	1.2	t	18.7	205.0	19.9	205.0	7.0	192.0	2.3	64.0
≠ Telone @ 3.6 gal/A	7.4	7.1	t	t	118.0	210.0	118.0	210.0	105.1	197.0	35.0	65.6
≠ Telone @ 36.0 gal/A	6.6	6.7	4.0	t	90.0	245.0	94.0	245.0	81.1	232.0	27.0	77.3
≠ Telone @ 360.0 gal/A	7.4	7.0	t	t	13.3	131.7	13.3	131.7	0.4	118.7	0.1	39.5

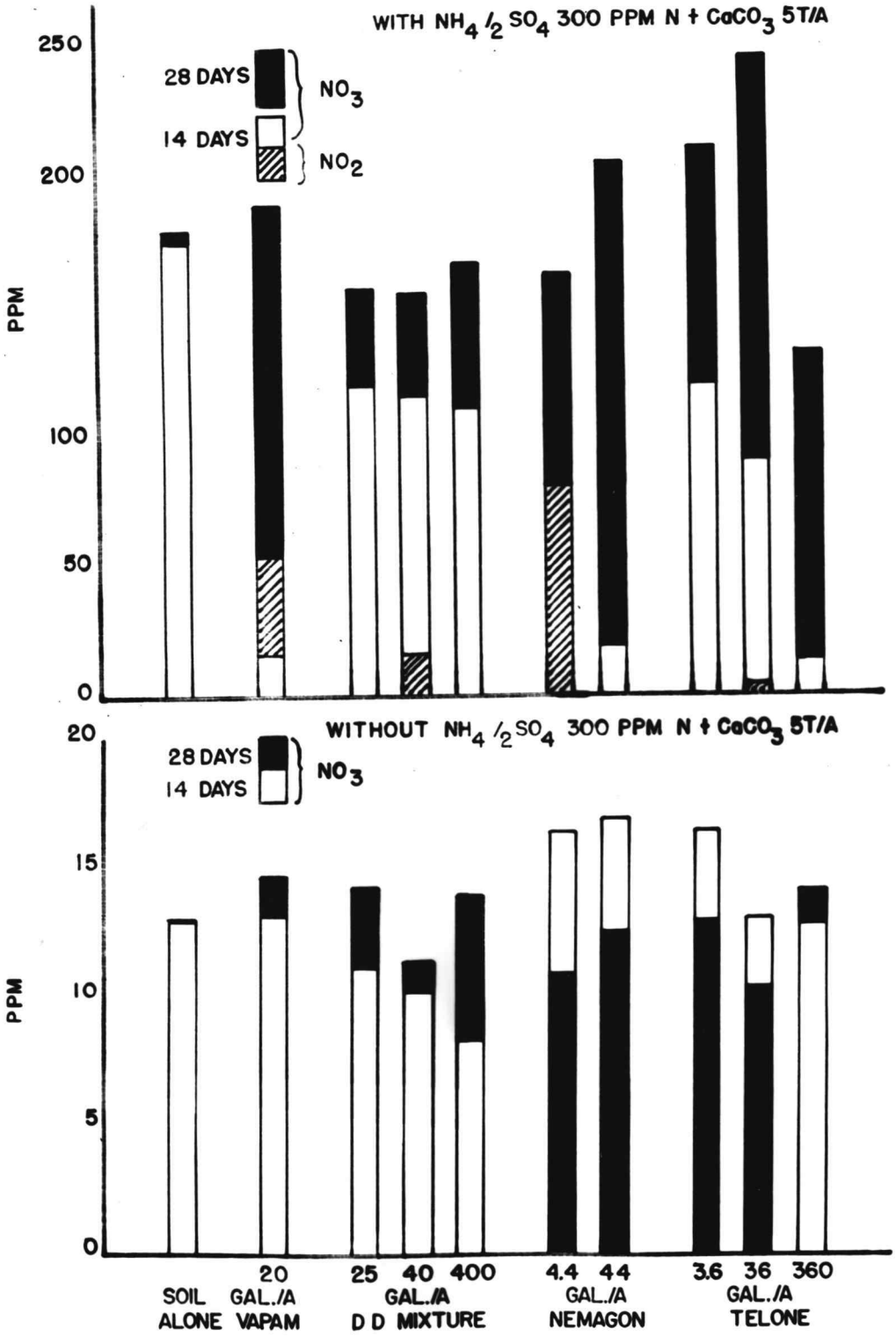

FIGURE: 12 INFLUENCE OF VARIOUS FUMIGANTS ON NITRIFICATION IN CHEHALIS SILTY CLAY LOAM SOIL.

shaker. A small portion of the suspension was used for pH readings, after which it was returned to the bottle. To determine NO_3^-- N and NO_2^-- N clarification of the soil suspension was made as follows: one gram of cupric acetate and 0.4 grams of calcium hydroxide were added, followed by vigorous shaking for one minute. The copper hydroxide thus formed functions as an effective clarifying agent. If the supernate showed a greenish-blue color, an excess of base was necessary to remove the last traces of copper. After a few minutes the solid in the bottles began to settle down, leaving a more or less clear liquid on top; this liquid was filtered through a dry Whatman No. 1 paper into a clean container. To the clear solution one gram of ammonium carbonate was added to remove any excess of calcium hydroxide. Once more filtration with a dry Whatman paper was required. The resultant liquid was clear and ready for nitrite-nitrogen and nitrate-nitrogen colorimetric determinations, which was accomplished according to the methods previously employed and mentioned on page 19.

Results and Discussion

Nitrification results are shown in Table 9 and Figure 12, pages 58 and 59, respectively.

The various fumigants had little influence on nitrification of the native nitrogen content of the soil. Only traces of nitrite were found at 14 and 28 days, and the differences in nitrate were not significant. Fumigant additions at different concentrations

caused no changes at the end of 14 and 28 days incubation period.

When ammonium sulfate @ 300 ppm N and calcium carbonate at 5 T/A were added to the soil marked differences occurred with the different fumigants. At 14 days soils with $NH_4/_2SO_4$ and no fumigant showed 54 per cent nitrification, while in the fumigant-treated soils, the nitrification ranged from 0.1 per cent with Telone at 360 gal/A (ten times the field rate) to 40.1 per cent with D-D Mixture at 40 gal/A. In some cases these low nitrification values were due to nitrite-nitrogen accumulation; Vapam and Nemagon especially were more inhibitive to nitrification than to nitrosofication. However this inhibitory property was lost within 28 days. At this time only traces of NO_2^-- N appeared and the previously low levels of NO_3^-- N increased, in some cases exceeding the control. Only D-D Mixture and Telone at 360 gal/A appreciably retarded the over-all nitrification. Nemagon at 44 gal/A and Telone at the lower rates, definitely stimulated formation of nitrates.

Sulfur Oxidation

Experimental Methods

Pint milk bottles and 80 gram soil samples were used as in the Ammonification and Nitrification experiments. Soil treatments consisted of flour sulfur addition at 1000 ppm and various fumigants at different rates. Incubation time was at 28° C. for 30 days. Loss of weight was made up by the addition of distilled water at 5-day intervals. At the end of the incubation period a 1/5 suspension of a

ten gram portion of soil, dry basis, was used for pH readings; after which it was returned to the respective bottle. Sulfate sulfur was then determined in a 1/5 extract of the entire sample clarified as explained under Nitrification, page 57, using the modified turbidimetric procedure of Schreiner and Failyer (34).

Results and Discussion

The results are shown in Table 10 and Figure 13, pages 63 and 64, respectively.

Soils without added sulfur but exposed to various fumigants at different concentrations showed no significant differences from the control in sulfate sulfur, which averaged 24.4 ppm. Sulfur added to the soil at 1000 ppm was 62.3 per cent oxidized in 30 days. D-D Mixture, Nemagon and Vapam added to the soil with sulfur had no appreciable effect on the extent of the oxidation. Telone at field and below field rates slightly inhibited sulfate production, while at ten times their field rate sulfur oxidation was greatly inhibited. From the practical standpoint it may be considered that any of these fumigants used at field rates will not significantly influence sulfur oxidation.

Table 10. Influence of Various Fumigants on Sulfur Oxidation in Chehalis Silty Clay Loam Soil with and without Sulfur at 1000 ppm

Treatments	pH	Sulfate S	Increase over control	S Oxidized
		ppm	ppm	%
Soil Alone	6.2	24.3	-	-
+ Vapam @ 20 gal/A	6.0	29.2	4.9	-
+ D-D Mixture @ 25 gal/A	6.0	33.3	9.0	-
+ D-D Mixture @ 38 gal/A	6.0	17.2	-7.1	-
+ D-D Mixture @ 41 gal/A	6.0	26.7	2.4	-
+ Telone @ 3.6 gal/A	6.2	21.3	-3.0	-
+ Telone @ 36 gal/A	6.0	20.7	-3.6	-
+ Telone @ 360 gal/A	5.8	22.0	-2.3	-
+ Nemagon @ 4.4 gal/A	5.9	24.3	-	-
+ Nemagon @ 44 gal/A	5.8	24.7	0.4	-
Soil + Sulfur @ 1000 ppm	4.6	646.8	622.5	62.3
+ Vapam @ 20 gal/A	4.5	690.0	665.7	66.6
+ D-D Mixture @ 25 gal/A	4.5	675.0	650.7	65.1
+ D-D Mixture @ 38 gal/A	4.5	723.0	698.7	69.9
+ D-D Mixture @ 41 gal/A	4.6	710.0	685.7	68.6
+ Telone @ 3.6 gal/A	4.8	583.0	558.7	55.9
+ Telone @ 36 gal/A	4.9	531.7	507.4	50.7
+ Telone @ 360 gal/A	4.8	79.2	54.9	5.5
+ Nemagon @ 4.4 gal/A	4.8	647.0	622.7	62.3
+ Nemagon @ 44 gal/A	4.9	648.0	623.7	62.4

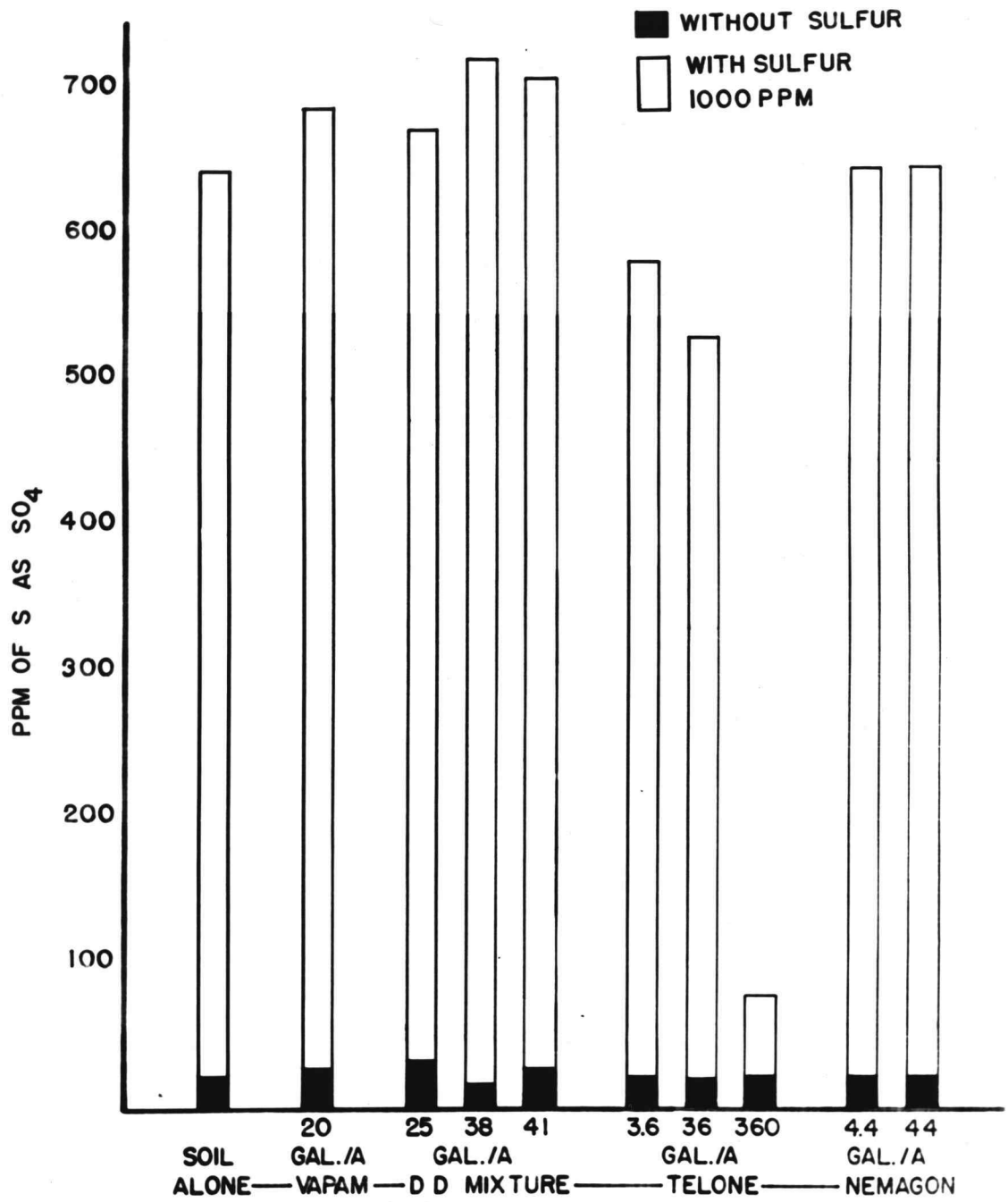

FIGURE: 13 INFLUENCE OF VARIOUS FUMIGANTS ON SULFUR OXIDATION IN CHEHALIS SILTY CLAY LOAM SOIL TREATED WITH AND WITHOUT SULFUR AT 1000 PPM.

PART IV. ZONES OF MICROBIAL INHIBITION

Experimental Methods

To further investigate the effects of fumigants on microbial activities the modified paper disc method devised by Vincent and Vincent (44, vol. 55, p. 162-164) was used. From stock cultures maintained in the Department of Bacteriology at Oregon State College, the organisms indicated in Tables 11 and 12 were selected as representatives of important morphological and physiological groups. One ml of a suspension from a 24-hour-old culture was transferred to 200 ml of an appropriate melted agar medium. Approximately 20 ml aliquots of the seeded medium were poured into 100 mm diameter petri dishes. Filter paper discs 1/2 inch in diameter were sterilized in the autoclave. The paper discs were immersed in aqueous solutions of the fumigants diluted to 10, 100, 1,000 and 100,000 ppm. When saturated they were removed from the liquid with sterile forceps; after shaking off the excess liquid the discs were placed on the hardened agar plates seeded with organisms. Special care was observed in placing the discs to avoid smearing the surface of the medium. Four discs were placed on each plate, each one representing a different fumigant concentration, the intervening space being ample to avoid any interference. This general procedure was followed for each of the organisms involved, as well as for each of the fumigants used. Triplicate plates of each treatment and controls were made. Plates were incubated at 28° C. The incubation time

Table 11. Microorganisms and the Types of Media used for the Determination of Inhibition Zones caused by the use of Fumigants at Different Concentrations

Microorganisms	Broth	Agar
Bacteria		
Azotobacter chroococcum	legume*	legume*
Rhizobium leguminosarum	legume*	legume*
Pseudomonas flourescens	nutrient**	nutrient**
Aerobacter aerogenes	nutrient**	nutrient**
Bacillus subtilis	nutrient**	nutrient**
Escherichia coli	nutrient**	nutrient**
Actinomycetes		
Streptomyces griseus	nutrient plus 0.5% glucose and traces of yeast extract	
Fungi		
Aspergillus niger	Sabourad's***	Sabourad's***
Penicillium chrysogenum	Sabourad's***	Sabourad's***
Torula cremoris	Sabourad's***	Sabourad's***
Mucor racemosus	Sabourad's***	Sabourad's***
Rhizopus sp.	Sabourad's***	Sabourad's***

* Legume medium was prepared according to the following recipe: sucrose 10 g., mannite 2 g., K_2HPO_4 0.5 g., $MgSO_4$ 0.1 g., NaCl 0.1 g., $CaSO_4$ 0.1 g., $CaNO_3$ 0.1 g., $CaCO_3$ 0.5 g., Agar 15.0 g. (agar omitted for broth), carrot juice 10 cc., yeast water 10 cc. and water 1,000 cc.

** Difco Manual, 9th edition, p. 238. 1953.

*** Difco Manual, 9th edition, p. 29, 32. 1953.

Table 12. Zone of Microbial Inhibition due to Vapam*

Microorganisms	Width of Inhibition Zone after 18 Hours	
	1,000 ppm	100,000 ppm
	mm	mm
Control	-	-
Bacteria		
Azotobacter chroococcum	1	8
Rhizobium leguminosarum	-	-
Pseudomonas flourescens	-	2
Aerobacter aerogenes	3	3
Bacillus subtilis	-	6
Escherichia coli	-	1
Actinomycetes		
Streptomyces griseus	2	6
Fungi		
Aspergillus niger	-	-
Penicillium chrysogenum	-	-
Torula cremoris	2	6
Mucor racemosus	-	-
Rhizopus sp.	-	-

* 10 and 100 ppm did not show any inhibition zones.

varied from 18 hours to 4 days, according to the organisms. The zones of inhibition were measured in mm from the edge of the microbial growth to the edge of the paper discs.

Results and Discussion

Since fumigant concentrations of 10 and 100 ppm showed no inhibitory zone effects, and since D-D Mixture, Telone and Nemagon produced no inhibitory zones at any concentration even after 10 days, these results are not tabulated. Only results with Vapam at 1,000 and 100,000 ppm are shown in Table 12, page 67.

Vapam showed inhibitory properties which started at 18 hours with some organisms and as late as 4 days with Azotobacter chroococcum and Rhizobium leguminosarum. Whatever the inhibition starting time, the effect was rapidly lost by microbial overgrowth which in most cases appeared more vigorous over the original zone of inhibition than elsewhere in the plate. It was suspected then that reasons for this behaviour were due, first, to acquired tolerance which involved rapid adaptation of the organisms to Vapam effects on the environment or, second, to rapid volatilization of the fumigant. The first assumption was discarded when the overgrowth around the disc was transferred to a new plate under the same conditions and repeated the results of an early inhibition zone followed by rapid overgrowth. The second assumption was also discarded when the same trend of behaviour was observed with cultures grown in a synthetic medium and to which Vapam was added as carbon

source. Volatilization of Vapam did not occur because the liquid efficiently retained the fumigant. It is most likely then that a condition of temporary "shock" occurred when the organisms were exposed to the effects of Vapam. The stimulatory effects which resulted afterwards could be ascribed to utilization of Vapam as a more favorable carbon source.

PART V. FUMIGANTS AS A CARBON SOURCE

Some stimulating effects of the fumigants on the general activity of soil microorganisms gave an indication that some of these chemicals might serve as possible source of carbon. If such were the case a possible explanation could be given for the rapid disappearance of certain fumigants from the soil by other than simple volatilization and could provide evidence for stimulatory effects observed in previous experiments.

Experimental Methods

The synthetic liquid medium of Ayers, Rupp and Johnson (3) was used as the base to which 1 per cent of the fumigants were added for the carbon source. The medium with 1 per cent dextrose was used as standard of comparison. Nutrient broth was used for control cultures. Escherichia coli, Bacillus subtilis, Streptomyces griseus and Penicillium chrysogenum were selected as representative soil microorganisms for the tests. Pure cultures of each of these organisms were grown in nutrient broth at 28° C. for 18 hours and the resulting growth was washed three times by centrifugation with sterile saline water. The washed organisms were resuspended in 15 ml of normal salt solution. Three drops of the suspension of each organism was used to inoculate tubes of the following:

1. Nutrient broth, to test the viability of the organisms after being washed and resuspended in saline solution.

2. Basal medium without source of carbon, to determine if the inoculum carried enough carbon source to permit growth.

3. Basal medium with dextrose as the carbon source, to determine the extent of growth in a favorable medium.

4. Basal medium with 1 per cent dextrose and 1 per cent addition of fumigant, to determine the effect of fumigant on microbial growth in the presence of a favorable source of carbon.

5. Basal medium with 1 per cent addition of fumigant without other carbon source, to determine availability of fumigant carbon.

All cultures were made in triplicate and uninoculated tubes were incubated as controls. Incubation was at 28° C. for 10 days. Observations were made daily for surface growth, turbidity and change in reaction.

Results and Discussion

The results are shown in Table 13, page 72.

All the test organisms grew vigorously in nutrient broth. Only Penicillium chrysogenum grew in the basal medium without added carbon source, the slight amount of growth indicating that some available carbon was carried in the inoculum.

Vapam - Results of the previous experiment, on zones of microbial inhibition indicated that Vapam might be a source of carbon for microbial growth. Vapam as the sole carbon source in basal medium was slightly inhibitory to all organisms at 18 hours but gave even better growth than dextrose alone at 5 and 10 days. Where Vapam and

Table 13. Fumigants as Carbon Sources

Medium	Blank	Escherichia coli			Bacillus subtilis			Streptomyces griseus			Penicillium chrysogenum		
		18 hrs	5 days	10 days	18 hrs	5 days	10 days	18 hrs	5 days	10 days	18 hrs	5 days	10 days
Nutrient broth	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Basal medium	-	-	-	-	-	-	-	-	-	-	+	+	+
B. M. + C	-	++	++	++	++	++	++	++	++	++	+++	+++	+++
B. M. + C + Vapam	-	++	++	+++	++	+++	+++	-	++	++	+	+++	+++
B. M. + C + D-D Mixture	-	+	++	++	+	+	+	-	-	-	+	++	++
B. M. + C + Nemagon	-	-	+	+	-	-	+	-	-	++	+	+	+
B. M. + C + Telone	-	+	+	+	-	+	+	-	-	-	+	+	++
B. M. + Vapam	-	-	+++	+++	-	+++	+++	-	+++	+++	-	+++	+++
B. M. + D-D Mixture	-	-	-	-	-	-	-	-	-	-	+	+	+
B. M. + Nemagon	-	-	+	+	+	+	+	-	-	+	-	-	+
B. M. + Telone	-	-	-	-	-	-	-	-	-	-	-	-	+

B. M. = Basal medium
C = carbon source as dextrose

+ = slight microbial growth
++ = moderate growth
+++ = heavy growth

- = indicates no growth

dextrose were added together a slight inhibitory effect was observed with Streptomyces griseus at 18 hours while the other organisms showed some growth. At 5 and 10 days the growth of all the organisms increased; however, in the case of Escherichia coli and Streptomyces griseus, this growth was not as abundant as in nutrient broth or with Vapam as the sole carbon source. This might indicate a certain degree of interference between Vapam and other sources of carbon.

D-D Mixture - When this fumigant was used as a sole source of carbon no growth of microorganisms occurred. The slight growth observed with Penicillium chrysogenum could not be attributed to D-D Mixture since the same occurred in the basal medium control. In the basal medium with dextrose, D-D Mixture caused complete inhibition of Streptomyces griseus and greatly retarded the growth of Escherichia coli, Bacillus subtilis and Penicillium chrysogenum.

Nemagon - Supported slight microbial growth when used as the sole source of carbon; however, it did not stimulate growth in the basal medium to which dextrose was added.

Telone - As a sole source of carbon did not support growth of any of the organisms. With glucose it retarded growth of Escherichia coli, Bacillus subtilis and Penicillium chrysogenum and was completely inhibitory to Streptomyces griseus.

Vapam and to some extent Nemagon can evidently support growth of microorganisms in the presence or absence of other carbon sources. Nemagon, D-D Mixture and Telone were more or less inhibitory. Of the

four fumigants used, Telone was the most germicidal, followed by D-D Mixture and Nemagon.

CONCLUSION

Microbial counts on soil to which Vapam was added reveal early stimulatory effects followed by an apparent toxicity manifested in lower counts. Respiration studies indicated no significant effects on decomposition of native soil organic matter, but when organic materials were added to the soil significant depressions in carbon dioxide evolution occurred. Field rates of Vapam, 20 gal/A, increased ammonification at 3 days while ten times this concentration decreased it. Vapam treatments of 20 and 200 gal/A inhibited ammonification of peptone; however, this inhibitory effect disappeared at 7 days. Nitrification of ammonium sulfate was temporarily inhibited by Vapam. Sulfur oxidation was unaffected by Vapam.

The various studies suggest that Vapam as a fumigant would support growth of the general soil microflora in a native environment but that it would interfere with oxidation of certain added energy sources. Inhibition zone studies on agar plates supported these views; the results indicated that Vapam definitely exerted stimulatory effect on the growth of some important soil microorganisms. Further studies established this fumigant as being a good source of carbon in a synthetic medium. With dextrose, however, Vapam caused some inhibition of Streptomyces griseus. It appears that a certain degree of interference exists if Vapam and other energy sources are used together.

D-D Mixture followed the general trend of early depression and late stimulatory effect, although these indications were not

consistent. Respiration experiments showed a late stimulation of decomposition of native soil organic matter, but with added dextrose a definite retardation of carbon dioxide production occurred. Ammonification depression in soils containing peptone by D-D Mixture was evident at 3 days but at 7 days the depressive effects were overcome. Of more significance is the fact that nitrification of ammonium sulfate was retarded by D-D Mixture, even up to 28 days. No effect on sulfur oxidation occurred. Inhibition studies with agar media were inconclusive. D-D Mixture used as a sole carbon source did not support microbial growth, while with dextrose the chemical completely inhibited the growth of Streptomyces griseus and significantly retarded the growth of Escherichia coli, Bacillus subtilis and Penicillium chrysogenum. From these experiments, it can be deduced that D-D Mixture is toxic to some important soil microorganisms, although this toxicity in the soil is not permanent.

Nemagon caused no appreciable effects on microbial numbers. It significantly stimulated organic matter decomposition as evidenced from respiration studies. It did not affect ammonification of peptone in Chehalis silty clay loam soil. Nemagon was temporarily inhibitive to nitrification; the inhibition disappeared within 28 days. Sulfur oxidation was unaffected. Inhibition zone studies showed no toxic effect to the organisms tested. Nemagon used as a sole carbon source in the synthetic medium supported slight microbial growth; however, some inhibition resulted when in combination with dextrose.

Plate counts for total numbers of microorganisms of soil treated with Telone were reduced in the first five days of the incubation period but later the organisms re-established themselves in greater numbers. Decomposition of native organic matter was enhanced by Telone. The fumigant significantly increased carbon dioxide evolution in soils to which dextrose was added; on the other hand depression was obtained with the less readily decomposable wheat straw. Similar trends were observed with soils in which the C/N ratio was brought up to 20/1 with ammonium nitrate. Ammonification was not affected by the fumigant at field rates; significant depression resulted with treatments above field rates but these effects disappeared in 7 days. Nitrification in soils to which ammonium sulfate was added was greatly retarded by Telone at ten times the field rate, even at 28 days. Sulfur oxidation inhibition with Telone at field rates was slight; however, higher concentrations proved to be significantly depressive. Zone inhibition studies with Telone resulted in no significant effects on the pure cultures tested. Toxicity of the fumigant was evidenced when it retarded the growth of Escherichia coli, Bacillus subtilis and Penicillium chrysogenum and completely inhibited the growth of Streptomyces griseus in a synthetic medium to which glucose was added.

SUMMARY

Investigations were undertaken to determine the influence of Vapam, D-D Mixture, Nemagon and Telone upon the soil microflora and their activities. Several microbial physiological processes were studied in Chehalis silty clay loam soil, and representative soil microorganisms were isolated and exposed directly to the fumigants. The fumigants were used at field rates and also in concentrations ten times lower and ten times higher.

The results indicate that the fumigants at field rates did not significantly interfere with the various microbial activities important in soil fertility. Higher fumigant concentrations in some cases caused temporary detrimental effects. Vapam and Nemagon enhanced microbial activities, while D-D Mixture and Telone caused temporary depressive effects.

It can be concluded that these fumigants can safely be used at field rates without detriment to soil microorganisms involved in transformations essential to soil fertility.

BIBLIOGRAPHY

1. American Public Health Association. Standard method for the examination of water, sewage and industrial wastes. New York, the Association, 1958. 522 p.

2. Association of Official Agricultural Chemists. Official and tentative methods of analysis. Washington, D. C., 1955. 108 p.

3. Ayers, S. H., P. Rupp and W. T. Johnson. Study of the alkali forming bacteria found in milk. Washington, 1919. 39 p. (U. S. Department of Agriculture. Professional paper No. 782)

4. Baines, R. C. Results with Vapam to control nematodes, fungi. California Citrograph 42:192. April 1957.

5. Bollen, W. B. Soil respiration studies on the decomposition of native organic matter. Iowa State College Journal of Science 15:353-374. 1941.

6. Bollen, W. B., H. E. Morrison and H. H. Crowell. Effect of field and laboratory treatments with BHC and DDT on nitrogen transformations and soil respiration. Journal of Economic Entomology 47(2):302-312. 1954.

7. Carter, W. A. A promising new soil amendment and disinfectant. Science 97:383-384. 1943.

8. _____ Soil treatments with special reference to fumigation with D-D Mixture. Journal of Economic Entomology 38:35-44. 1945.

9. Cooper, S. C. The mixed indicator bromcresol green methyl red for carbonates in water. Industrial Engineering Chemistry (Analytical Edition) 13:466-470. 1941.

10. Christie, J. R. Soil fumigation for control of nematodes and other soil inhabiting organisms. Plant Disease Report, Supplement 170:170-189. 1947.

11. Dalton, F. H. and C. H. Hurwitz. Effect of volatile disinfectants on survival of microflora in the soil. Soil Science 66:233-238. 1948.

12. Dransfield, M. The effects of tetrachloronitro-benzene on the soil microflora. Transaction of the British Mycology Society 40:165. 1957. (Abstracted in Soils and Fertilizers 21(1):32. 1958)

13. Eno, C. F. and P. H. Everett. Effect of soil applications of ten chlorinated hydrocarbon insecticides on soil microorganisms and the growth of Stringless Black Valentine Beans. Soil Science Society of America, Proceedings 22(3):235-238. 1958.

14. Fenwick, D. W. et al. Effect of repeated field injections of D-D Mixture against potato-root eelworm. Annual of Applied Biology 40:208-214. 1953.

15. Gamble, S. J., C. J. Mayhew and W. E. Chappell. Respiration rates and plate counts for determining effects of herbicides on heterotropic soil microorganisms. Soil Science 74:347-350. 1956.

16. Godfrey, G. H. Control of soil fungi by soil fumigation with Chloropicrin. Phytopathology 26:246-256. 1936.

17. Harper, H. J. The accurate determination of nitrates in soils. Journal of Industrial and Engineering Chemistry 16:180-183. 1924.

18. Hauke-Pacewiczowa, T. The effect of the insecticide BHC on the soil microflora. Roczniki Nauk Rolniczych 76:640-657. 1957. (Abstracted in Soils and Fertilizers 21(3):175. 1958.)

19. Johnson, M. O. The pineapple. Honolulu, Hawaii, Paradise of the Pacific Press. 1935. 306 p.

20. Jones, L. W. Are insecticides toxic to soil microorganisms? Farm and Home Science, September 1950, p. 58-59.

21. Kincaid, R. R. and G. M. Volk. Effect of soil fumigation on cigar-wrapper tobacco and soil nitrogen. Gainsville, Florida, 1952. (Florida. Agricultural Experimental Station. Bulletin 490)

22. Klemmer, H. W. Response of bacterial, fungal and nematode population of Hawaiian soils to fumigation and liming. Bacteriological Proceedings 57:12. 1957. (Abstracted in Soils and Fertilizers 21(1):33. 1958.)

23. McClellan, W. D., J. R. Christie and N. L. Horn. Effect of temperature and moisture on the efficiency of soil fumigants. Phytopathology 37:440. 1947.

24. MacLeod, D. J. and J. L. Howatt. Soil treatment in control of certain soil borne diseases of potatoes. American Potato Journal 11:60-61. 1934.

25. Martin, J. P. Effects of fumigation and other soil treatments in the greenhouses of the fungus population of old citrus soil. Soil Science 69:107-122. 1950.

26. Martin, J. P. and D. G. Aldrich. Effect of fumigation on soil aggregation. Soil Science Society of America, Proceedings 16: 201-203. 1952.

27. Miller, P. R. D-D fumigation of soil for control of parasitic nematodes. Agricultural Chemicals 11:53. 1956.

28. Nelson, R. T. Studies of microbial activity, chlorate reduction and chlorate toxicity in soil treated with sodium chlorate. Journal of Agricultural Research 68:221-237. 1944.

29. Newman, A. S. Herbicides and the soil. Journal of Agricultural and Food Chemistry 6(5):352. 1958.

30. New Stauffer pesticide. Agricultural Chemicals 10:117. 1955.

31. Olsen, S. R. et al. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. Washington, 1954. 19 p. (Department of Agriculture. Circular no. 939)

32. Ross, H. F. Effect of DDT, chlordane and aldrin on nitrification and ammonification in arredondo fine sand. Soil Science Society of Florida 11:58-61. 1952.

33. Schollenberger, C. J. and R. H. Simon. Determination of exchange capacity and exchangeable basis in soils. Soil Science 59:13-24. 1945.

34. Schreiner, Oswald and George H. Failyer. Colorimetric, turbidity and titration methods used in soil investigation. Washington, 1906. 60 p. (U. S. Department of Agriculture. Bureau of Soils. Bulletin no. 31)

35. Shrikhande, J. G. Determination of ammonical and nitrate nitrogen in decomposed plant materials. Industrial Engineering Chemistry (Analytical Edition) 13:187-188. 1941.

36. Smith, Nathan R., Virginia T. Dawson and Marie E. Wenzel. The effects of certain herbicides on soil microorganisms. Soil Science Society of America, Proceedings 10:197-201. 1945.

37. Stark, F. L. Investigations of Chloropicrin as a soil fumigant. Ithaca, New York, Cornell University, 1948. 61 p. (New York. Agricultural Experimental Station. Memoir 278)

38. Stark, F. L., J. B. Smith and F. L. Howard. Effect of Chloropicrin fumigation on nitrification and ammonification in soil. Soil Science 48:433-442. 1939.

39. Stark, F. L. and B. Lear. Miscellaneous greenhouse tests with various soil fumigants for the control of fungi and nematodes. Phtyopathology 37:698-711. 1947.

40. Starkey, R. L. and S. A. Waksman. Partial sterilization of the soil, microbiological activities and soil fertility. Soil Science 16:137-157. 1923.

41. Stoklasa, J. A. A contribution to the knowledge of the determination of soil fertility. International Review of Science and Practical Agriculture 4(3):589-599. 1926.

42. Tam, R. K. The comparative effects of a 50-50 mixture of 1:3 dichloropropane and 1:2 dichloropropene (D-D Mixture) and of Chloropicrin on nitrification in the soil and on growth of the pineapple plant. Soil Science 59:191-205. 1945.

43. Teater, R. W., J. L. Mortensen and P. F. Pratt. Effect of certain herbicides on rate of nitrification and carbon dioxide evolution in soil. Journal of Agricultural and Food Chemistry 6(3):214-216. March 1958.

44. Vincent, James C. and Helen Whitergrove Vincent. Filter paper disc modification of the Oxford Penicillin Cup penicillin determination. Society for Experimental Biology and Medicine 55:162-164. 1944.

45. Waksman, S. A. Principles of soil microbiology. New York, Williams and Wilkins, 1932. 894 p.

46. Waksman, S. A. and E. B. Fred. A tentative outline of the plate method for determining the number of microorganisms in the soil. Soil Science 14:27-28. 1922.

47. Wilson, J. K. and R. S. Choudri. Effects of DDT on certain microbiological processes in the soil. Journal of Economic Entomology 39:537-538. 1946.

48. _____ The effect of Benzene Hexachloride on soil organisms. Journal of Agricultural Research 77:25-32. 1948.

49. Woodruff, C. M. Testing soils for lime requirement by means of a buffered solution and the glass electrode. Soil Science 66:53-64. 1948.