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Title: EFFECT OF ENDOTHAL AND CERTAIN OTHER SELECTIVE HERBICIDES ON MICROBIAL ACTIVITY IN SIX DIFFERENT SOILS

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The effect of Endothal (3, 6-endoxyhexahydrophthalic acid), sodium pentachlorophenate (Na-PCP) and TD-47 (di-N, N-dimethyl-cocoamine salt of Endothal) on the microbial populations, ammonification, nitrification and respiration in six different soils was investigated. It was found that Endothal at 20 and 200 ppm had no adverse effect on the microbial population, ammonification, nitrification and respiration, whereas treatments with Na-PCP at 40 ppm and combined with Endothal were found to be suppressive. TD-47 at 100 ppm had a stimulatory effect on molds and bacteria in sandy soils. Microbial respiration was measured by three methods: continuous aeration, periodic aeration, and oxygen uptake by Warburg respirometers. Essentially, similar pattern of results were obtained from these methods. In sandy soils Endothal at 20, 200 and 2000 ppm had a

bimodal inhibiting effect on oxygen uptake.

Two bacteria and one fungus, capable of utilizing Endothal and TD-47 as a sole carbon source, were isolated, characterized and identified.

Using four unialgal species to evaluate eight herbicides as potential algicides, it was found that Na-PCP, TD-47 and Diuron (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea) were most effective on Chlamydomonas reinhardti Dangeard, Chlorella pyrenoidosa Chick, Anabaena cylindrica Lemmerman, and Nostoc muscorum Kütz. Endothal was not significantly inhibitive.

EFFECT OF ENDOTHAL AND CERTAIN OTHER SELECTIVE HERBICIDES ON MICROBIAL ACTIVITY IN SIX DIFFERENT SOILS

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I. INTRODUCTION

The control of weeds has progressed from crude unsophisticated compounds, such as common salt, to the more complex selective herbicides represented by 2, 4-dichlorophenoxyacetic acid and Endothal. The usage of herbicides in the United States has rapidly increased, where in 1962 over 70 million acres were treated. The chemical control of weeds has lowered production costs, reduced the amount of hand labor, increased mechanized production and raised the productivity on nontilled croplands. These results are reflected in our strong economy and better standard of living. However, problems unknown before the advent of herbicides have occurred with the increased usage of these chemicals.

Foremost is the residue of the herbicide in the soil. Associated problems with soil residues are: movement by leaching or volatization; retention of active or inactive forms of the herbicide; chemical reaction between the herbicide and different soil components; the rate and nature of herbicidal degradation, chemical, biological or physical; and the persistence and effect of the herbicide under different environmental conditions on successive crops and plants.

In this thesis, the primary interests are the effects of Endothal

on the biological activities in six different soils and the identification of microorganisms which can utilize Endothal as a carbon source. Two other herbicides, sodium pentachlorophenate and TD-47, a derivative of Endothal, were also studied.

II. REVIEW OF LITERATURE

Soil is a dynamic-complex system, abounding with microscopic life which plays important roles in soil fertility. It is therefore imperative to ascertain any influence on the soil microbiological processes when pesticides are incorporated into this regime.

The present review considers only the following aspects:

1. Influences of herbicides on microbial activities:
   a. groups of microorganisms
   b. microbial processes.
2. Chemical and biological influences on herbicides:
   a. inactivation of herbicides
   b. activation of herbicides
   c. potentiation or retardation of herbicidal action.
3. Influences of soil properties on the persistence of the herbicides:
   a. Endothal
   b. Sodium pentachlorophenate (Na-PCP)
   c. Penco Herbicide TD-47 (di-N, N-dimethylcocoamine salt of Endothal 47).
4. Herbicides and algae:
   a. effect of herbicides on algae
   b. relationship of algae to other microorganisms in the soil.

Influences of Herbicides on Microbial Activities

When an herbicide is added to soil a variety of responses ranging from stimulative to depressive effects on soil microorganisms and microbial processes may result. Such anomalies are not uncommon and have already been affirmed by several workers (Smith, Dawson, and Wenzel, 1945; Elkan and Moore, 1960; Virag, 1959; Chandra and Bollen, 1961; Corden and Young, 1965).

Excellent reviews are available on the interaction between pesticides and soil microorganisms (Bollen, 1961), on soil fungicides (Domsch, 1964), and on the influence of pesticidal residues on the properties of soil (Martin, 1963).

Groups of Microorganisms

An example of an herbicide exerting many influences on groups of soil microorganisms is 2, 4-D (2, 4-dichlorophenoxyacetic acid). At concentrations up to 100 ppm, it was found to stimulate the growth of molds and bacteria (Egorova, 1955; Andersen and Baker, 1950; Newman and Downing, 1958; Roberts and Bollen, 1955; Paixao and Dobereiner, 1955). After spraying the soil with 2, 4-D at 5 lbs. per acre, Nair, Menon, and Mariakulandai (1957) noted that there was a marked increase in microbial numbers 15 to 30 days after treatment and that the effect persisted for some 45 days. However, Stevenson

and Mitchell (1945) discovered that 800 ppm of 2, 4-D in agar medium prevented the growth of Bacillus subtilis and Staphylococcus aureus, whereas the growth of Fusarium and Penicillium sp. was not noticeably affected. Besides these expressed effects, Smith, Dawson and Wenzel (1945) demonstrated that concentrations of 2, 4-D ranging from 0.5 to 500 ppm added to a sandy soil of good fertility did not greatly influence the microbial population. Baldacci and Amici (1954) and Baldacci (1955) upon testing some forty-one strains of Actinomyces concluded that only eight strains failed to grow in the presence of 2, 4-D at 400 ppm.

It is generally agreed that 2, 4-D applied at the prescribed agricultural rates have no adverse effects on the total number of microorganisms in the soil (Teater, Mortensen and Pratt, 1958; Kratochvil, 1950, 1951).

Microbial Processes

Koike and Gainey (1952), and Newman and Downing (1958) found that at recommended rates of application 2, 4-D had an initial inhibitory effect on nitrification but that this was followed by recovery. Fleig (1952) ascertained that under favorable conditions for microorganisms 2, 4-D was inactivated within a few weeks in soils and that ammonification and nitrification were unaffected. However, in nutrient solutions nitrification was severely checked by 3 ppm of

2, 4-D, but the addition of soil almost completely counteracted this effect. Slepecky and Beck (1950), studying the effect of 2, 4-D on nitrification by the soil percolation method, observed that the conversion of ammonium nitrogen to nitrate nitrogen was initially completely inhibited by 50 ppm of 2, 4-D. However, prolonged percolation resulted in reappearance of the nitrification reaction to a level as high as the control. This recovery was either a result of adaptation of the nitrifying bacteria to 2, 4-D or of destruction of the herbicide by microbiological activity in the soil. Shaw and Robinson (1960) discovered that 2, 4-D applied at rates of 5 and 50 ppm on Cumberland silt loam soil did not inhibit nitrification. At 100 ppm 2, 4-D Smith, Dawson and Wenzel (1945) reported that nitrification was repressed, but recovery occurred within 19 to 40 days. This indicated a possible bacteriostatic action.

Wolcott, et al., (1960) found that Telone fumigant treatments caused inhibitions resulting in reduced nitrate levels and large accumulation of ammonia. There was a lag period of seven to eight weeks in nitrification; the significance of this was an alteration in the seasonal distribution of ammonium and nitrate nitrogen in the field.

Though nitrification was unaffected at field rates of silvex (2-(2, 4, 5-trichlorophenoxy)propionic acid), oxygen uptake was inhibited at concentrations up to 200 ppm (Hale, et al., 1957). Whiteside and Alexander (1960), using Warburg respirometers, noted that

2, 4-D and 2, 4, 5-T(2, 4, 5-trichlorophenoxyacetic acid) at concentrations up to 100 ppm did not prevent oxygen uptake in a silt loam soil. Determinations of gas pressure evolved through the activity of the soil-borne bacteria and fungi were made by Kratochvil (1951) on a silt loam soil treated with 2, 4-D and 2, 4,5-T at several rates of application. He concluded that these compounds had no significant influence on the relative microbial activity of the soil. Teater, et al., (1958) reported that normal field rates of 2, 4-D, CIPC (isopropyl N-(3-chlorophenyl) carbamate, CDEC (2-chloroallyl diethyldithiocarbamate) and CDEA (2-chloro-N, N-diethylacetamide) had little effect on nitrification and carbon dioxide evolution, and that higher rates inhibited nitrification but increased carbon dioxide evolution from the soil. They explained this inverse relationship between nitrate accumulation and carbon dioxide evolution as a "stimulative" effect by the selectivity of these compounds. When herbicides were added to the soil they probably caused a bacteriostatic action on some organisms, allowing more tolerant organisms to multiply more rapidly. This increased activity, in an otherwise resting population, could cause a temporary net increase in the carbon dioxide evolution because organisms in an active state of reproduction and growth have greatly increased respiration rates with a concomitant immobilization of nitrogen. The latter occurrence is noted by a reduction in nitrate accumulation.

Chandra, et al., (1960), studying the effect of 5 and 100 ppm of Diuron (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea), 2-chlorobenzoic acid, and ethyl N, N-di-n-propylthiolcarbamate on respiration in nine widely different soils, reported that CO_2 evolution was decreased for at least 28 days, that this inhibition was reduced considerably at the end of 56 days, and that the results were significantly influenced by soil type. Pochon, et al., (1960) established that CO_2 evolution was not significantly affected by field rates of Simazine (2-chloro-4, 6-bis(ethylamino)-s-triazine) and other aminotriazine herbicides.

Nematocides have been reported to greatly increase the number of bacteria and Streptomyces after an initial depression; to inhibit the growth of molds for long periods; and to promote ammonfication and depress nitrification (Teuber and Poschendrieder, 1964).

In laboratory experiments with loess and sandy loam soils, Strzelczyk and Strzelczyk (1963) showed that certain insecticides and fungicides applied at the usual agricultural rates had no harmful effect on the number of soil bacteria and streptomycetes, but the fungicides considerably reduced the number of soil fungi. Insecticides at doses ten times higher than normally used stimulated bacterial and streptomycete development, whereas fungicides at similar doses inhibited the development of soil fungi and streptomycetes. Fungicides generally decreased the number of ammonifying and nitrifying bacteria.

Chemical and Biological Influences

On Inactivation of Herbicides

Herbicides are lost or become inactivated in soil through such non-biological means as volatilization and temperature fluctuations, cropping, leaching, pH changes, and adsorption by organic matter and clay.

Hartley (1964) presents an excellent review on the loss of herbicides by non-biological decomposition, evaporation and cropping. He stated that slow hydrolysis was a common reaction in non-biological decomposition; for example, the amide (-CO-NH-) grouping of the phenylureas and -C-Cl, -C-O-CH_3 and -C-S-CH_3 groupings of the substituted triazines were subject to hydrolysis in sterile solution. The reactions proceeded at a significant speed only under strong acid or alkaline conditions, but these substances in moist soils mainly exist adsorbed on clay or organic matter surfaces. Adsorption can either retard or accelerate chemical reaction. Also, the extensive contact with atmospheric oxygen could lead to more rapid oxidation, a type of reaction more likely, than hydrolysis, to be affected by adsorption. The significance of adsorption with implications concerning bioactivity of herbicides is clearly presented by Bailey and White (1964). Schatz, et al., (1964 a, b) proposed a theory of chelation between organic matter in soil and an ion added to the soil,

resulting in formation of a metallo-humic complex which activates or inactivates the compound.

Adsorption of Neburon (1-n-butyl-3-(3, 4-dichlorophenyl)-1-methyl urea) by soil was highly correlated with organic matter (Obien, et al., 1966). Dubey, et al., (1966), investigating the effect of soil properties on the persistence of Linuron (3-(3, 4-dichlorophenyl)-1-methoxy-1-methylurea) and Diphenamid (N, N-dimethyl-2, 2-diphenyl-acetamide), concluded that organic matter was the greatest factor accounting for the disappearance of these herbicides in soils. McKaren, et al., (1963) discovered that organic herbicides were broken down most rapidly in soils rich in organic matter and under conditions of high moisture and high temperature. Thiegs (1955) demonstrated with soils of different texture that Dalapon (Sodium 2, 2-dichloropropionate) at 50 ppm decomposed more rapidly with increasing temperature and moisture. A similar pattern for TCA (trichloroacetic acid) was noted by Loustalot and Ferrer (1950) in which inactivation was most rapid at warmer temperatures and higher moisture in a sandy soil. The significant influence of temperature was clearly shown by McCormick and Hiltbold (1966) for the decomposition of Atrazine (2-chloro-4-ethyl-6-isopropylamine-s-triazine), which doubled at each 10^{o} rise in temperature from 10^{o} to 30^{o}C., paralleling the response of soil organic-matter decomposition.

Microorganisms play a major role in the detoxification of herbicides in the soil (Thiegs, 1962). Several genera of fungi, including Penicillium roqueforti and the Aspergillus flavus group were reported to degrade herbicides and Quercitrin (quercetin-3-L-rhamnoside) (Jensen, 1957, and Westlake, 1962). Rogoff and Reid (1954) isolated a Corynebacterium which decomposed 1000 ppm of 2, 4-D within three days. Audus (1949), by a soil percolation technique, showed that the detoxification of 2, 4-D was due almost entirely to the activity of microorganisms and not to chemical oxidation. Several Pseudomonas species were reported to attack substituted urea herbicides (Douros, 1956). In pure culture experiments, Chacko, et al., (1966) detected that Streptomyces aureofaciens degraded pentachloronitrobenzene (PCNB). Hirsch and Alexander (1960) isolated and characterized five strains of Norcardia which decomposed Dalapon (2, 2-dichloropropionic acid).

Holstum and Loomis (Weeds, 1956) pointed out the importance of indirect factors which influence the decomposition of herbicides. They reported that the decomposition of Dalapon in soils was primarily a function of the microbial population; factors influencing the decomposition rate, such as temperature, moisture, pH, and soil type, were considered to act indirectly by affecting the activity of the microorganisms in the soil. Low moisture, low pH, temperatures below 20°C., and large additions of organic matter inhibited

the destruction of the herbicide. Donaldson and Foy (1965) concluded that Amiben (3-amino-2, 5-dichlorobenzoic acid) was more rapidly decomposed by microbes in organic soil than in clay or sandy soil. The influence of soil type on the effectiveness of Atrazine and Simazine on soil microflora was reported by Mashtov and his associates (1962): herbicides added to a peat-podzol soil brought about marked quantative changes, and particularly qualitative alterations of certain groups of bacteria, actinomycetes and fungi. A marked inhibition was seen in the fluorescent bacteria group. Atrazine and Simazine at rates of 75 kg. per hectare were observed to be non-toxic for the microflora in peat-podzolic soil, and they increased the activity of the main groups of microorganisms. Therefore, they concluded that the character and rate of such an effect are influenced by the soil type. Morita and Aoki (1952) discovered that 2, 4-D sometimes suppressed and sometimes stimulated ammonification, depending on the soil type.

Activation of Herbicides

Microorganisms, besides being affected by a pesticide, may activate it, as in 2, 4-DES (Sodium 2-2, 4-dichlorophenoxyethyl sulfate). 2, 4-DES itself is not active as an herbicide but is converted in the soil by Bacillus cereus var. mycoides to either 2, 4-D or to 2-(2, 4-dichlorophenoxy)-ethanol which then prevents the growth of weeds.

Sodium 2-2, 4, 5-trichlorophenoxyethyl sulfate (2, 4, 5-TES) resembles 2, 4-DES in that it is converted to an active form in the soil. Gamble, et al., (1952) reported that 2, 4-DES at 2 lbs. per acre had no effect on the respiration rate nor on the number of soil microorganisms when the soil was sampled at one month after treatment. Kratochvil (1950, 1951) found that at rates up to 8 lbs. per acre 2, 4-DES had a stimulatory effect on soil microorganisms. Hale, et al., (1957) also reported increased oxygen uptake by soil microorganisms with 2, 4-DES concentrations from 50 to 150 ppm.

Fields and Hemphill (1966) found no adverse effect of Zytron (0-2, 4-dichlorophenyl-0-methyl isopropyl phosphoramidothioate), an herbicide, on molds, actinomycetes and soil bacteria. At levels above 10 ppm, its degradation product, 2, 4-dichlorophenol, was toxic to molds. Aspergillus clavatus degraded both Zytron and 2, 4-dichlorophenol; another degradation product, Sodium o-methyl isopropyl phosphoramidothioate, stimulated the growth of a species of Penicillium.

Potentiation or Retardation of Herbicidal Action

Certain herbicides have been reported to be more effective when applied in combination than singly. Kaufman (1966) noted that the microbial degradation of Dalapon in soil was inhibited in the presence of Amitrole (3-amino-1, 2, 4-triazole), which rapidly

disappeared. Phytotoxic residues persisted longer in soils when the herbicides were applied together than when they were applied alone. Dalapon did not affect the behavior of Amitrole in muck soil, but affected the availability or persistence of Amitrole applied at higher rates to a silty clay loam soil. In laboratory studies Koike and Gainey (1952) found no appreciable effect on nitrifying bacteria and plate counts when CADE (concentrated activated diesel emulsion) consisting of 67 percent diesel oil and 33 percent pentachlorophenate, 2, 4-D, and combinations were applied at field rates in loam soils. Nitrification was inhibited from 8 to 16 weeks at rates of 50 lbs. of 2, 4-D and 5000 gallons of CADE. Total plate counts of bacteria were greatly increased by 2, 4-D at 25 and 50 lbs., CADE at 500 to 5000 gallons, and their combinations at 1000 gallons per acre. Klyuchnikov and Petrova (1960) applied four applications of 2, 4-D, two of fuel kerosine and one of 2 M-4Kh to a chernozem soil. They found that the compounds did not affect the number of non-spore forming bacteria in the oak rhizosphere but decreased the number of actinomycetes and increased the total mold count. The predominant fungus in the rhizosphere was Trichoderma in the control plots and Penicillium in the treated plots.

Influence of Soil Properties on the Persistence of the Herbicides

Endothal (3, 6-endoxyhexahydrophthalic acid)

Comes, et al., (1961), using three Wyoming soils, sandy clay loam, fine sandy loam and clay loam, studied the movement pattern and persistence of Endothal as influenced by soil texture, temperature and moisture content. They found that Endothal was more resistant to leaching in clay loam and sandy clay loam than in sandy loam soil. The two finer textured soils retained a greater amount of Endothal in the surface two inches than at any other depths tested. Soil moisture content was a highly critical factor in the disappearance of Endothal. Air-dried soils which were treated with 6 lbs. of Endothal per acre and held in an air-dried condition for 8 weeks before the planting of flax, yielded no flax when irrigated. Results were similar for all temperatures employed. Temperature was very influential on the inactivation rate of Endothal in soils that were wetted to 80 percent of field capacity. Inactivation was 12 times more rapid at 20° than at 15°C., one week after treatment. There was no significant difference in the persistence of Endothal at 20, 25 and 30°C., nor between the two lower temperatures, 10 and 15°C. Accordingly, the disappearance of Endothal was slower in clay loam soil than in sandy loam and sandy clay loam soils which were wetted.

A sufficient breakdown occurred within 14 days after treatment in sandy soils held at 20°C. and higher, to allow weed seeds to germinate and grow. Endothal in clay loam soil did not exhibit any measurable degree of inactivation until 28 days after treatment. Inactivation of the herbicide was completed 42 days after treatment in all soils and at all temperatures employed.

Horowitz (1966), studying the breakdown of Endothal on a Neve-Yaar clay soil of Israel, observed that the residual activity of Endothal persisted considerably longer on dry soil than on moist soil. This agreed with the results of Comes and associates (1961). In his experiment on temperature effect, he noted that the rate of degradation was slower at 30°C. than at 10°C. which was the reverse from that reported by Comes, et al., (1961). Horowitz explained this difference by the possibility that the optimum temperature for the development of microorganisms involved in the breakdown of Endothal in the Neve-Yaar soil might be lower than in the Wyoming soils, as well as the possible involvement of adsorption-desorption relationships proposed by Freed, Montgomery and Nance (1963). In another experiment, the effect of repeated application of Endothal on the final herbicidal activity was studied. It appeared that this activity was markedly higher in the soil which received one treatment than in the soil treated twice. This finding could possibly be explained by the phenomenon of microbiological adaptation or to a proliferation of

degrading microorganisms in response to the first application of Endothal. The second application was more rapidly inactivated than on soil treated once (Audus, 1960).

In laboratory experiments, Freed and Montgomery (1963) demonstrated that under optimum conditions for microbiological activity, Endothal was completely degraded within seven to fourteen days. With C^{14} label Endothal and a chromatogram paper scanning technique they (1964) studied the metabolic products in soil. They found that little, if any, Endothal remained after 48 hours. At this time there appeared to be at least two products containing the C^{14} label which were further metabolized, as evidenced by the appearance of new radioactive compounds at the 96 and 192 hour sampling times. The presence of these new radioactive compounds indicated that Endothal was readily degraded. It was proposed that these compounds were amphoteric, and most likely were amino acids. Jensen (1964 a, b) observed that Endothal at 500 to 1000 ppm was rapidly decomposed in soil by ten strains of Arthobacter globiformis, which utilized a wide range of carbon and nitrogen sources, and grew from 2 to 37°C. and from pH 5 to 7.5.

Novogrudskaya, et al., (1966) discovered that in the first six weeks after Endothal application the total number of microorganisms growing on meat-peptone agar increased 1.5 to 2 times. During two months after application, nitrifying bacteria were stimulated, thus

improving the nitrogen nutrition of beet plants. However, Kratochvil (1951) reported that Endothal had no significant effect on microbial activity in a silt loam soil. The herbicidal activity was greatly inhibited in a soil containing 10.2 percent organic matter (Robocker and Canode, 1965).

Endothal has been used also as an algicide. Its persistence as an aquatic herbicide was found to be less with an increase in the amount of silt and plant debris present (Hiltibran, 1962).

Sodium Pentachlorophenate (Na-PCP)

Pentachlorophenol belongs to the general class of substituted phenols which also include 2-methyl-4, 6-dinitrophenol, 4, 6-dinitro-2-s-butylphenol, and 4, 6-dinitro-2-s-pentyl-phenol. PCP at normal field application rates has an initial depressing effect followed later by a stimulatory effect on the soil microflora. Kratochvil (1951) found that Na-PCP at 4, 8, or 16 lbs. per acre reduced the gas output of treated soils when measurements were made 52, 56, or 60 hours after treatment. Kutuzov (1958) and Pochon, et al., (1951) agreed that Na-PCP had a stimulatory effect on the soil microflora. Na-PCP at field application rate of 20 lbs. per acre inhibited plant pathogens such as Sclerotium rolfsii, Phytophthora parasitica var. nicotianae, Helminthosporium victoriae, Fusarium oxysporum f. lycopersici and f. conglutinans (Chappell and Miller, 1956). Furthermore, Na-PCP

was shown to prevent the formation of sporodochial mats of Sclerotinia laxa and to kill the conidia of Coryneum beijerinckii (Wilson, 1953).

Akai and Oku (1956) reported that 50 ppm Na-PCP added to soil decreased the number of bacteria; that it was selective in action as determined by methylene blue staining technique; and that the nitrifying bacteria were most susceptible to PCP. Hale, et al., (1957), testing several herbicides against nitrifying bacteria, found that Na-PCP was most toxic; also that a reduction of respiration resulted with as low a concentration as 2.5 ppm, and that almost complete inhibition of oxygen uptake occurred at 200 ppm.

Field application of Na-PCP upon microorganisms in soil and water under water-logged conditions was reported by Ishizawa and Matsuguchi (1966) to produce a slight stimulation in bacterial count and ammonification; and also a growth depression of nitrogen-fixing blue-green algae. The influence of organic matter on the effectiveness of PCP was investigated by Ishizawa and his associates (1961a) who found that in the absence of organic matter fungi, actinomycetes, and bacteria were depressed even by the low level of application (2000 ppm). This effect was greater with the heavy treatment (20,000 ppm). Moisture content of the soil exerted little influence upon the trend. In the presence of organic matter, Na-PCP at 20,000 ppm exerted no distinctive effect on bacteria but was inhibitory, though not

extensively, to fungal growth. Also in the presence and absence of organic matter, nitrate accumulation and CO_2 production were not affected by Na-PCP at 20,000 ppm (Ishizawa, et al., 1961b). Tsunoda (1965a, b) pointed out that PCP adsorption was correlated positively with soil organic matter, pH and cation-exchange capacity and negatively with clay content. In more adsorptive soils Na-PCP was likely to become inactive. Consequently, higher rates of application were necessary than on less adsorptive soils. Like Mashtov (1962), Tsunoda found that much more Na-PCP could be applied on peat soils than on sandy soils before the same degree of PCP toxicity was reached.

Studying the decomposition of PCP when applied as a residual pre-emergence herbicide, Young and Carrol (1951) showed that decomposition was more rapid when moisture content of the soil was near the moisture equivalent (20 percent) and when the soil temperature approached the optimum (24°C.) for microbial activity.

In his work on the decomposition of PCP derivatives in soil, Tsunoda (1965c) observed that amine salts of PCP, decomposed more readily in sunlight than in the dark; the alcoholic derivatives decomposed little in three weeks, even in sunlight.

TD-47 (Di-N, N-dimethylcocoamine salt of Endothal)

Very little information about this herbicide has been published.

TD-47 is a fatty acid amine derivative of Endothal acid and is a highly effective aquatic herbicide and algicide for use in irrigation and drainage canals, lakes, ponds, and other problem areas to control the following weeds and algae: Najas, Elodea, Coontail, Milfoil, Pondweeds, Zannichellia, Water Stargrass, Cabomba, Cattail, Burr Weed, Arrowhead, and Chara. Blackburn (1963), evaluating herbicides against aquatic weeds, reported that this compound at 1 ppm in still water killed 85 percent of the test plants, which were Elodea densa, Najas guadalupensis and Cerotophyllum demersum. At 5 and 10 ppm a 100 percent kill was obtained with this compound. Blackburn and Weldon (1964) noted that recovery of Najas guadalupensis occurred three months after treatment with 3 ppm TD-47.

Herbicides and Algae

The Effect of Herbicides on Algae

Ukeles (1962) investigated the effect of 17 toxicants, including bactericides, fungicides, herbicides and insecticides, on the growth of five species of algae. She found that of eight different classes of toxicants tested, substituted urea compounds and a mercuric compound were most effective in inhibiting growth of all algal species at the lowest concentrations.

Evaluating the toxicity of six chemical compounds to thirty

cultures of algae, Maloney and Palmer (1956) found that 2, 3 DNQ (2, 3 dichloronaphthoquinone) at 0. 5 ppm was the most selective in controlling the growth of some blue-green algae, and that DAC (dodecylacetamido dimethyl benzyl ammonium chloride) at 0. 5 ppm was most toxic to green algae.

A nutrient-perlite medium was used by Jansen, et al., (1958) to screen potential algicides and determine the algicidal properties of several substituted-urea and s-triazine compounds. Of 14 chemicals, four substituted urea herbicides and seven s-triazine derivatives showed considerable algicidal potential. All of these compared very favorably in performance with copper sulfate. The dominant algae, isolated from soil and used in each of three experiments, were representative of the genera Chlamydomonas, Hormisceum, Protococcus and Oscillatoria.

Monuron (3-(p-chlorophenyl)-1, 1-dimethylurea) at 1 ppm was toxic to Stichococcus bacillaris (Nag) in liquid media (Rand, et al., 1959).

Relationship of Algae to Other Microorganisms in the Soil

Ecological and physiological studies have confirmed the importance of algae to other microorganisms.

Allison, et al., (1937) found that Nostoc muscorum Ag. ex Born et Flak, fixed an average of 9. 6 mg. nitrogen per gram of

glucose in the dark, with a maximum value of 14.8 mg. under ordinary atmospheric conditions. This is a higher production than generally reported for nitrogen-fixing bacteria. They noted that the competition for mineral foods which existed between the algae and higher plants, harmful at times, was more likely to have an over-all beneficial effect through the prevention of leaching and the retention of a reserve supply of mineral elements in a semi-available form used by higher plants. However, Stokes (1940) found that blue-green algae made appreciable growth in cultures with Azotobacter chroococcum where the sole limiting factor was the available supply of carbon and energy. Little or no available organic matter was supplied to Azotobacter by the algae. He concluded that the majority of soil algae did not fix atmospheric nitrogen. Stokes is partially correct in his conclusion but his selection on non-nitrogen-fixing soil algae was unfortunate. Contrary to Stokes' findings, Singh (1939) found that Aulosira fertilissima Ghose, growing near the surface, obtained both their carbon and nitrogen from the air. Lund (1947) reported that Myxophyceae in general were characteristic of soils which were not deficient in bases, gave a positive reaction for nitrates, and were rich in available phosphates. Algal population had been reported to be about 10,000 to 100,000 per gram of soil, its weight almost equal to that of bacteria (Petersen, 1935).

Besides the importance of algal fixation of nitrogen, the study

of algal excreta were of value. Some investigators have noted that blue-green algae were a source of Vitamin B_{12} (Darken, 1953; Sundara Rao, 1963). In this connection a higher incidence of oak rhizosphere organisms responding to Vitamin B_{12} was found when algae or algae plus Azotobacter were present in the rhizosphere than when they were absent (Sundara Rao, 1937). Algal excreta also favored bacterial growth. Polypeptides, excreted by Anabaena, were good solubilizers of tricalcium phosphate, making more phosphorous available, as well as being good chelators (Provasoli, 1958). More recently, Taha, et al., (1962) determined that Nostoc commune fixed 5.4 mg. nitrogen per 100 mg. of algal cells, dry basis, and that 13 percent of this cellular nitrogen was present in soluble form. The extracellular nitrogen amounted to 30 percent of the total fixed nitrogen. Glutamic and aspartic acids were the only free amino acids in the culture solution. These two acids are important in the metabolic pathway of nitrogen.

The relationship of the narrow carbon-to-nitrogen ratio of 10:1 of blue-green algae to the bacterial decomposition of organic matter, resulting in nitrogen becoming available for higher plants, has been mentioned by Fogg (1962). Bjalfve (1962) showed that a relatively rapid and large nitrogen fixation, 5.42 mg. nitrogen per 100 ml. medium, occurred when Bacillus megaterium and Nostoc calcicola were mixed together. Other mixtures of Nostoc calcicola with

Agrobacterium radiobacter and Streptomyces albus increased nitrogen fixation. An experiment by Okuda, et al., (1955) showed the significant role of blue-green algae and photosynthetic bacteria in fixing nitrogen in paddy soils. When ample amounts of organic matter were present the nitrogen fixation was dependent upon heterotrophic bacteria which could utilize the carbon source; in the absence of organic matter, the heterotrophic process was repressed. The fixation by organisms such as blue-green algae and photosynthetic bacteria which utilize the energy of light markedly increased nitrogen fixation.

The influence of soil bacteria on the decomposition of blue-green algae was reported by Watanabe and Kiyohara (1960). They found that about 40 percent of the algal nitrogen was converted to ammonium by a strain of Bacillus subtilis within 10 days incubation.

Investigations of symbiotic relationships with algae have been made. Jacobs, et al., (1963) isolated an alga from the corolloid roots of Cycas revoluta and found that in addition to nitrogen fixation, Vitamin B_{12} and the free amino acids, aspartic acid, glutamic acid, and alanine, were liberated. Watanabe, et al., (1963) concluded that the blue-green algae Nostoc punctiforme, N. sphaericum and N. cycadae, isolated from their respective host plants, could fix atmospheric nitrogen in free-living states as well as in symbiosis with the host. Symbiotic algae are a source of thiamine for their fungal

partner in lichens (Zehnder, 1949).

Green algae are important. Allison, et al., (1932) considered them to play an indirect role in the fixation of nitrogen by living in association with nitrogen-fixing bacteria and furnishing them with energy sources. In culture solutions exposed to light, green algae have been found to exert a protective effect on tobacco roots against infection by fungi (Engle and McMurtrey, 1940). Gaumann and Jaag (1950) showed that several unicellular soil algae produced the thiamine needed by phytopathogenic fungi. A necessary ingredient for Vitamin B_{12}, cobalamin, has been found in Chlorella vulgaris (Brown, 1956). The biotic relationship between soil algae and other microorganisms was investigated by Parker, et al., (1961), who found that in soil-water cultures, Streptomyces sp. enhanced the growth and mobility of Chlamydomonas sp. The filtrate from Chlamydomonas promoted the growth of Chlorella in situations where Chlorella could not grow (Provosoli, 1958). Based on results of single and mixed cultures of Chlorella and Azotobacter, Eschrichia coli and Urobacillus, Nakamura (1963) proposed the possiblity of a nutritional relationship between Chlorella and these bacteria in an eco-system.

Recently several Russian workers have mentioned the beneficial relationship of soil algae to other microorganisms. Perminova (1964) found that unialgal cultures isolated from soil of the Kirov region contained Azotobacter, Clostridium pasteurianum and

oligonitrophiles. Furthermore, introduction of algae into the soil increased the numbers of aerobic and anaerobic nitrogen-fixing microorganisms. Shtina (1963) noted that in soil-manure composts blue-green nitrogen-fixing algae proliferated. Their presence increased the fertilizing value of the compost directly by nitrogen fixation and accumulation of organic matter, and indirectly by stimulating the activity of useful microorganisms. Shtina, et al., (1963) inoculated soddy-podzolic soil with soil algae from the same soil and found that green and blue-green algae increased the effectiveness of Azotobacter and stimulated the spontaneous development of Azotobacter, Clostridium sp., and other oligonitrophiles.

From these various reports it is apparent that herbicides may be inactivated by microbial decomposition and such factors as organic matter, soil type, soil moisture, volatilization, temperature, cropping, chelation, pH, and non-biological hydrolysis and oxidation. Beneficial relationships between algae and bacteria have been demonstrated. Algae were found to be selectively inhibited by toxicants. These findings are pertinent to the following thesis.

III. MATERIALS AND METHODS

Soils and Herbicides

Soils

Six soils were used: three from Oregon and one each from Illinois, Nebraska and Colorado. The Oregon soils were Willamette silt loam from the Hyslop Farm, Klamath loamy sand from the Klamath Falls Branch Experiment Station, and Ephrata fine sand from Umatilla Branch Experiment Station. The Illinois soil, from the Drug and Horticulture Experiment Station, Downer's Grove, was of a clay loam texture. The texture of the Colorado and the Nebraska samples were sandy clay loam and sandy loam, respectively, from the Great Western Experiment Station at Longmont, Colorado, and from Mitchell, Nebraska. Sugar beets were grown on these last named three soils. The soil samples were received in 1962 through the courtesy of the Pennsalt Chemical Company Corporation, Agricultural Chemical Division, Tacoma, Washington.

Properties of these soils used in the following studies with Endothal, TD-47 and Na-PCP, are presented in Table 1. The microbial populations of the original samples are given in Table 2.

Table 1. Properties of soils used in studies with Endothal, TD-47 and Na-PCP.

Soil	Willamette	Klamath	Ephrata	Illinois	Colorado	Nebraska
Water %	3.2	2.2	1.1	15.4	2.9	2.6
Water-holding capacity %	51	35	29	63	37	39
pH	5.73	6.45	7.25	6.55	8.36	8.20
Lime requirement T/A	2	1/2	--	1-1/2	0	0
Nitrogen						
Ammonium ppm	33	10	23	28	13	13
Nitrite ppm	0.14	0.14	0.10	0.20	0.43	0.10
Nitrate ppm	27.50	25.67	10.38	21.92	6.85	7.25
Kjeldahl %	0.135	0.06	0.045	0.53	0.089	0.049
Phosphate, as P, ppm	29.0	24.5	--	10.5	41.0	11.3
Cation exchange capacity, me/100g	22.2	8.9	--	41.8	12.3	8.2
Exchangeable cations						
K me/100g	0.80	1.06	--	0.58	0.84	0.78
Ca me/100g	9.8	4.4	--	19.4	9.3	4.8
Mg me/100g	5.7	2.2	--	10.3	3.5	2.2
Na me/100g	0.51	0.37	--	0.48	0.16	0.20
Total carbon, as C, %	2.40	0.70	0.37	6.45	0.76	0.5
C/N ratio	17.78	11.67	8.22	12.17	8.54	10.20
Texture	silt loam	loamy sand	fine sand	clay loam	sandy clay loam	sandy loam

Table 2. Microbial populations of the original samples.

Soil	Molds					Bacteria	
	Total thousands	mucors %	Penicilli %	Aspergilli %	Trichoderma %	Total millions	Streptomyces %
Willamette silt loam	23	99	1	0	0	2	22
Klamath loamy sand	4	90	10	0	0	3	23
Ephrata fine sand	5	26	63	3	0	4	45
Illinois clay loam	7	8	33	39	0	3	33
Colorado sandy clay loam	3	11	16	5	16	5	33
Nebraska sandy loam	2	0	8	8	0	2	21

Herbicides

The three herbicides studied were Endothal, TD-47 and Na-PCP, furnished by Pennsalt Chemical Corporation. In determining the algicidal potential, five additional herbicides were used besides those mentioned. These were Diquat (1, 1'-ethylene-2, 2'dipyridylium dibromide), Paraquat (1, 1'-dimethyl-4, 4'-dipyridylium dichloride), furnished by California Chemical Company, San Francisco, California; Picloram (Tordon 22K) (4-amino-3, 5, 6-trichloropicolinic acid), Amiben (3, amino-2, 5-dichlorobenzoic acid), and Diuron (3, 4-dichlorophenylurea), obtained from the Department of Farm Crops, Oregon State University, Corvallis, Oregon. The properties of Endothal, TD-47 and Na-PCP are discussed in detail.

Sodium Endothal. This is disodium 3, 6-endoxohexahydropthallate, also designated as disodium 7-oxabicyclo-(2, 2, 1) heptane-2, 3-dicarboxylate ($C_8H_8O_5Na_2$, molecular weight-230. 1; melting point-263-266°C.). It forms anhydride readily at 85-90°C. (m. p. 116°C.). This compound has been used primarily as the water soluble sodium salt, as a pre- and post-emergence herbicide at rates of four pounds per acre. It has defoliant and dessicant properties at two pounds per acre. Sugar beets have shown a useful tolerance to pre-emergence applications to control a number of annual weeds, including Polygonum spp., Papaver spp., and Capsella bursa-pastoris. Endothal has,

however, a high mammalian toxicity.

A purified crystal form was used for the experiments reported in this thesis.

According to Freed (1964) the development of a suitable quantitative assay for Endothal has been especially intractable. The colorimetric method of Goldberg and Spoerri (1958) which involved the conversion of the parent acid to a hydroxamic acid, followed by treatment with ferric chloride for development of a violet-purple color, is applicable only for samples of several milligrams or larger. Hiltibran (1962) developed a method of detection in which the root elongation of flax seed (Linum usitatissium L. var. Bolley) was sensitive to small quantities of Endothal. Freed (1964) mentioned that gas chromatographic methods have been developed to assay for Endothal.

Brian (1964) reported that virtually nothing is known of Endothal's mode of action. Mitotic effects were noted but these could not be fully explained on the basis of the high toxicity. Wilson and his associates (1956) found that Endothal exhibited mitotic effects similar to those of ionizing radiations which induced chromosome fragmentation, followed by structural rearrangements.

In the present studies powdered "purified" disodium salt of Endothal was used at rates of 20 and 200 ppm.

TD-47. The literature contains very little information on the characteristics of TD-47, the di N, N-dimethylcocoamine salt

of Endothal. Technical TD-47 (29.2 percent Endothal acid equivalent) was used in these studies as a liquid concentrate soluble in water. It is a fatty acid amine derivative of Endothal and a highly effective herbicide and algicide. As an algicide, it has been used in drainage and irrigation ditches at 3 ppm (acid equivalent), in ponds and lakes at 0.5 and 2.5 ppm, and in cooling towers at 3 ppm (acid equivalent).

The molecular complexity of this herbicide was readily accounted for by Lindaberry (1965) in his report on the tertiary amine salts of 3, 6-endo-oxohydro-o-phthalic acids. He mentioned that the acids may be substituted for by halogens, C_{1-4} alkyl, haloalkyl or alkoxy, aryl, aryloxy, NO_2^-, and CN^-. Futhermore, the tertiary amine moiety had one C_{12-18} and two C_{1-4} alkyl radicals; e.g., N, N-dimethylcocoamine. Perhaps the closest insight available on the preparation of this type of compound was provided by the works of Reck and associates (1965). An oil-soluble amine salt mixture of 3, 6-endoxohexahydrophthalic acid was prepared by melting 21 g. of Armeen 2C (an amine from cocoa fatty acids) at 46°C. and pouring it into 400 ml. of isopropanol. This mixture was added to 10.25 g. of 90.8 percent pure 3, 6-endo-oxohexahydrophthalic acid, warmed on a steam bath until a solution occurred, cooled, and the mixed amine salts of 3, 6-endoxohexahydrophthalic acid recovered by filtration.

The mixed amine salts were used in the present study at 10 and 100 ppm.

Na-PCP. Pentachlorophenate has been known for many years to have herbicidal properties similar to those of the dinitrophenols; but only recently have its potentialities been investigated. PCP and its sodium salt are not translocated and can be used as contact herbicides and as pre-emergence treatments for the control of germinating weed seeds. Na-PCP is also used as a pre-harvest dessicant for leguminous seed crops (Woodford and Evans, 1965). Na-PCP has been extensively tested for its effect on phytopathogenic fungi (Fletcher, 1960). It was found inhibiting at 10 ppm.

In the following experiments powdered sodium pentachlorophenate, Na-PCP, (C_6Cl_5ONa, 90 percent active ingredient; molecular weight-266.4; melting point 190-191°C.) was used at 40 ppm in conjunction with Endothal.

Properties of the herbicides used in this study are presented in Table 3.

Procedures

Preparation and Treatment of Soil Samples

Soils as received were spread on kraft paper, to air-dry and then screened through a 10-mesh sieve. They were put into plastic bags and stored in friction-top cans at room temperature.

In all studies the basic soil treatments were: Soil alone; Soil

Table 3. Properties of herbicides used.

Herbicide	Chemical name	Empirical formula	Active ingredient %	m. wt.	Melting point °C.	Source of herbicide
Endothal	Disodium 3, 6-endoxohexahydro-phthallate	$C_8H_8O_5Na_2$	100	230.1	263-266	Pennsalt Chemical Corporation, Tacoma, Washington
TD-47	Di N, N-dimethyl-cocoamine salt of Endothal	----------	29.2 (acid equi-valent)	-	-	Pennsalt Chemical Corporation, Tacoma, Washington
Na-PCP	Sodium penta-chlorophenate	C_6Cl_5Na	90	266.4	190-191	Pennsalt Chemical Corporation, Tacoma, Washington
Diquat	1, 1'-ethylene-2, 2'-dipyridylium dibromide	$C_{12}H_{12}N_2Br_2$	100	344	335-340	California Chemical Company, San Francisco, California
Paraquat	1, 1'-dimethyl-4, 4'-dipyridylium dichloride	$C_{12}H_{14}N_2Cl_2$	100	257	approx. 300	California Chemical Company, San Francisco, California
Picloram (Tordon 22K)	4-amino-3, 5, 6-trichloropicolinic acid	$C_6H_2O_2Cl_3N_2K$	24.9	241.5	209.5-210	Department of Farm Crops, OSU, Corvallis, Oregon
Amiben	3, amino-2, 5-benzoic acid	$C_7H_5O_2Cl_2$	23.4	206	200-201	Department of Farm Crops, OSU, Corvallis, Oregon
Diuron	3, 4-dichlorophenyl-urea	$C_9H_{10}ON_2Cl_2$	80	233.1	158-159	Department of Farm Crops, OSU, Corvallis, Oregon

plus Endothal at 20 ppm and 200 ppm; TD-47 at 10 ppm and 100 ppm; Na-PCP at 40 ppm; Endothal plus Na-PCP at 20 ppm and 40 ppm, and 200 ppm and 40 ppm, respectively. Additives used in nitrification and other experiments are listed in the appropriate sections. In all herbicide treatments the herbicides were dissolved in water and then applied to the soil at the desired concentration.

Unless otherwise stated, fifty grams of soil, oven-dry basis, were added in four portions into pint milk bottles. After addition of each portion, approximately one-fourth of the amount of distilled water or chemical solution required to bring the moisture to 50 percent of the water-holding capacity was distributed on the surface. The bottles of prepared soil were capped with milk covers perforated for aeration. The bottles were weighed, recorded and incubated at 28°C. for a specified time. Restoration of moisture was made every three days.

All treatments were duplicated except where specifically stated otherwise. Results are expressed on the oven-dry basis.

Chemical Analyses

All chemical analyses were made in duplicate. Concentrations of available phosphorous was determined by extraction with sodium bicarbonate solution. Color intensity, developed by ammonium molybate and stannous chloride solutions, was read at 560 mμ,

With N ammonium acetate solution as an extractant, exchangeable magnesium, calcium, sodium and potassium were measured by flame photometer at wavelengths of 383, 554, and 768 mμ, respectively.

The ammonium acetate method of Schollenberger and Simon (1945) was used to determine cation exchange capacity. Using ten grams of soil, cations were replaced by N ammonium acetate and the excess ammonium ions were washed out with 95 percent ethanol. The soil then was washed with 0. N HCl to replace the adsorbed ammonium ions which were then determined by Kjeldahl procedure.

The procedure of Allison, Bollen and Moodie (1965) was employed for determination of total carbon, using 1,000 g. oven-dry basis, of -100 mesh air-dry soil samples.

Woodruff's (1948) method was used to measure lime requirement. The pH was determined on a 1:2 soil:water paste. To this a given volume of Woodruff buffer solution was added and the pH again read. Each one-tenth drop of pH from 7.0 corresponded to 1,000 lbs. of $CaCO_3$.

The weight difference between drained water-saturated and then oven-dried soil was used to measure the water holding capacity. The soil was placed in a Gooch crucible, wetted from below until it was saturated with water, and drained to constant weight in a moist atmosphere. Loss in weight after drying 24 hrs. at 105°C.

represented the water-holding capacity.

Water content was ascertained from loss in weight by drying 100 gram samples of soil for 24 hours at 105°C.

Samples of -10 mesh soil, oven-dry basis, were used for pH, NO_2^--N and NO_3^--N determinations. Distilled water was added to give a 1:5 suspension which was shaken on a mechanical shaker for 15 minutes. After allowing the coarse particles to settle, readings were made with a model N Beckman glass electrode pH meter. Cupric acetate and calcium hydroxide were then added and the mixture was shaken. After the copper hydroxide floc had settled the clear supernatent was filtered off. Ammonium carbonate was added to the clear filtrate to remove excess calcium, which was then precipitated as the carbonate and removed by filtration. Aliquots of the filtrate were analyzed for NO_2^--N by using sulfanilic acid, 1-naphthylamine and sodium acetate solution (American Public Health Association, 1955), and for NO_3^--N by the phenoldisulfonic acid method of Harper (1924). Readings for NO_2^--N and NO_3^--N were made on a Klett-Summerson photoelectric colorimeter with a 540 mμ. and a 420 m . filter, respectively. Concentrations were evaluated from standard curves.

Ammonium nitrogen was determined by steam-distilling 10 grams of soil, oven-dry basis, with 30 ml. phosphate buffer solution, pH 7.4, and 300 ml. distilled water. Approximately 100 ml. of

distillate was collected in 30 ml. of saturated boric acid solution, which was then titrated with N/14 H_2SO_4, using methyl red-bromcresol green mixed indicator (Nichols and Foote, 1931).

A modified AOAC procedure (Association of Official Agricultural Chemists, 1960) was used for Kjeldahl determination in which 35 ml. H_2SO_4, Hibbards' mixture and a selenized granule were added for the digestion. The digest was diluted with 300 ml. distilled water, made alkaline with an excess of 70 percent NaOH and steam distilled into a flask containing 30 ml. saturated boric acid solution. The absorbed ammonia was titrated with N/14 H_2SO_4, using methyl red-bromcresol green indicator.

All results are calculated on the oven-dry basis.

Total Microbial Counts

Soil samples treated with Endothal and Na-PCP were incubated for 7 days and 30 days, after which total microbial counts were made. Samples treated with TD-47 were incubated for 30 days only. Data obtained from this 30-day incubation proved sufficient for information on the effect of this endothalic derivative.

Dilutions were prepared from 20 grams of soil, oven-dry basis, added to sufficient sterile water to give a 1:5 suspension. This was vigorously shaken on a mechanical shaker for 10 minutes. Subsequently, 1:500 and 1:5,000 dilutions were prepared and plated

in triplicate for molds with peptone-glucose agar; 1:50,000 and 1:500,000 dilutions were prepared and plated in triplicate for bacteria and Streptomyces with sodium albuminate agar (Fred and Waksman, 1928).

Molds were counted after 2 days and differentiated after 7 days. Bacteria and Streptomyces were counted and differentiated after 14 days. The arithmetic means of the triplicate counts were used to calculate numbers of microorganisms per gram of soil.

Ammonification

In the Soil. Peptone and herbicide were added to the soil to observe their effect on the ammonifying ability of the soil. Sixty-mesh Bacto peptone at 1000 ppm N was added in the dry state and mixed with the soil. Soil treatments were as given in "Preparation and Treatment of Soil Samples". Controls, including peptone only, were included.

The preparations were incubated for 3 days and 5 days, then analyzed for pH, ammonium, nitrite, and nitrate nitrogen as described under "Chemical Analyses".

In Solution. One percent peptone water in 3/4 x 6 inch test tubes was used. The medium was prepared as follows: Bacto peptone 1 percent, sodium chloride 0.5 percent, distilled water 1 liter, pH adjusted to 7.0, and autoclaved at 15 lbs. for 15 minutes.

One milliliter of 1:1,000 soil suspension was added to 8.5 ml. of the peptone solution and the herbicides tested were added at the rate of 0.5 ml. of solutions to give concentrations as follows: Endothal, 20, 200, 2000 ppm; Na-PCP, 40 ppm; TD-47 10, 100, 1000 ppm; Endothal plus Na-PCP, 20 ppm and 40 ppm, 200 ppm and 40 ppm, and 2000 ppm and 40 ppm, respectively. The tubes were incubated at 28°C. and observations for turbidity as signs of growth were made after 3, 5 and 14 days. Nessler's solution as a testing reagent was unsuitable especially in the presence of TD-47 because of interference of a milky-white precipitate.

All treatments were made in triplicate.

Nitrification

Because efficient nitrification is generally associated with good soil fertility, the effect of herbicides on nitrification in the different soils was investigated.

The additives were ammonium sulfate solution at 200 ppm N, with and without the herbicides at the concentrations previously mentioned. The variously treated samples were incubated for 30 days and analyzed for pH, nitrite and nitrate nitrogen as stated under "Chemical Analyses".

Utilization of Herbicides as a Carbon Source by Soil Isolants

Isolation Method. Isolants were obtained from Endothal-treated soils by plating with peptone-glucose and sodium albuminate agars. Isolants were also obtained from one percent peptone water incubated with the herbicide and soil suspensions by plating one ml. and one-tenth ml. with nutrient agar.

Purification Method. Twenty-four hour cultures of each isolate, grown in nutrient broth, were thoroughly shaken and three successive series of dilution plates were made in the conventional manner with nutrient agar. Sub-cultures were made from isolated colonies. According to McNew (1938), this technique involving three successive platings gives essentially single-cell cultures.

Utilization of Herbicide. Purified isolants were inoculated into a synthetic basal medium containing various concentrations of glucose and herbicides. The basal medium had the following composition: $NaNH_4HPO_4 \cdot 4H_2O$-1.0g; KCl-1.0g; $MgSO_4 \cdot 7H_2O$-0.2g; Bromthymol blue-0.01g; distilled water-1 l; pH adjusted to 7.0. This was dispensed either 9.3 ml. or 9.8 ml. in small test tubes to give 9.0 ml. or 9.5 ml. after autoclaving at 15 lbs. for 15 minutes. Five-tenths ml. each of glucose and herbicide solutions were added to the basal medium so that the final concentrations of glucose and herbicide were: Endothal 20, 200, 2000 ppm; TD-47 10, 100, 1000 ppm;

glucose 10, 20, 100, 200, 1000, 2000 ppm; glucose plus Endothal 20 and 20 ppm, 200 and 200 ppm, 2000 and 2000 ppm, respectively; and glucose plus TD-47 10 and 10 ppm, 100 and 100 ppm, 1000 and 1000 ppm, respectively. The glucose and the herbicide solutions were sterilized by filtration.

The inoculum was prepared by carefully taking 2 loops of surface growth from a 24-hour old nutrient agar culture and dispersing in 10 ml. of sterile synthetic basal medium. One ml. was removed and added into 50 ml. of basal medium from which two drops (approximately 0.05 ml.) were transferred into each test tube of the solutions to be tested.

All treatments were triplicated and incubated at 28°C. Observations were made every 3 to 5 days for 60 days for turbidity and for development of a yellow color indicating acid production.

Identification Methods. Standard bacteriological methods were used to characterize organisms found to utilize the herbicide as sole carbon source (Manual of Microbiological Methods, 1957). Keys used in the identification of the microorganisms were "Bergey's manual of determinative bacteriology" (1957) and "A manual of the Aspergilli" (Thom and Raper, 1945).

Inhibition Tests by Seeded Plate Method

One-tenth ml. of a 24-hour nutrient broth culture of Bacillus

megaterium, and of B. cereus var. mycoides, and of a spore suspension of Aspergillus niger was added and spread on the surface of solidified nutrient agar. Paper discs, 12.7 mm diameter, were dipped into the test solutions, wiped of excess by contact with the inside of the container, and placed on the seeded agar surface. Four discs were used per plate. The test solutions were: Control, sterile water only; Endothal at 20, 200, 2000 ppm, and TD-47 at 10, 100, 1000 ppm. Endothal and TD-47 were sterilized by filtration. Triplicate plates were made in each case. The plates were inverted and incubated at 28°C., and observed each day for two days for zones of inhibition around the discs. The zones were measured in millimeters from the edge of the disc to the edge of the bacterial or fungal growth. The results are expressed as the means of the triplicate plates.

Soil Respiration

The effects of the herbicides on soil respiration were studied by three different methods: a. continuous aeration, using Bollen's (1941) modification of the Potter and Synder apparatus (1916); b. periodic aeration (Bartha and Pramer, 1965); and c. oxygen uptake by Warburg respirometers.

Continuous Aeration. In this experiment, 100 grams of soil, oven-dry basis, were used for each treatment. The soils studied were Willamette silt loam, Colorado sandy clay loam, and Klamath

loamy sand. In addition to the herbicides added alone at the rates of Endothal 20 ppm and 200 ppm, TD-47 at 10 ppm and 100 ppm, Na-PCP at 40 ppm, Endothal and Na-PCP at 20 and 40 ppm, and 200 and 40 ppm, an aqueous solution of glucose at 800 ppm C. was added to soil alone, and to soil plus the herbicides.

In measuring carbon-dioxide evolved from the soil under continuous aeration CO_2-free air was passed over the soil under a slight pressure so that the evolved CO_2 was absorbed in 10 ml. of N NaOH in 3/4" x 6" test tubes. Test tubes were changed after 1-1/2, 4, 8, 12, 20, 30 days incubation. Determination of absorbed CO_2 was made by double titration (Cooper, 1941) using a Beckman model K automatic titrimeter. Results are expressed as cumulative carbon evolved as CO_2, milligrams per 100 grams of soil.

Periodic Aeration. By the method of Bartha and Pramer (1965) fifty grams of Willamette soil, oven-dry basis, was added in portions to a 250-cc. Erlenmeyer flask fused to a 50-cc. tube with a round bottom. The soil was moistened to 50 percent of its water-holding capacity and the flask closed with a rubber stopper upon which was mounted an Ascarite filter provided with a stopper and stopcock. The side tube was sealed with a rubber stopper, fitted with a 15-gauge needle 15 cm. long and with a 4 mm. glass stem 7 cm. long. The glass tube and needle were capped with a rubber policeman. The point of the needle was covered with a short length of polyethylene

tubing which touched the bottom of the side tube. The unit was charged with alkali by injection; the policeman was replaced by a calibrated syringe containing N NaOH; the filter stopper was removed and stopcock opened; 10 ml. of alkali was introduced through the needle to the side tube; the stopcock was closed; the syringe was removed, and the policeman and filter stopper were then returned to their initial positions. Carbon dioxide produced by the soil was absorbed by the alkali, which, after selected time intervals, was removed with the syringe, replaced, and the absorbed CO_2 determined by double titration (Cooper, 1941).

With a limited number of flasks, only these treatments were made: soil alone; soil with Endothal at 20 ppm; TD-47 at 20 ppm; Na-PCP at 40 ppm; glucose at 800 ppm C.; and Endothal plus Na-PCP at 20 ppm and 40 ppm. Treatments were in duplicates and incubated at 28°C. Alkali was changed after 1-1/2, 4, 6, 10, 21, 30 days incubation. Prior to charging the unit with new alkali, the round base test tube was rinsed with 10 ml. distilled water and this added to the alkali previously removed. The unit was flushed with CO_2-free air. To insure an adequate amount of CO_2-free air in the flask, a water displacement apparatus was devised to draw in 300 ml. of air freed of CO_2 by passage through Ascarite. Two one-quart milk bottles were joined by a length of rubber tubing and arranged so that when one was lowered 300 ml. of water flowed into it from the upper

bottle, thus replacing the atmosphere of the flask with a fresh supply of CO_2-free air. Results are expressed as cumulative carbon evolved as CO_2, milligrams per 50 grams of soil.

Oxygen Uptake by Warburg Respirometers

The procedure of Webley (1947) was adapted as a means of rapidly assessing the significance of the effect of herbicides on microbial respiration on each of the three soils.

Four-gram portions of 10-mesh soil, oven-dry basis, were funnelled into Warburg flasks. Treatments in duplicates were: soil only; soil with Endothal at 20, 200, 2000 ppm; TD-47 at 10, 100 ppm; Na-PCP at 40 ppm; and Endothal plus Na-PCP at 20 and 40 ppm, respectively. These treatments were repeated with glucose at 80 ppm C added in solution plus sufficient water to moisten the soil to 50 percent of its water-holding capacity. Three-tenths ml. of 20 percent NaOH and a fluted strip of Whatman No. 1 filter paper was added to the center well of each flask. To further increase carbon dioxide absorption, cuts 5 to 8 mm. in length were made in the folds of paper. The rim of the center well was thinly coated with vaseline to prevent the alkali from creeping over the wall of the well. The flasks were attached to their respective manometers and the manometers placed statically in a water-bath, thermostatically controlled at 30°C. After allowing a period of 20 to 30 minutes for equilibrium

to be established between the flasks and the bath, the manometer stopcocks were closed. Readings of oxygen uptake were taken at periodic intervals during 96 hours.

In the presence of soil, the following equation (Webley, 1947) was used for determining the oxygen constant (K_{O_2}) of the manometer flasks:

$$K_{O_2} = \frac{V-(V_s + V_f)\frac{273}{T} + V_f \, \alpha O_2}{P_o}$$

where V = total volume of the vessel + manometer up to the 150 mm mark,

V_s = $\frac{\text{mass of soil in g.}}{\text{(particle) density of soil}}$

V_f = volume of fluid in the vessel

T = absolute temperature of the water-bath,

P_o = normal pressure expressed in mm of the manometric fluid (10,000 mm)

α = solubility of gas, under observation in the fluid in the vessel.

This is based on the assumptions that there is neither interaction of oxygen with the soil nor solubility of this gas in the soil over the period of the experiment.

Particle density of soil was determined by the procedure of Blake (1965).

Results are expressed in microliters of oxygen uptake per gram of soil per hour.

Algicidal Properties of Endothal, TD-47, Na-PCP and Other Toxicants on Four Different Algae

The method of Jansen, et al., (1958) was modified to study eight herbicides for their algicidal properties on four algal species. The herbicide treatments (Table 4) were made in duplicate. The test organisms were obtained from Dr. Richard C. Starr, Indiana University, Bloomington, Indiana. These were No. 89-Chlamydomonas reinhardti Dangeard, No. 251-Chlorella pyrenoidosa Chick, No. B 629-Anabaena cylindrica Lemmerman, and No. 486-Nostoc muscorum Kutz. Algal streaks, tested for bacterial or fungal contaminants using eight selective media, showed no macroscopic evidence of contamination.

Table 4. Concentration of herbicides used in experiment with algae.

	Rate		
Herbicides	Low ppm	Medium ppm	High ppm
Endothal	10.	20.	200.
TD-47	5.0	10.	100.
Na-PCP	10.	40.	100.
Amiben	0.5	1.0	10.
Tordon 22K	0.5	1.0	10.
Diuron	0.25	0.5	5.0
Diquat	0.025	0.25	2.5
Paraquat	0.025	0.25	2.5

Petri dish bottoms (13 mm deep x 90 mm diameter) were filled with a fine grade of perlite, commercially used as a plaster ingredient. Fifteen grams of perlite were used per dish and 45 ml. of the appropriate nutrient solution was added to saturate the perlite.

The green and blue-green algae were grown at room temperature (22°C.) in 500 ml. flasks containing 200 ml. sterilized solutions of modified Knop's medium (Myers, 1957) and also in Medium C of Kratz and Myers (1955), respectively. Four 40W DLX warm-white fluorescent tubes 18 inches above the flasks were used for illumination. Air containing five percent carbon dioxide was bubbled into the cultures. After 6 days, each algal suspension was centrifuged, resuspended in 15 ml. of modified Knop's or Medium C media, and used as inoculum for the tests.

The nutrient solution, together with the herbicides at the desired concentrations, were gently applied on the perlite and four drops (approximately 0.2 ml.) of inoculum were added at the center of each plate. The dishes were placed on metal racks in a greenhouse bench converted to a water bath kept at 20°C. by running water. Uninoculated dishes served as checks for contamination.

Five dishes were selected at random every 2 days and weighed. On the assumption that evaporative water loss was equal in all dishes, sufficient water was replaced in each dish to return it to saturation, using the calculated loss of 60 grams minus average weight of

five dishes.

Development of algal growth proceeded in a more or less circular pattern from the inoculated center. Cultures were allowed to develop until the growth on untreated check dishes reached the periphery of the perlite surface; or lacking this amount of growth the experiment was terminated after 30 days. Two diametric measurements were made of each growth. The average of these two measurements, the longest and shortest, was recorded as the mean diameter of the growth. The results were statistically analyzed, using Duncan multiple range test at the five percent level of significance. Statistical analyses were made by Dr. Donald Guthrie, Jr., Statistics Department, Oregon State University, Corvallis, Oregon.

The experiment was made from mid-September to mid-October.

IV. RESULTS AND DISCUSSION

Total Microbial Counts

The results on the effect of Endothal, Na-PCP and TD-47 on total microbial counts are presented in Tables 5 to 11.

1. With Endothal and Na-PCP treatments the following were observed.

Willamette Silt Loam. After seven days incubation, molds were slightly decreased with increasing Endothal concentration but the total bacteria count was increased by 200 ppm Endothal and the percentage of Streptomyces was lower than with Endothal at 20 ppm. Treatments of Na-PCP alone and with Endothal at 20 and 200 ppm resulted in lower mold counts. However, the combination of Na-PCP and 200 ppm Endothal resulted in a greater mold reduction over Na-PCP alone or with 20 ppm Endothal. Na-PCP alone and with 20 ppm Endothal lowered the bacterial count. Na-PCP and Endothal at 200 ppm increased numbers of bacteria more than did the other treatments. All treatments with Na-PCP decreased Streptomyces percentage.

After 30 days the influence of Endothal at 200 ppm in decreasing molds was still evident whereas at 20 ppm it was not. The greatest reduction in molds continued to occur with treatments of Na-PCP alone and with Endothal. Bacterial numbers were decreased with

Table 5. Effect of Endothal and Na-PCP on soil microbial numbers in Willamette silt loam after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	175	74	11	0	0	126	28
20	0	153	81	19	0	0	82	44
200	0	145	86	7	1	0	121	20
0	40	73	73	16	2	0	82	16
20	40	139	76	12	0	0	51	19
200	40	54	68	21	0	0	146	13
30 days								
0	0	114	94	4	0	0	91	61
20	0	124	57	3	3	0	88	41
200	0	104	88	5	0	0	63	64
0	40	95	70	17	2	0	58	46
20	40	85	69	10	2	0	72	42
200	40	50	70	3	0	0	60	37

Table 6. Effect of Endothal and Na-PCP on soil microbial numbers in Illinois clay loam after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	320	0	20	78	2	93	62
20	0	295	1	12	86	1	128	68
200	0	204	4	20	72	1	130	71
0	40	249	0	21	76	1	81	66
20	40	423	0	14	86	0	105	67
200	40	249	1	14	80	3	73	48
30 days								
0	0	214	21	16	53	0	104	84
20	0	326	0	12	84	1	134	81
200	0	168	0	18	80	0	141	46
0	40	388	0	14	83	0	88	86
20	40	320	0	23	74	0	94	67
200	40	295	1	19	77	0	107	76

Table 7. Effect of Endothal and Na-PCP on soil microbial numbers in Colorado sandy clay loam after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	17	5	15	11	4	27	21
20	0	12	4	3	9	13	30	17
200	0	9	4	11	6	15	51	10
0	40	4	0	15	20	12	62	3
20	40	3	5	23	35	5	53	4
200	40	5	0	14	56	8	68	6
30 days								
0	0	7	13	5	13	15	3	60
20	0	8	0	15	13	8	7	51
200	0	8	4	4	8	6	6	43
0	40	1	0	15	0	0	3	16
20	40	1	0	48	0	11	4	21
200	40	1	0	41	0	41	4	13

Table 8. Effect of Endothal and Na-PCP on soil microbial numbers in Ephrata fine sand after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	27	6	82	1	0	35	24
20	0	17	16	82	0	2	36	19
200	0	11	9	87	0	0	27	19
0	40	2	0	57	0	0	40	25
20	40	2	0	67	0	0	37	12
200	40	1	0	63	0	11	41	30
30 days								
0	0	14	3	87	0	0	11	40
20	0	8	26	55	0	0	9	43
200	0	14	4	87	0	0	11	32
0	40	1	0	35	0	0	16	8
20	40	1	0	13	0	0	12	10
200	40	1	0	23	0	0	14	9

Table 9. Effect of Endothal and Na-PCP on soil microbial numbers in Klamath loamy sand after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	10	58	42	0	0	27	19
20	0	12	9	74	0	5	27	21
200	0	15	14	77	0	0	35	10
0	40	9	4	87	0	0	27	4
20	40	7	27	48	0	0	25	5
200	40	7	2	88	0	0	25	4
30 days								
0	0	24	19	65	0	0	7	29
20	0	18	14	65	0	0	8	30
200	0	16	21	65	0	0	15	10
0	40	6	15	61	0	0	11	4
20	40	6	0	80	0	0	12	1
200	40	3	0	32	0	0	14	4

Table 10. Effect of Endothal and Na-PCP on soil microbial numbers in Nebraska sandy loam after 7 and 30 days incubation.

Treatments		Molds					Bacteria	
Endothal	Na-PCP	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	ppm	thousands	%	%	%	%	millions	%
7 days								
0	0	8	0	12	0	31	33	8
20	0	7	0	21	0	21	50	5
200	0	10	0	16	0	16	72	5
0	40	7	0	5	0	2	62	2
20	40	12	0	10	0	0	79	2
200	40	11	0	1	0	0	64	2
30 days								
0	0	5	0	22	0	18	11	20
20	0	17	0	8	1	10	12	23
200	0	9	0	17	0	7	12	19
0	40	3	0	0	0	0	14	4
20	40	7	0	2	2	2	12	5
200	40	4	0	2	2	2	13	3

Table 11. Effect of TD-47 on soil microbial numbers in six soils after 30 days incubation.

Treatments	Molds					Bacteria	
TD-47	Total	mucors	Penicillia	Aspergilli	Trichoderma	Total	Streptomyces
ppm	thousands	%	%	%	%	millions	%
Willamette silt loam							
0	120	58	4	0	4	88	55
10	110	74	4	0	0	42	79
100	105	73	4	0	0	36	85
Illinois clay loam							
0	27	4	37	43	4	60	65
10	42	2	16	64	1	70	62
100	37	3	15	72	2	83	70
Colorado sandy clay loam							
0	8	44	25	0	6	7	60
10	6	15	38	8	8	9	56
100	10	52	21	5	5	16	78
Ephrata fine sand							
0	14	4	86	0	0	8	66
10	22	62	33	0	0	8	39
100	16	45	39	0	0	10	59
Klamath loamy sand							
0	15	77	20	0	0	5	47
10	11	68	32	0	0	5	42
100	20	72	26	0	0	9	66
Nebraska sandy loam							
0	8	6	62	0	19	8	40
10	10	5	63	0	5	7	45
100	10	20	50	0	10	14	45

increasing Endothal concentration. Slightly lower bacterial and Streptomyces counts were obtained from treatments of Na-PCP alone and with Endothal.

It appeared that the combination of Na-PCP and Endothal at 200 ppm was most effective in reducing mold and Streptomyces numbers.

Illinois Clay Loam. After seven days total mold count was decreased with an increasing concentration of Endothal. Total bacteria and Streptomyces numbers were slightly increased by greater concentration of Endothal. Treatments of Na-PCP alone and with Endothal decreased the bacteria count.

After 30 days Endothal at 200 ppm continued to reduce total molds. Treatments with Na-PCP alone and with Endothal generally increased total mold count and decreased slightly numbers of bacteria. Increasing Endothal concentration increased total bacteria with a concomitant decrease in Streptomyces.

It appeared that all treatments with the incorporation of Na-PCP had a depressive effect on the bacterial population, but none on molds.

Colorado Sandy Clay Loam. After seven days the total mold count was decreased by 47 percent with increasing Endothal concentration. A lower total mold count resulted from treatments with Na-PCP alone and with Endothal. However, an increase in Aspergilli was observed with these combined treatments over that of the control and Endothal alone. A general increase in total bacteria

from 27 to 68 million occurred with an increasing concentration of Endothal and with the combinations of Na-PCP and Endothal. However, the opposite effect on Streptomyces occurred.

After 30 days the effect of Na-PCP in decreasing mold and Streptomyces numbers was still prevalent. However, treatments with Endothal at 20 and 200 ppm had no adverse effect on total molds. Endothal at 20 and 200 ppm increased total bacteria but decreased Streptomyces by as much as 60 percent.

Therefore, the combination of Na-PCP and Endothal decreased molds and Streptomyces in the sandy clay loam soil.

Ephrata Fine Sand. After seven days, a decrease of 60 percent in total molds occurred with increasing Endothal concentration, the greatest decrease being nearly 100 percent for the Na-PCP treatments. Mucors were completely absent in treatments with Na-PCP alone and combined with Endothal. A decrease of 20 percent in total bacteria and Streptomyces occurred with Endothal at 200 ppm. Treatments with Na-PCP alone and with Endothal generally increased total bacteria and Streptomyces numbers by 15 percent and 25 percent, respectively.

After 30 days, Endothal at 20 ppm reduced total molds, Penicillia, and total bacteria by 43 percent, 32 percent and 18 percent, respectively. Endothal at 200 ppm decreased Streptomyces by 20 percent in comparison with the control. Treatments with Na-PCP

decreased total molds by nearly 100 percent and Streptomyces by 80 percent. Mucors and Penicillia also were decreased by Na-PCP additions.

The effectiveness of Na-PCP addition in decreasing mold counts was quite obvious and the effect of Endothal at 20 ppm in decreasing total molds and bacteria numbers was still evident after 30 days.

Klamath Loamy Sand. After seven days, total molds and Penicillia were increased with the increasing Endothal concentrations by 50 percent and 83 percent, respectively. Concomitantly, mucors were decreased by 85 percent. Endothal at 200 ppm produced a 33 percent increase in total bacteria over the control and the 20 ppm Endothal treatments. Streptomyces were decreased by 200 ppm Endothal. Addition of Na-PCP caused a reduction in total molds and Streptomyces.

After 30 days a decrease of 33 percent on total molds by both Endothal treatments was observed. This was the inverse pattern of the seven days incubation period. Also in all treatments a pattern similar to the seven days incubation occurred for the total bacteria and Streptomyces. Mucors were decreased by Na-PCP and by Endothal at 20 and 200 ppm.

The effectiveness and persistency of Na-PCP was quite evident in both incubation periods.

Nebraska Sandy Loam. After seven days total molds were generally increased by 25 percent to 50 percent with increased additions of Endothal and Na-PCP. Penicillia and Trichoderma numbers were decreased by nearly 100 percent. A similar pattern occurred with the total bacteria count as with the total molds. However, Streptomyces were decreased by Endothal at 20 and 200 ppm, and by Na-PCP treatments by 38 percent and 75 percent. Penicillia were decreased by Na-PCP and by 200 ppm Endothal treatment.

After 30 days a pattern similar to that of the seven day period occurred in which total molds and bacteria were increased over the control. Treatment with Na-PCP alone decreased total molds by 60 percent. Penicillia and Streptomyces also were decreased by Na-PCP additions.

From these data the relatively consistent and persistent effectiveness of Na-PCP alone or combined with Endothal in decreasing molds, bacteria and Streptomyces in the six soils is remarkably striking, particularly in view of the many differing opinions regarding Na-PCP on the soil population. Na-PCP at normal field application rate has been observed to initially depress the soil microflora, becoming stimulatory (Fletcher, 1960). Pochon, et al., (1951) and Kutuzov (1958) found that PCP exerted a stimulatory effect on the soil microflora. On the other hand the effectiveness of Na-PCP in depressing fungi, bacteria and Streptomyces was observed by

Ishizawa, et al., (1961a) and by Akai and Oku (1956) who found that Na-PCP decreased numbers of soil bacteria. Treatment with Endothal at 200 ppm caused a reduction in total molds after seven and 30 days in the finer textured soils, Willamette silt loam and Illinois clay loam. This may be indicative of the relative persistency of Endothal effects on the mold population. Persistency of Endothal in finer textured soils was also observed by Comes, et al., (1961). However, Endothal applied at the field rate of 20 ppm generally had no prolonged adverse effect on molds, bacteria or Streptomyces. Under optimum conditions for microbial activity, it was reported that the herbicidal effectiveness of Endothal was lost within seven to 14 days (Freed and Montgomery, 1963).

2. With TD-47 after 30 days incubation the following were observed.

Willamette Silt Loam. With increasing concentration of TD-47 total molds and bacteria were decreased by as much as 12 percent and 60 percent, respectively. Trichoderma was totally absent with both herbicide treatments. Streptomyces were increased by 55 percent with increasing concentration of TD-47.

Illinois Clay Loam. Total molds were increased by TD-47 at 10 and 100 ppm by 36 percent and 27 percent, respectively. Aspergilli were increased with increasing herbicide concentration whereas Penicillia were decreased. Total bacteria were increased 17 percent

and 24 percent by TD-47 at 10 and 100 ppm, respectively. Streptomyces were decreased slightly by 10 ppm TD-47 and increased 8 percent with 100 ppm.

Colorado Sandy Clay Loam. Total molds were decreased 25 percent by 10 ppm TD-47 and increased 50 percent by 100 ppm TD-47. Aspergilli were greatly increased by both herbicide treatments. Total bacteria increased with increasing herbicide concentration. Streptomyces were decreased slightly by 10 ppm TD-47 and increased 30 percent by 100 ppm.

Ephrata Fine Sand. Total molds were increased 63 percent by 10 ppm TD-47 and 14 percent by 100 ppm. Mucors were greatly increased by both herbicide treatments. Penicillia were decreased 62 percent by 10 ppm TD-47. Both herbicide concentrations decreased Streptomyces.

Klamath Loamy Sand. Total molds were increased 33 percent by 100 ppm TD-47 and decreased 27 percent by 10 ppm TD-47. A slight decrease in mucors resulted from the herbicide treatments. TD-47 at 100 ppm increased total bacteria and Streptomyces.

Nebraska Sandy Loam. TD-47 at 10 and 100 ppm increased total molds by 25 percent. Trichoderma was decreased 74 percent by the lower herbicide concentration. Mucor numbers were increased 230 percent by 100 ppm TD-47. A slight decrease in total bacteria occurred with 10 ppm TD-47 and a 75 percent increase

with 100 ppm. Each concentration of the herbicide increased Streptomyces by 13 percent.

From these data it can be inferred that generally the soil microbial population would not be adversely affected by these concentrations of TD-47. However, in the sandy soils there is indication that a stimulatory influence on the total mold and bacteria population may result from the higher herbicide treatment.

Ammonification

In the Soil. Results for ammonification on six soils with Endothal, Na-PCP and TD-47 are presented in Tables 12 to 23.

1. With Endothal and Na-PCP treatments, the following results were found.

Willamette Silt Loam. After three days, suppression of ammonification from 2 percent to 6 percent resulted with all treatments except for an increase of 4 percent with Endothal at 20 ppm.

After five days, ammonification was suppressed from 11 percent to 39 percent by all treatments. Endothal at 200 ppm after three and five days suppressed ammonification more than did 20 ppm.

Illinois Clay Loam. After three days a slight suppression in ammonification from 2 percent to 5 percent resulted from all the treatments. After five days ammonification was not depressed by any treatment, but was increased by Endothal at 200 ppm and by

Table 12. Effect of Endothal and Na-PCP on ammonification of peptone in Willamette silt loam after three and five days incubation.

Treatments								
Peptone-N	Endothal	Na-PCP	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm	ppm		ppm	ppm	ppm	%	%
	3 days							
0	0	0	6.0	52	0.20	36	6	1
1000	0	0	7.2	415	0.27	38	36	35
1000	20	0	7.2	455	0.57	36	40	4
1000	20	40	6.9	346	0.90	37	30	- 6
1000	200	0	7.3	402	0.54	33	34	- 2
1000	200	40	7.0	349	1.17	34	30	- 6
1000	0	40	7.0	352	0.38	34	30	- 6
	5 days							
0	0	0	6.0	63	0.20	35	7	3
1000	0	0	7.8	710	0.55	30	64	61
1000	20	0	7.4	595	0.50	31	53	-11
1000	20	40	7.0	319	0.34	31	25	-39
1000	200	0	7.5	435	0.49	29	37	-27
1000	200	40	7.0	490	0.39	30	42	-22
1000	0	40	7.4	484	0.52	24	41	-23

Table 13. Effect of Endothal and Na-PCP on ammonification of peptone in Illinois clay loam after three and five days incubation.

Treatments								
Peptone-N	Endothal	Na-PCP	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm	ppm		ppm	ppm	ppm	%	%
	3 days							
0	0	0	6.6	40	0.28	25	1	0
1000	0	0	7.2	304	0.52	25	26	26
1000	20	0	7.2	284	0.26	24	24	- 2
1000	20	40	7.1	274	0.29	21	23	- 3
1000	200	0	7.2	284	0.23	22	24	- 2
1000	200	40	7.1	260	0.44	23	22	- 4
1000	0	40	6.9	250	0.91	25	21	- 5
	5 days							
0	0	0	6.6	17	0.34	26	1	0
1000	0	0	7.4	310	0.40	28	29	29
1000	20	0	7.4	306	0.38	26	29	0
1000	20	40	7.5	363	0.45	21	34	5
1000	200	0	7.5	394	0.45	26	38	9
1000	200	40	7.5	400	0.40	21	38	9
1000	0	40	7.4	307	0.17	31	29	0

Table 14. Effect of Endothal and Na-PCP on ammonification of peptone in Colorado sandy clay loam after three and five days incubation.

Treatments								
Peptone-N	Endothal	Na-PCP	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm	ppm		ppm	ppm	ppm	%	%
	3 days							
0	0	0	8.5	18	0.26	13	2	0
1000	0	0	9.0	469	8.73	12	47	47
1000	20	0	9.0	458	9.50	12	46	- 1
1000	20	40	8.8	334	5.93	17	33	-14
1000	200	0	9.0	461	9.23	10	46	- 1
1000	200	40	8.9	312	5.30	8	30	-17
1000	0	40	8.5	415	1.76	12	40	- 7
	5 days							
0	0	0	8.4	118	0.28	13	3	0
1000	0	0	8.9	505	28.70	13	52	0
1000	20	0	8.9	559	27.27	11	57	5
1000	20	40	8.8	425	18.80	11	43	- 9
1000	200	0	8.9	506	26.80	10	52	0
1000	200	40	8.9	597	14.93	12	60	8
1000	0	40	9.1	462	8.24	9	45	- 7

Table 15. Effect of Endothal and Na-PCP on ammonification in Ephrata fine sand after three and five days incubation.

Treatments								
Peptone-N	Endothal	Na-PCP	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm	ppm		ppm	ppm	ppm	%	%
	3 days							
0	0	0	7.8	8	1.88	11	5	1
1000	0	0	9.0	535	1.37	3	52	51
1000	20	0	9.0	490	1.37	3	47	- 5
1000	20	40	8.9	478	4.40	5	47	- 5
1000	200	0	9.0	535	1.50	3	52	0
1000	200	40	8.9	538	4.90	4	53	1
1000	0	40	8.9	454	3.15	17	45	- 7
	5 days							
0	0	0	7.4	10	1.70	13	5	2
1000	0	0	9.0	525	0.71	4	50	48
1000	20	0	9.0	506	0.90	4	49	- 1
1000	20	40	8.9	440	1.30	7	42	- 8
1000	200	0	9.0	489	0.80	3	47	- 3
1000	200	40	8.9	455	1.60	8	44	- 6
1000	0	40	8.9	472	2.10	4	45	- 5

Table 16. Effect of Endothal and Na-PCP on ammonification in Klamath loamy sand after three and five days incubation.

Treatments								
Peptone-N	Endothal	Na-PCP	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm	ppm		ppm	ppm	ppm	%	%
	3 days							
0	0	0	6.5	18	0.20	26	7	1
1000	0	0	8.8	524	5.75	18	50	49
1000	20	0	8.9	508	1.66	16	48	- 2
1000	20	40	8.6	374	11.30	19	36	-14
1000	200	0	8.9	556	3.05	8	52	2
1000	200	40	8.5	364	13.50	14	35	-15
1000	0	40	8.9	443	2.35	9	41	- 9
	5 days							
0	0	0	6.5	14	0.20	30	7	1
1000	0	0	8.9	555	5.53	25	52	51
1000	20	0	8.9	564	2.00	24	54	2
1000	20	40	8.9	420	15.70	16	41	-11
1000	200	0	9.1	556	1.33	20	53	1
1000	200	40	8.9	455	14.10	15	44	- 8
1000	0	40	9.0	520	10.50	10	50	- 2

Table 17. Effect of Endothal and Na-PCP on ammonification in Nebraska sandy loam after three and five days incubation.

Treatments								
Peptone-N ppm	Endothal ppm	Na-PCP ppm	pH	NH_4^+-N ppm	NO_2^--N ppm	NO_3^--N ppm	Ammonification %	Ammonification Increase %
	3 days							
0	0	0	7.9	15	0.20	14	6	2
1000	0	0	9.0	483	3.06	3	46	44
1000	20	0	9.0	523	3.75	3	50	4
1000	20	40	8.9	491	1.10	3	47	1
1000	200	0	9.0	494	0.45	2	47	1
1000	200	40	9.0	461	0.70	2	43	- 3
1000	0	40	8.5	381	1.32	3	36	-10
	5 days							
0	0	0	7.8	3	0.25	18	4	0
1000	0	0	9.0	574	4.15	6	56	56
1000	20	0	9.0	538	6.35	5	53	- 3
1000	20	40	8.9	470	4.90	4	46	-10
1000	200	0	9.0	516	5.85	5	51	- 5
1000	200	40	9.0	530	3.80	4	52	- 4
1000	0	40	9.2	561	4.33	4	55	- 1

Table 18. Effect of TD-47 on ammonification in Willamette silt loam after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	6.2	60	0.64	23	6	2
1000	0	7.0	528	0.24	2	45	43
1000	10	7.0	530	0.40	2	45	0
1000	100	7.1	536	0.40	3	46	1
5 days							
0	0	6.2	58	0.20	26	6	2
1000	0	7.6	540	0.72	26	48	46
1000	10	7.6	512	0.90	18	46	- 2
1000	100	7.5	579	0.90	13	51	3

Table 19. Effect of TD-47 on ammonification in Illinois clay loam after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	6.6	29	1.60	9	1	0
1000	0	6.9	422	0.10	3	38	38
1000	10	6.9	374	0.10	3	34	- 4
1000	100	6.9	400	0.10	2	36	- 2
5 days							
0	0	6.8	11	0.50	18	1	0
1000	0	7.5	402	1.00	17	39	0
1000	10	7.4	366	1.00	22	36	- 3
1000	100	7.4	444	0.81	23	44	5

Table 20. Effect of TD-47 on ammonification in Colorado sandy clay loam after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	8.3	15	1.00	17	4	1
1000	0	8.8	570	5.00	4	56	55
1000	10	8.7	544	4.20	3	53	- 3
1000	100	8.7	560	1.45	2	54	- 2
5 days							
0	0	8.4	10	0.24	14	2	0
1000	0	8.9	518	48.00	14	56	56
1000	10	9.0	516	38.00	11	55	- 1
1000	100	9.1	539	18.00	7	54	- 2

Table 21. Effect of TD-47 on ammonification in Ephrata fine sand after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	7.4	8	1.93	10	4	1
1000	0	9.0	535	0.90	7	52	51
1000	10	9.1	496	0.90	4	48	- 4
1000	100	9.0	505	0.53	2	49	- 3
5 days							
0	0	7.5	8	1.80	14	5	2
1000	0	8.9	490	0.90	3	47	45
1000	10	9.0	538	0.44	5	52	5
1000	100	9.0	493	0.60	2	47	0

Table 22. Effect of TD-47 on ammonification in Klamath loamy sand after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	6.7	11	0.40	22	6	- 1
1000	0	9.0	541	4.60	11	52	53
1000	10	9.0	578	5.80	10	56	4
1000	100	8.9	519	7.00	5	50	- 2
5 days							
0	0	6.6	15	0.41	17	5	- 1
1000	0	9.1	540	4.50	13	52	53
1000	10	9.1	532	3.90	12	52	0
1000	100	9.0	593	2.00	11	57	5

Table 23. Effect of TD-47 on ammonification in Nebraska sandy loam after three and five days incubation.

Treatments							
Peptone-N	TD-47	pH	NH_4^+-N	NO_2^--N	NO_3^--N	Ammonification	Ammonification increase
ppm	ppm		ppm	ppm	ppm	%	%
3 days							
0	0	7.7	5	0.60	13	4	0
1000	0	8.8	539	1.03	2	52	52
1000	10	8.8	514	0.94	2	50	- 2
1000	100	8.8	498	0.32	2	48	- 4
5 days							
0	0	7.9	4	0.13	13	4	0
1000	0	9.1	500	1.05	3	49	49
1000	10	9.1	516	6.10	3	51	2
1000	100	9.1	479	1.14	2	46	- 3

the combination with Na-PCP.

Colorado Sandy Clay Loam. After three days a noticeable suppression in ammonification occurred in the combined treatments of Na-PCP and Endothal at 20 and 200 ppm, being reduced 14 percent and 17 percent, respectively. Na-PCP alone also had a suppressive influence, 7 percent. Endothal at 20 and 200 ppm slightly decreased ammonification.

After five days, only the treatments with Na-PCP alone and combined with Endothal at 20 ppm showed any suppressive influence. However, this was less than at three days. Ammonification was increased by the other treatments.

Ephrata Fine Sand. After three days Endothal alone at 20 ppm and combined with Na-PCP caused a 5 percent suppression. Endothal alone at 200 ppm and combined with Na-PCP exhibited no such effect. Na-PCP alone had the greatest suppresssion of 7 percent.

After five days all the herbicide treatments suppressed ammonification from 1 percent to 8 percent. The greatest decreases occurred with the combinations of Na-PCP and with Endothal at 20 and 200 ppm.

Klamath Loamy Sand. After three days, the greatest decreases resulted from Na-PCP and Endothal at 20 and 200 ppm, being 14 percent and 15 percent, respectively. Na-PCP alone was suppressive by 9 percent. Endothal at 20 ppm was only slightly suppressive,

2 percent, while Endothal at 200 ppm slightly increased ammonification by 2 percent.

After five days Na-PCP and Endothal at 20 and 200 ppm decreased ammonification by 11 percent and 8 percent, respectively. Na-PCP alone was only slightly suppressive. Endothal at 20 and 200 ppm produced only minor increases.

Nebraska Sandy Loam. After three days, treatments with Na-PCP alone and with 20 ppm Endothal decreased ammonification by 10 percent and 3 percent, respectively. Slight increases in ammonification resulted in the other treatments. After five days all treatments reduced ammonification slightly; the largest increase, 10 percent, occurred with Na-PCP combined with Endothal at 20 ppm.

It would appear that in all of the soils the combinations of Na-PCP and Endothal at 20 and 200 ppm were the most suppressive. This combined effect of the herbicides was also noted by Kaufman (1966). He observed that the phytotoxic residues of Dalapon and Amitrole persisted longer in soils when the herbicides were applied together than when they were applied alone. Endothal at 20 and 200 ppm generally did not suppress ammonification to any extent. However, the apparent increase in the suppression of ammonification in Willamette silt loam soil after five days may have been due to a more prolonged influence of Endothal and Na-PCP on the ammonifying organisms in this soil type. Such anomalies have been observed by

Morita and Aoki (1952) with 2, 4-D. They noted that, depending on the soil type, ammonification was sometimes suppressed and sometimes stimulated by 2, 4-D.

2. With TD-47 treatments, the following results were obtained.

In most of the soil samples ammonification after three days was suppressed either by one or by both herbicide concentrations from 2 percent to 4 percent. However, this effect generally disappeared within five days.

In Solution. Table 24 shows the results of the effect of Endothal, TD-47, and Na-PCP on ammonification in suspensions of the six different soils in one percent peptone water.

Ammonification was not affected by Endothal during the length of the experiment. Similar results were obtained with TD-47 at 10 ppm. Except for the Willamette and Colorado soils, which were not affected, ammonification was completely inhibited throughout the 14 days by 100 ppm TD-47. Total inhibition resulted with 1000 ppm TD-47. In treatments with Na-PCP a slight suppression resulted after three days. However, after five and 14 days, this effect was no longer evident, the results were little different from the control and Endothal only treatments.

From all these experiments it can be concluded that under the usual rates of application, ammonification in soils is not likely to be inhibited by Endothal, TD-47 and Na-PCP. Even at rates far in

Table 24. Ammonification* in suspensions of six different soils in one percent Peptone water as influenced by Endothal, TD-47, and Na-PCP.

Days	3						5						14					
Soil Suspension / Treatment	Willamette	Illinois	Colorado	Nebraska	Ephrata	Klamath	Willamette	Illinois	Colorado	Nebraska	Ephrata	Klamath	Willamette	Illinois	Colorado	Nebraska	Ephrata	Klamath
Peptone only	4	2	4	3	3	3	4	3	3	3	4	5	4	4	6	4	4	5
+ Endothal																		
20 ppm	4	2	4	3	3	3	4	3	3	3	5	5	4	4	6	4	5	5
200 ppm	6	2	4	3	3	3	4	4	3	3	4	4	4	4	6	4	4	5
2000 ppm	5	2	3	3	3	3	5	3	3	3	4	3	5	4	5	4	4	5
+TD-47																		
10 ppm	3	2	4	3	2	3	4	3	4	3	3	5	4	4	6	4	3	5
100 ppm	1	0	1	0	0	0	2	0	1	0	0	0	3	0	3	0	0	0
1000 ppm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+Na-PCP																		
40 ppm	2	2	1	3	1	2	4	4	3	4	1	3	4	4	3	4	3	3
+Na-PCP + Endothal																		
40+20 ppm	2	2	2	3	2	2	4	4	3	4	2	3	3	3	3	4	3	3
40+200 ppm	2	2	2	3	2	2	4	4	3	4	2	3	4	3	3	4	3	3
40+2000 ppm	2	2	1	3	2	2	4	4	3	3	1	3	4	4	4	4	3	3

* As indicated by appearance of turbidity: 0 none, 1 slight, 2 fair, 3 moderate, 4 good, 5 very good, 6 excellent

excess of field use the herbicides had only relatively minor effects.

Nitrification

The results on the effect of Endothal, Na-PCP and TD-47 on nitrification of the six soils are presented in Tables 25 to 28. Some nitrification of peptone is shown in Tables 12 to 23, discussed under "Ammonification". With peptone, however, in all cases there were no significant increases in nitrates in three or five days because (1) nitrification is a slow process, and (2) free ammonium inhibits nitrification, especially above pH 8.5. Nitrites generally were less than 1 ppm and need not be considered here.

In absence of the herbicides nitrification of the soil native nitrogen was relatively low. Only in the Colorado soil, where the pH was favorable, was nitrification of ammonium sulfate extensive. Otherwise, nitrate production from added ammonium sulfate was less, or little more, than in the controls. All the herbicides generally inhibited nitrification of ammonium sulfate in all the soils.

Utilization of Herbicides as a Carbon Source by Soil Isolants

The results in Tables 29 and 30 indicate that Endothal is more readily utilized as a carbon source and more easily degraded by several different microorganisms than is TD-47. It is interesting

Table 25. Effect of Endothal and Na-PCP on nitrification in Willamette silt loam and Illinois clay loam after 30 days incubation.

Treatments							
$(NH_4)_2SO_4$	Endothal	Na-PCP	pH	NO_2^--N	NO_3^--N	Nitrification	Nitrification increase
ppm	ppm	ppm		ppm	ppm	%	%
	Willamette silt loam						
0	0	0	5.9	0.40	41	3*	
200	0	0	5.7	0.32	29	14	- 6
0	20	0	5.9	0.62	43	3*	0
200	20	0	5.7	0.64	42	0	6
0	200	0	6.2	0.31	37	3*	0
200	200	0	5.9	0.20	28	- 7	- 1
0	0	40	6.2	0.21	31	2*	- 1
200	0	40	5.8	0.21	22	-10	- 3
0	20	40	6.2	0.24	41	3*	0
0	200	40	6.2	0.24	43	3*	0
	Illinois clay loam						
0	0	0	6.4	0.60	41	1*	
200	0	0	6.2	1.03	34	18	- 3
0	20	0	6.4	0.91	61	1*	0
200	20	0	5.9	1.10	52	6	9
0	200	0	6.4	1.60	59	1*	0
200	200	0	6.5	2.11	34	- 2	1
0	0	40	6.5	1.50	59	1*	0
200	0	40	6.5	2.72	33	- 2	1
0	20	40	6.8	2.00	36	1*	0
0	200	40	6.8	2.10	38	1*	0

* Based on Kjeldahl nitrogen (Table 1).

Table 26. Effect of Endothal and Na-PCP on nitrification in Colorado sandy clay loam and Ephrata fine sand after 30 days incubation.

Treatments							
$(NH_4)_2SO_4$ ppm	Endothal ppm	Na-PCP ppm	pH	NO_2^--N ppm	NO_3^--N ppm	Nitrification %	Nitrification increase %
	Colorado sandy clay loam						
0	0	0	8.3	0.43	29	3*	
200	0	0	7.0	0.40	155	78	63
0	20	0	8.2	0.20	23	3*	- 1
200	20	0	6.9	0.51	144	57	- 6
0	200	0	8.6	0.30	24	3*	- 1
200	200	0	7.2	0.40	141	56	- 7
0	0	40	8.4	0.91	26	3*	0
200	0	40	7.7	14.00	58	20	-42
0	20	40	8.4	1.54	23	3*	- 1
0	200	40	8.5	1.80	23	3*	- 1
	Ephrata fine sand						
0	0	0	7.1	0.24	33	7*	
200	0	0	5.8	0.40	55	27	11
0	20	0	7.2	0.31	26	6*	- 2
200	20	0	5.8	0.52	59	13	3
0	200	0	7.3	0.14	27	6*	- 1
200	200	0	6.0	1.20	45	7	- 4
0	0	40	7.6	0.24	17	4*	- 4
200	0	40	7.1	1.33	9	-11	-22
0	20	40	7.8	0.10	11	2*	- 5
0	200	40	7.8	0.32	9	2*	- 5

* Based on Kjeldahl nitrogen (Table 1).

Table 27. Effect of Endothal and Na-PCP on nitrification in Klamath loamy sand and Nebraska sandy loam after 30 days incubation.

Treatments							
$(NH_4)_2SO_4$	Endothal	Na-PCP	pH	NO_2^--N	NO_3^--N	Nitrification	Nitrification increase
ppm	ppm	ppm		ppm	ppm	%	%
	Klamath loamy sand						
0	0	0	6.1	0.43	43	7*	
200	0	0	5.2	1.94	93	48	26
0	20	0	6.3	0.43	39	7*	- 1
200	20	0	5.2	1.41	73	16	-10
0	200	0	6.6	0.41	35	6*	- 1
200	200	0	6.0	1.90	81	7	- 6
0	0	40	6.5	0.10	19	3*	- 4
200	0	40	6.5	1.40	70	5	-12
0	20	40	6.8	0.10	28	5*	- 2
0	200	40	6.8	0.10	25	4*	- 3
	Nebraska sandy loam						
0	0	0	7.8	0.31	25	5*	
200	0	0	5.8	0.52	81	41	28
0	20	0	7.7	0.40	16	3*	- 2
200	20	0	5.8	0.61	73	24	- 4
0	200	0	8.1	0.40	21	4*	- 1
200	200	0	6.2	0.42	78	27	- 2
0	0	40	7.9	0.94	20	4*	- 1
200	0	40	7.2	4.90	9	- 6	-34
0	20	40	8.1	1.21	5	1*	- 4
0	200	40	8.1	0.82	8	2*	- 3

* Based on Kjeldahl nitrogen (Table 1).

Table 28. Effect of TD-47 on nitrification of six soils after 30 days incubation.

Treatments $(NH_4)_2SO_4$ ppm	Treatments TD-47 ppm	pH	NO_2^--N ppm	NO_3^--N ppm	Nitrification %	Nitrification increase %
Willamette silt loam						
0	0	6.2	0.31	39	3*	
200	0	5.8	0.12	20	10	-10
0	10	6.2	0.20	34	3*	0
200	10	5.9	0.20	19	-10	- 1
0	100	6.1	0.20	36	3*	0
200	100	5.9	0.10	14	-12	- 3
Illinois clay loam						
0	0	6.5	3.90	29	1*	
200	0	6.3	4.92	19	12	- 4
0	10	6.7	4.70	23	1*	0
200	10	6.4	6.05	16	- 6	- 1
0	100	6.7	4.65	22	1*	0
200	100	6.4	6.05	20	- 4	1
Colorado sandy clay loam						
0	0	8.3	0.30	36	4*	
200	0	7.2	0.50	135	77	50
0	10	8.3	0.30	34	4*	0
200	10	7.0	0.54	134	49	0
0	100	8.3	0.45	34	4*	0
200	100	7.0	0.50	140	52	2

Table 28. (continued)

Treatments $(NH_4)_2SO_4$ ppm	Treatments TD-47 ppm	pH	NO_2^--N ppm	NO_3^--N ppm	Nitrification %	Nitrification increase %
Ephrata fine sand						
0	0	7.1	0.25	34	8*	
200	0	6.1	0.30	44	22	5
0	10	7.2	0.30	36	8*	1
200	10	6.0	0.40	35	1	- 4
0	100	7.2	0.40	37	8*	1
200	100	6.1	0.50	42	4	- 1
Klamath loamy sand						
0	0	6.2	0.45	43	7*	
200	0	5.8	0.70	44	22	1
0	10	6.3	0.65	48	8*	1
200	10	5.8	1.00	38	- 2	- 3
0	100	6.3	0.62	36	6*	- 1
200	100	5.8	0.85	38	- 2	- 3
Nebraska sandy loam						
0	0	7.8	0.24	25	5*	
200	0	6.2	0.40	57	29	16
0	10	7.8	0.36	18	4*	- 2
200	10	6.2	0.50	41	8	- 8
0	100	7.7	0.30	17	4*	- 2
200	100	6.2	0.51	50	12	- 4

* Based on Kjeldahl nitrogen (Table 1).

Table 29. Utilization of Endothal as sole carbon source by soil isolants in a synthetic liquid medium. *

Soil Isolants	Endothal											
	20 ppm				200 ppm				2000 ppm			
	days				days				days			
	6	12	30	40	6	12	30	40	6	12	30	40
Penicillium sp.	-	+	++	+++	-	+	++	+++	-	+	++	+++
Streptomyces sp.	-	-	-	+	-	-	-	+++	++	-	-	++++
Aspergillus niger	-	-	-	+	-	-	-	+++	+	-	-	++++
B. megaterium	-	-	++	++	-	-	++	++	-	-	++	++
B. cereus var. mycoides	-	-	+	+	-	-	-	-	-	-	-	-

* Appearance of turbidity as criteria for growth:
- - no growth
- + slight growth
- ++ good growth
- +++ better growth
- ++++ best growth

Table 30. Utilization of TD-47 as sole carbon source by soil isolants in a synthetic liquid medium. *

Soil Isolants	TD-47											
	10 ppm				100 ppm				1000 ppm			
	days				days				days			
	6	12	40	60	6	12	40	60	6	12	40	60
Penicillium sp.	-	-	-	-	-	-	-	-	-	-	-	-
Streptomyces sp.	-	-	+	+	-	-	-	-	-	-	-	-
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-
B. megaterium	-	-	-	+	-	-	-	-	-	-	-	-
B. cereus var. mycoides	-	-	-	+	-	-	-	-	-	-	-	-

* Appearance of turbidity as critera for growth:
- - no growth
- + slight growth

to note that Endothal at 2000 ppm was utilized by Streptomyces sp. and Aspergillus niger van Tiegham in six days, and that Endothal at 20 and 200 ppm was utilized after 40 days. However, Endothal at 20, 200, and 2,000 was utilized by a Penicillium sp. in 12 days and by B. megaterium in 30 days. Only at 20 ppm was Endothal utilized by B. cereus var. mycoides.

The relative ease in which Endothal is utilized and degraded has been also shown by Jensen (1964). He found that soils treated with Endothal under laboratory or field conditions became enriched with a microflora, which, under favorable conditions, would dissipate the phytotoxic effect of 500 ppm Endothal within a week. From soils treated with Endothal several Arthrobacter sp. were isolated on a soil extract-Endothal agar medium.

Only at 10 ppm was TD-47 slowly utilized by the Streptomyces and by the two bacteria. No visible growth of these microorganisms was observed at 100 and 1000 ppm TD-47 within 60 days. The apparent resistance of TD-47 to microbial utilization as a carbon source may be attributed to the changes made to the molecular structure of the parent molecule, Endothal. This relationship of resistance and molecular structure was shown to occur by DeRose and Newman (1947) in their work with the phenoxyacetic acid derivatives. They noted that resistance to microbial degradation increases in order from 2,4-D to 2-methyl-4-chlorophenoxyacetic acid to

2, 4, 5-trichlorophenoxyacetic acid. Audus (1951a) also reported that the substitution of a methyl for a chloro group in the ortho position of 2, 4-D or the addition of a third chlorine, greatly increased the resistance of the compound to bacterial attack.

Similar results were obtained with glucose and the herbicides added to the synthetic liquid medium.

Identification of Test Microorganisms. Descriptions for Bacillus megaterium, B. cereus var. mycoides and Aspergillus niger van Tiegham are presented in Charts 1, 2, and 3. Photographs of both bacterial species are also presented (Figures 1 to 10 at x5000).

Inhibition Tests by Seeded Plate Method

Results for the average size of inhibition zones of Bacillus megaterium, B. cereus var. mycoides and Aspergillus niger van Tiegham produced by different concentrations of Endothal and TD-47 on nutrient agar plates are presented in Table 31. Endothal was not inhibitory to any of these microorganisms. B. megaterium was slightly inhibited by TD-47 at 10 ppm. Inhibition zones of one, four, and five millimeters for A. niger, B. cereus var. mycoides and B. megaterium occurred at 100 ppm; at 1000 ppm distinctive zones of six, eleven and nine millimeters resulted and even persisted for more than a week. It would appear that only TD-47 is inhibitory to any appreciable degree and that the extent of inhibition varies with

Chart 1. Description of Bacillus megaterium.

12 (code number)	2003K (medium)	Trypticase soy agar	Soil in 1 percent peptone water (source)
Bacillus megaterium (name of organism)	28°C. (temperature)		Frederick H. F. Au (studied by)

Morphology: (24 hrs.)
rods, ends round; 1.1μ x 6.2μ; range-1.1μ to 2.3μ x 4.1μ to 9.2μ.

Gram Reaction:
positive at 18, 24, and 48 hrs.

Pasteurization Survival (85°C., 10 minutes):positive
Sporangia:rods
Endospores:oval, not swollen;central to subterminal; 1.4μ x 2.3μ;range-1.1μ to 1.7μ x 1.5μ to 3.2μ.

Cultural Characteristics:
Agar Stroke (1day): abundant, filiform, moist, viscid.
Colonies (6 days):
Macroscopic: 3mm., round, raised, contoured, compact.
Microscopic(100x): undulate, finely granular.
Optical properties: opalescent, opaque, pearl shell tint, CHM 3ba. (1958).
Nutrient Broth (3 days):
Amount of growth:abundant
Surface growth:none
Subsurface growth:none
Sediment:viscid
Odor:none
Soybean infusion agar (3 days):heavy thick growth.
Glucose-nitrate agar(3 days):good, filiform, moist adherent, white growth.
Anaerobic production of gas from nitrate (4 days):negative
Anaerobic growth in glucose broth (14 days):negative, pH 6.8.

Physiological Characteristics:
Relationship to O_2 (7 days):aerobic
Catalase (6 days):positive
Temp. Relationships (7 days):Growth at 10°C. +, 20°C. +, 28°C. +, 37°C. +, 45°C. +, 55°C. -.
Sole Carbon Sources:
Glucose (1 day):positive

Chart 1. Description of Bacillus megaterium (continued)

Sole Carbon Sources (continued)
- Sucrose (3 days):positive
- Mannitol (10 days):positive
- Xylose (1 day):positive
- Citrate (1 day):positive
- NH_4^+ as sole Nitrogen source (1 day):positive

Reductions:
- Nitrate (28 days):negative
- Methylene blue (14 days):negative
- Selenite (8 days):positive
- Tellurite (56 days):positive

Oxidative-Fermentative Reactions:
- Glucose (10 days):acid
- Sucrose (10 days):alkaline
- Lactose (10 days):acid
- Mannitol (10 days):acid

Hydrolysis:
- Gelatin (8 days):positive
- Casein (1 day):positive
- Fat (8 days):negative
- Starch (8 days):positive

Tolerances:
- Salt (7 days):positive at 2, 7, and 10 percent.

Litmus Milk Reactions (8 days):
- Reaction:neutral
- Curd: absent
- Peptonization:positive
- Reduction:positive

Other Reactions:
- NH_4^+ from peptone (1 day):negative
- Acetylmethylcarbinol (11 days):negative
- Indol (1 day):negative
- Methyl red (2 days):positive
- Tyrosine agar (14 days):browned.

Chart 2. Description of Bacillus cereus var. mycoides.

14 E201	Trypticase soy agar	Soil in 1 percent peptone water
(code number)	(medium)	(source)

Bacillus cereus var. mycoides	28°C.	Frederick H. F. Au
(name of organism)	(temperature)	(studied by)

Morphology: (24 hrs.)
rods, ends round, filaments; 1.0μ x 3.2μ; range 0.9μ to 1.2μ x 2.5μ to 4.2μ.

Gram Reaction:
positive at 18, 24, and 48 hrs.

Pasteurization Survival (85°C., 10 minutes):positive
Sporangia: rods
Endospores: cylindrical, not swollen; central to subterminal; 1.1μ x 2.3μ;range-0.9μ to 1.3μ x 1.8μ to 2.7μ.

Cultural Characteristics:
Agar Stroke (1 day): abundant, spreading, tough.
Colonies (6 days):
Macroscopic: 3mm., irregular, effuse, wrinkled, spreading.
Microscopic (100 x): lobed, fine granular.
Optical properties:irridescent, transparent, pearl shell tint, CHM 3ba. (1958).
Nutrient Broth (3 days):
Amount of growth:moderate
Surface of growth: pellicle
Subsurface growth:turbid
Sediment:flocculent
Odor:none
Gelatin stab (3 days):
Liquefaction: cratiform; fast rate.
Soybean infusion agar (3 days):heavy, spreading.
Glucose-nitrate agar (8 days):scanty to beady, spreading.
Anaerobic production of gas from nitrate (14 days):negative
Anaerobic growth in glucose broth (14 days):positive, pH 6.8

Physiological Characteristics:
Relationship to O_2(7 days):microaerophilic
Catalase (6 days):positive
Temp. Relationships (7 days): 10°C. +, 20°C. +, 28°C. +, 37°C. +, 45°C. +, 55°C. +.

Chart 2. Description of Bacillus cereus var. mycoides.(continued)

Physiological Characteristics (continued):

Sole Carbon Source:

- Glucose (1 day):positive
- Sucrose (3 days):positive
- Mannitol (1 day):positive
- Xylose (1 day):positive
- Citrate (3 days):positive
- NH_4^+ as sole Nitrogen source (1 day):positive

Reductions:

- Nitrate (1 day): NO_2^- positive
- Methylene blue (1 day):positive
- Selenite (8 days):positive
- Tellurite (56 days):positive

Oxidative-Fermentative Reactions:

- Glucose (10 days):acid
- Sucrose (10 days):acid
- Lactose (10 days):neutral
- Mannitol (10 days):acid

Hydrolysis:

- Gelatin (8 days):positive
- Casein (1 day):positive
- Fat (8 days):negative
- Starch (8 days):positive

Tolerances:

- Salt (7 days):positive at 2, 7 and 10 percent.

Litmus Milk Reactions (6 days):

- Reaction:neutral
- Curd:soft curd
- Peptonization:positive
- Reduction:positive

Other Reactions:

- NH_4^+ from peptone (1 day):negative
- Acetylmethylcarbinol (1 day):positive
- Indol (1 day):negative
- Methyl red (2 days):positive

Chart 3. Description of Aspergillus niger.

Name of organism: Aspergillus niger van Tiegham.

Culture No. 3. Date: 15 January 1963. Source: Dixonville soil.

Cultured 7 days at 25°C. on Czapek medium.

1. Colony Characters.
   Growth: spreading
   Diameter of colony: 4 mm. in 2 days.
   Texture: floccose.
   Margin: loose.
   Sporulation(asexual):abundant; azonate.
   Color: aerial white becoming brown-black in 3 days.
   substratum white becoming white in 3 days.
   Exudate: moderate, colorless.
   Odor: slightly, moldy.
2. Conidial Heads: black; globose, loose; dimensions 98μ x 115μ.
3. Conidiophore: continuous, smooth.
   Length: 1246μ (or 1.2 mm.); diameter 14.4μ.
4. Vesicle: globose, brown; dimensions 53μ x 50μ.
5. Foot-cells: dimensions 5μ x 38μ.
6. Sterigmata:
   Primary: colorless; club-shaped.
   Arrangement: 90 percent covering of vesicle; dimensions 4μ x 18μ.
   Secondary: club shaped; dimensions 0.7μ x 2μ.
7. Conidia: brown; echinulate; 5μ diameter.
8. Sterile hyphae (spicules): none.
9. Schlerotia: none.
10. Perfect stage: none.
11. Hulle cells: absent.

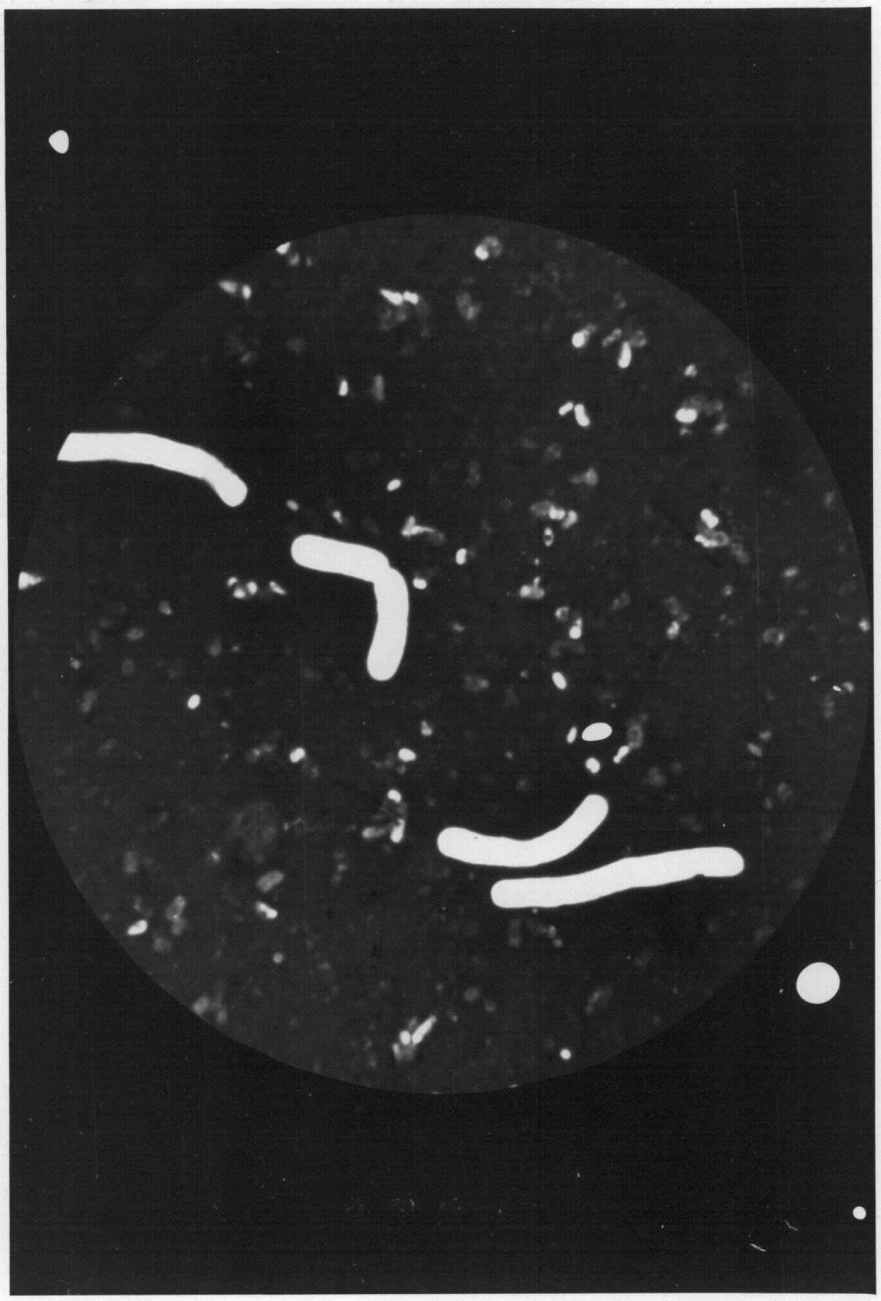

Figure 1. Nigrosin preparation of a 24 hour culture of Bacillus megaterium.

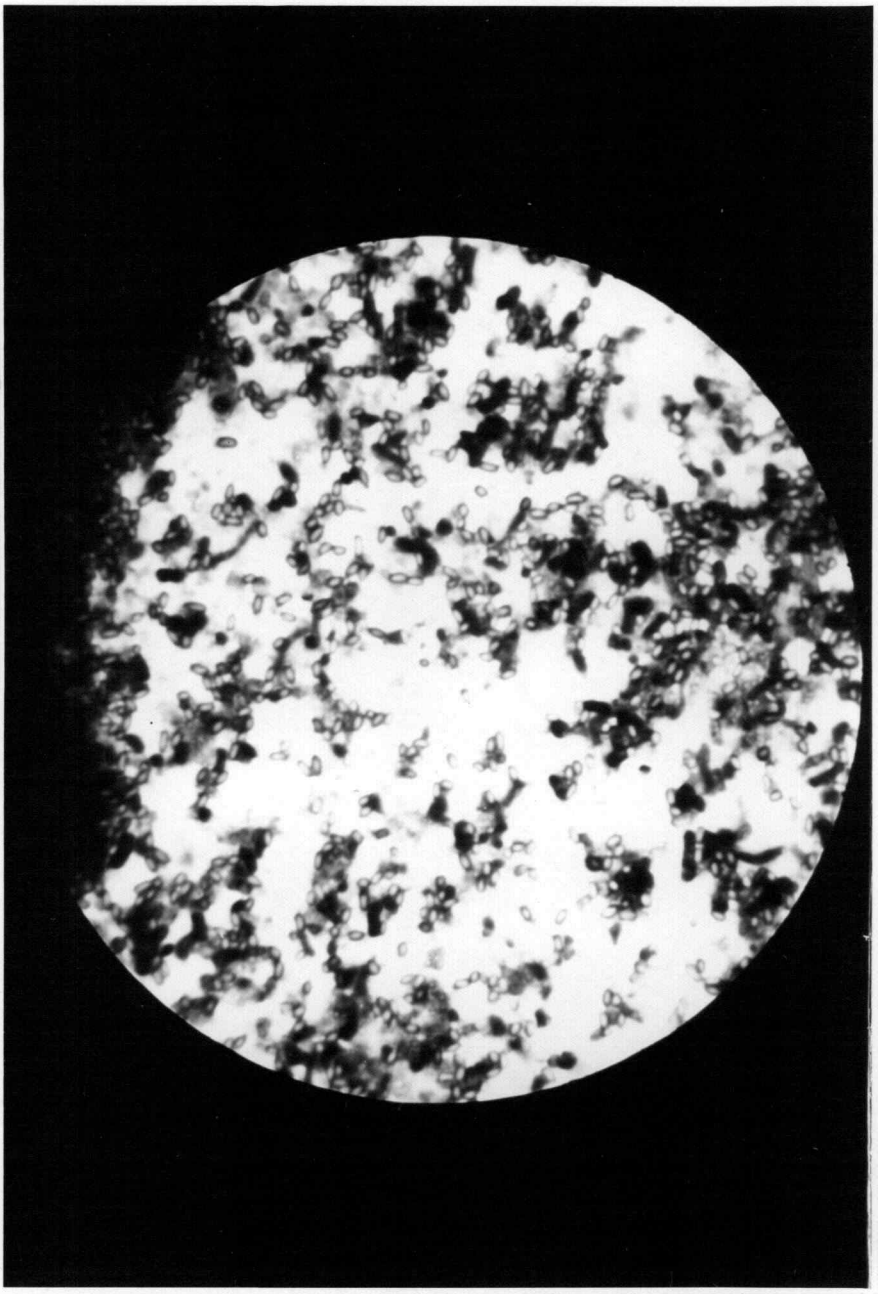

Figure 2. Methylene blue stain of a 72 hour culture of Bacillus megaterium.

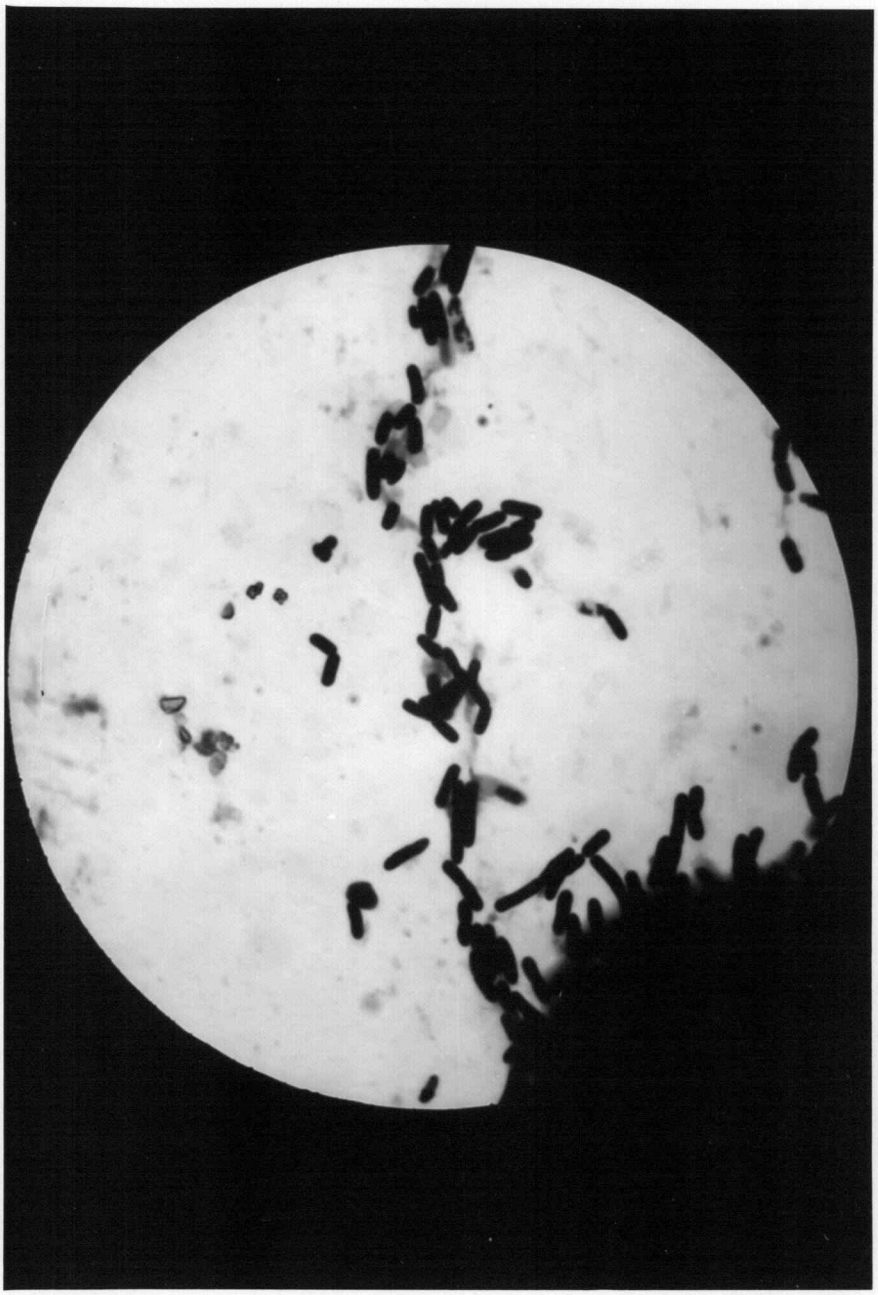

Figure 3. Gram stain of an 18 hour culture of Bacillus megaterium.

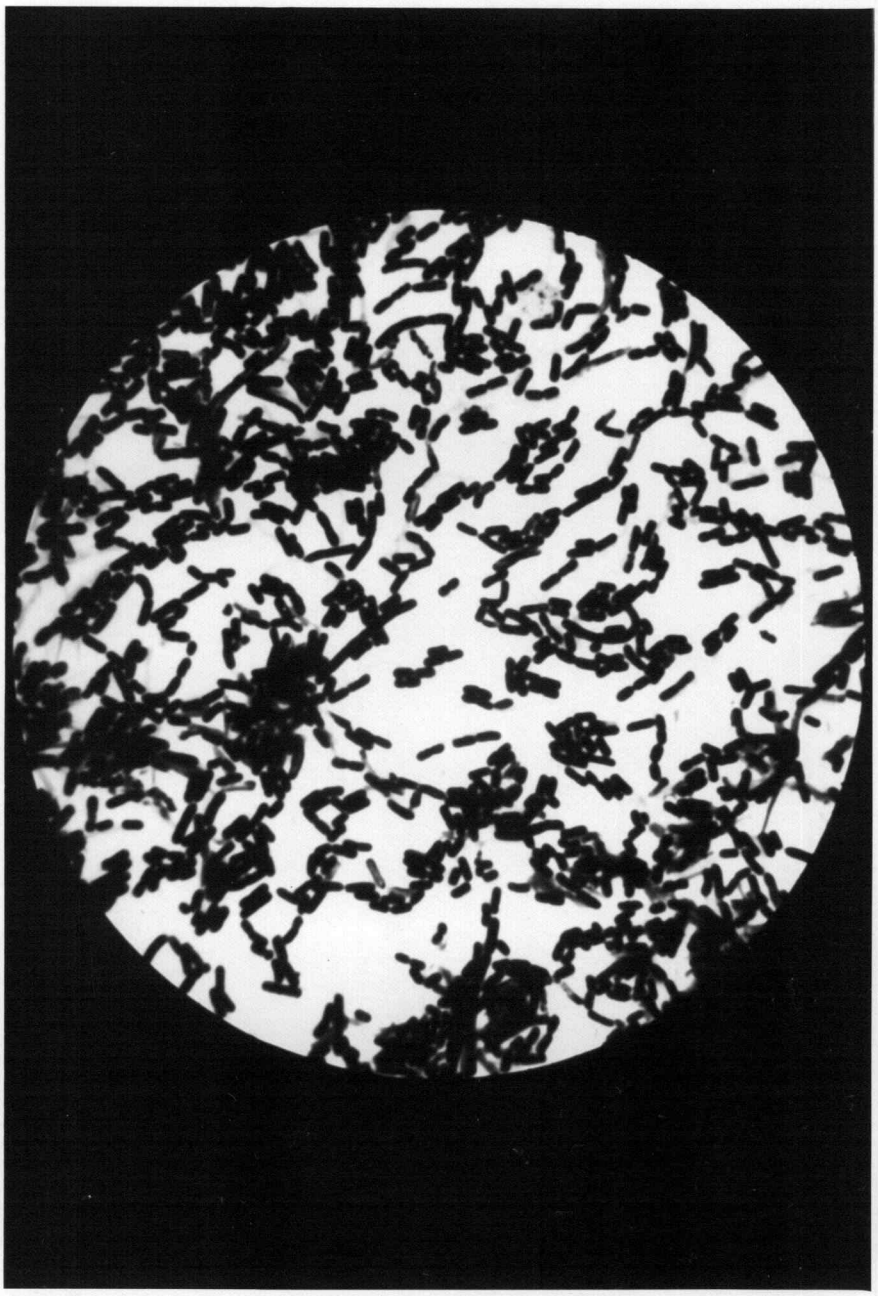

Figure 4. Gram stain of a 24 hour culture of Bacillus megaterium.

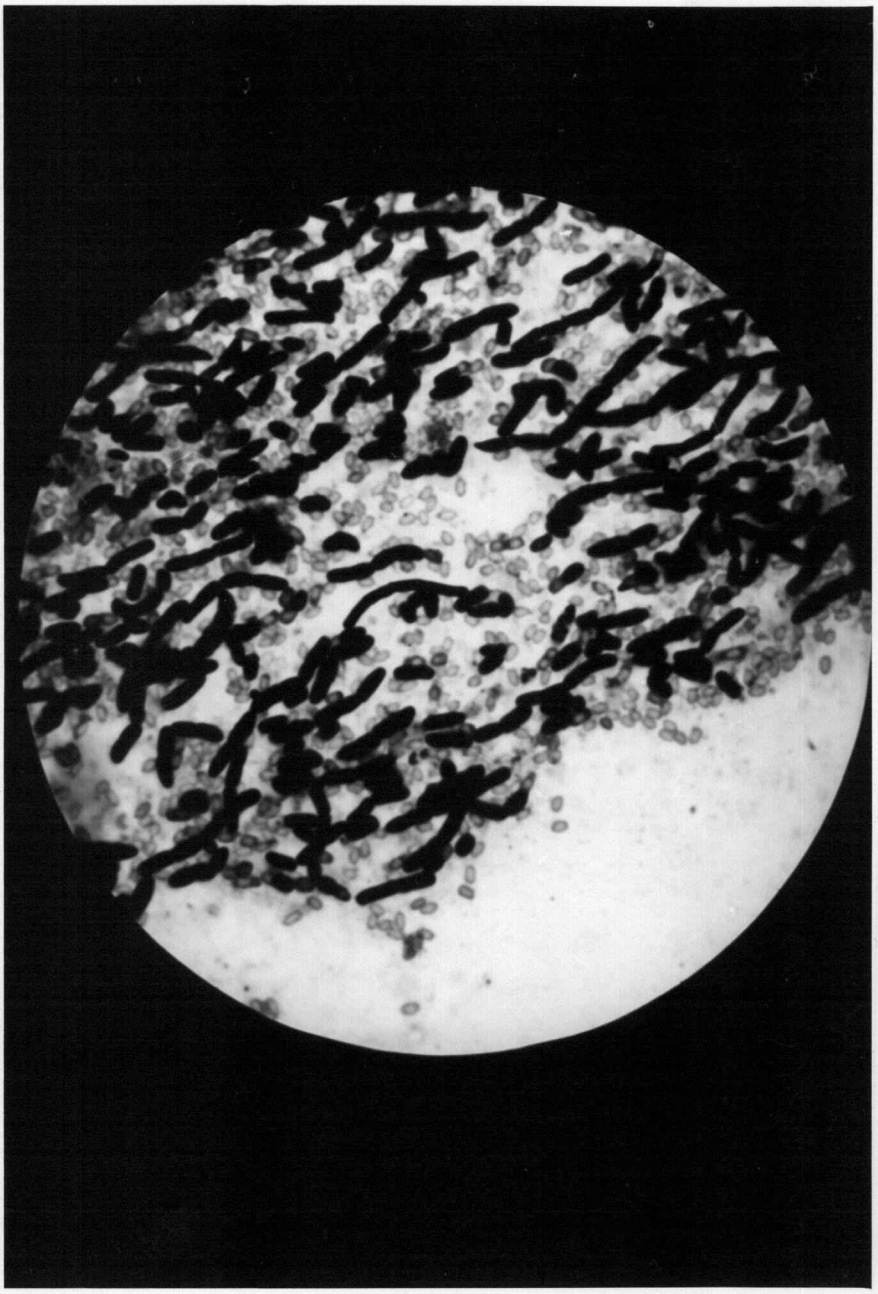

Figure 5. Gram stain of a 48 hour culture of Bacillus megaterium.

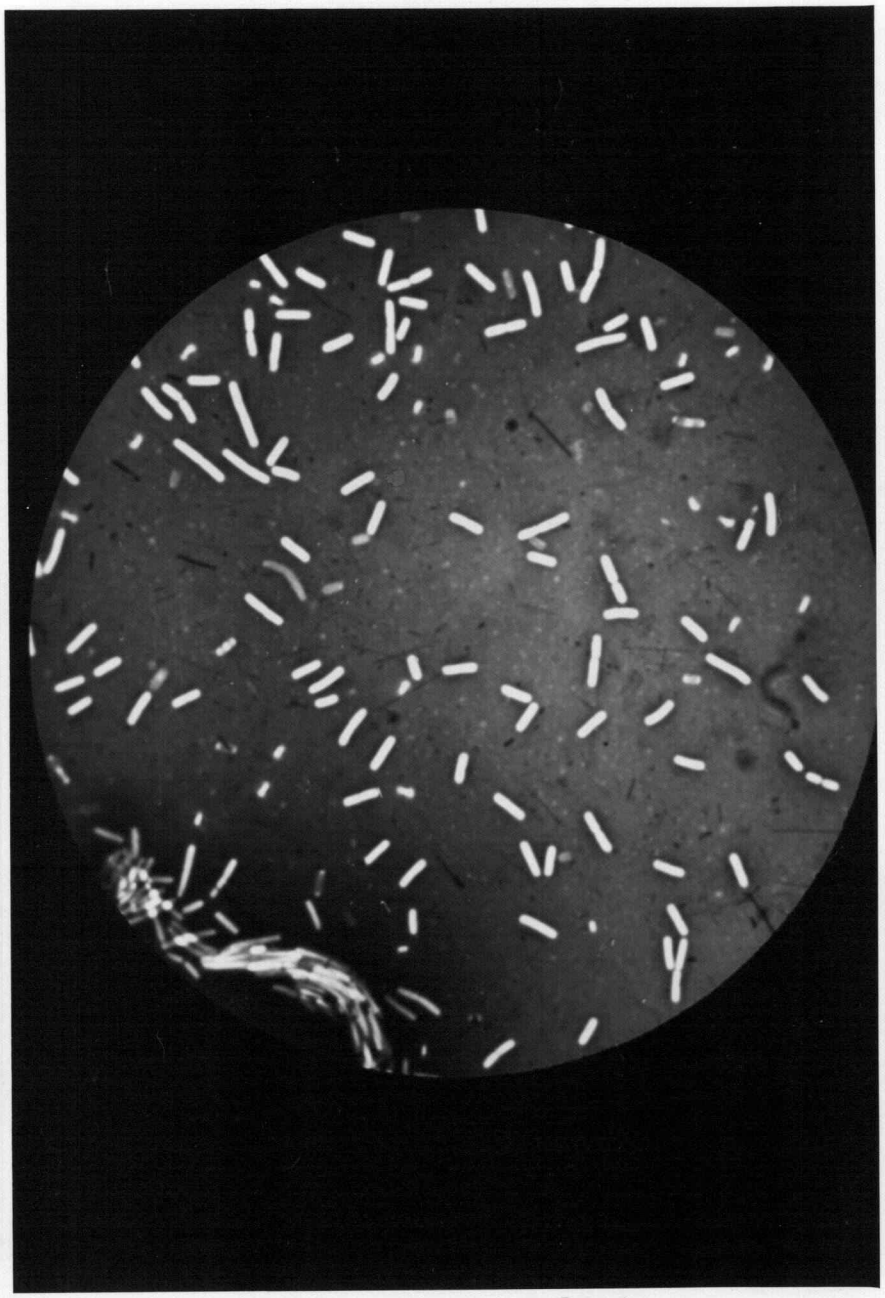

Figure 6. Nigrosin preparation of a 24 hour culture of Bacillus cereus var. mycoides.

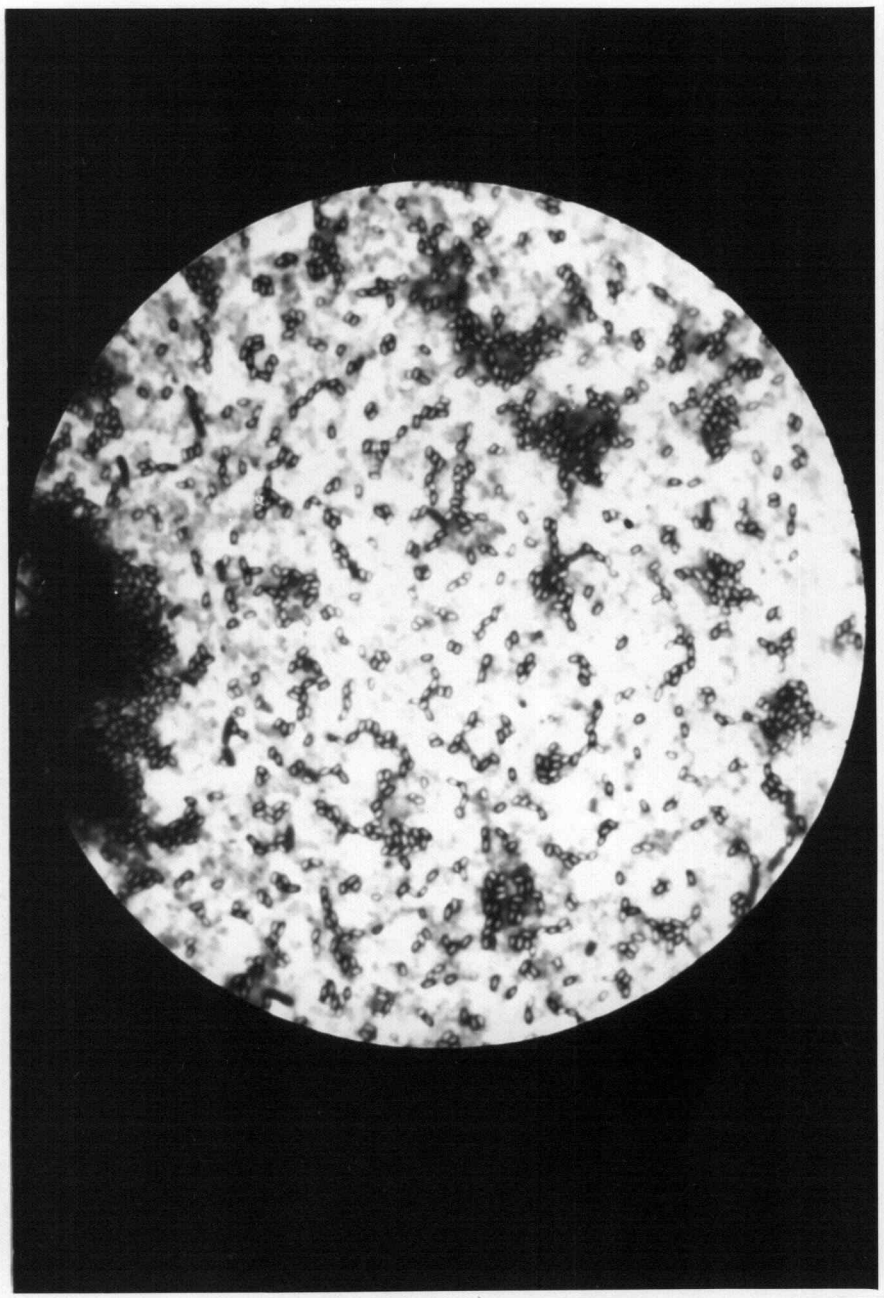

Figure 7. Methylene blue stain of a 72 hour culture of Bacillus cereus var. mycoides.

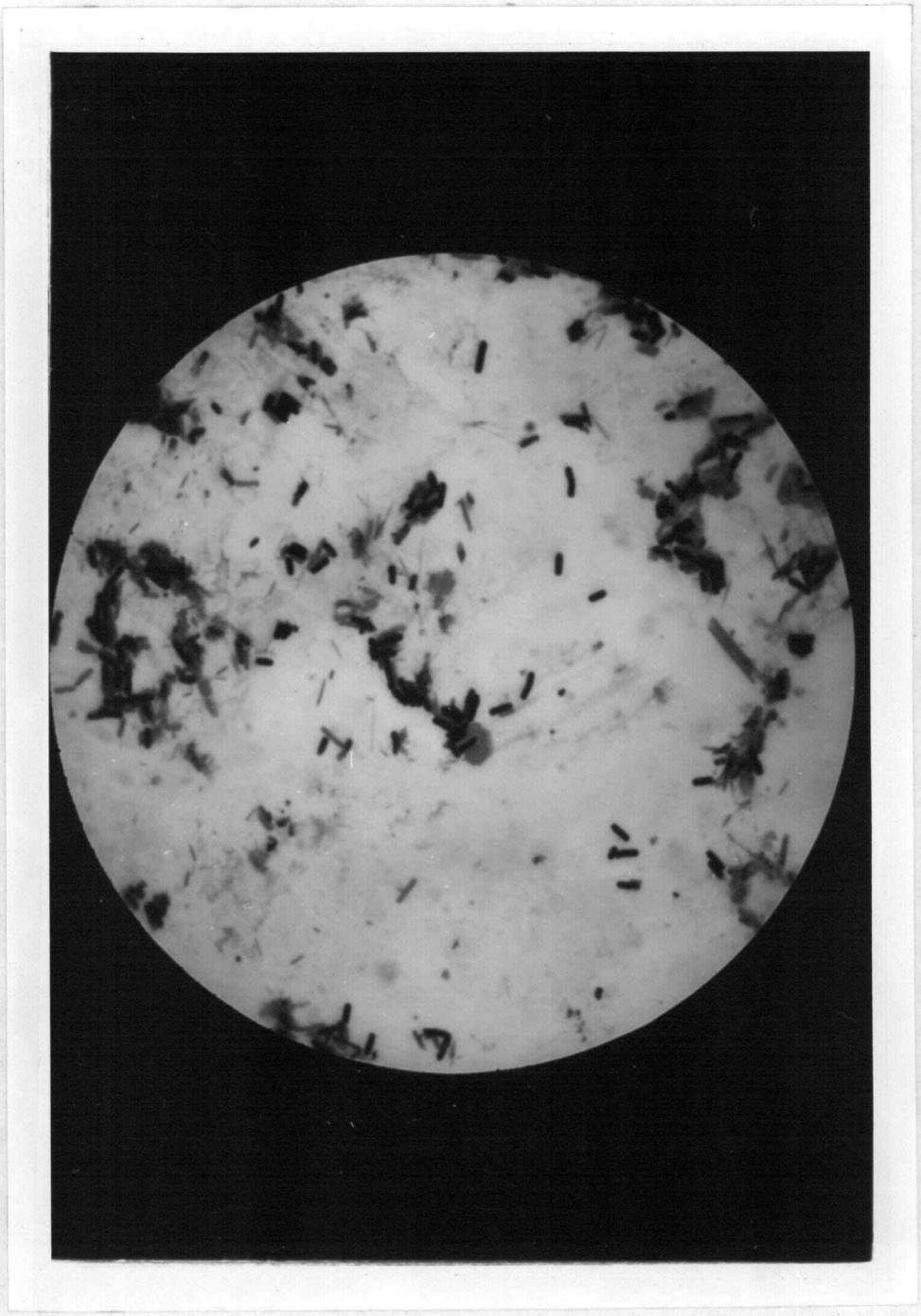

Figure 8. Gram stain of an 18 hour culture of Bacillus cereus var. mycoides.

Figure 9. Gram stain of a 24 hour culture of Bacillus cereus var. mycoides.

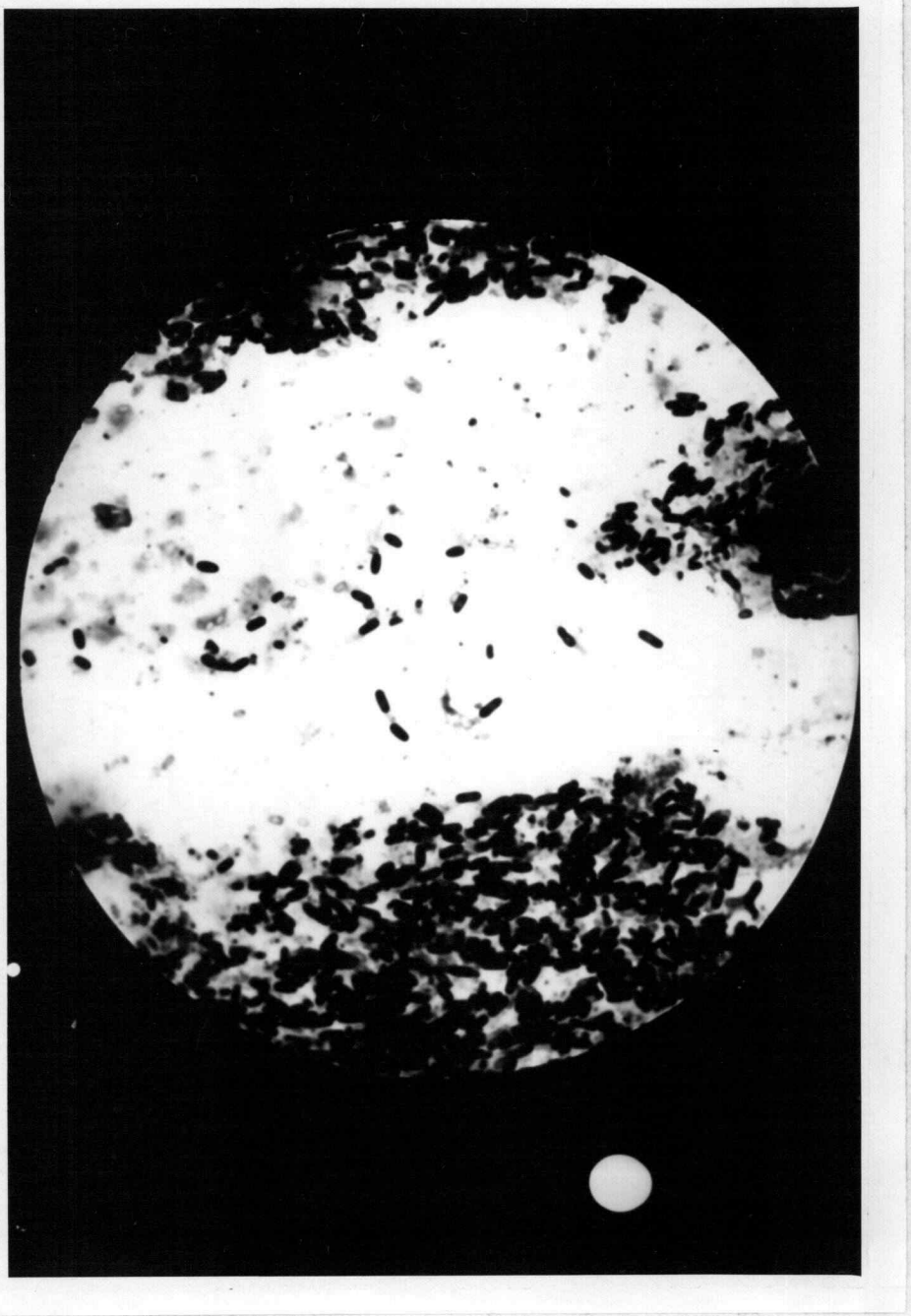

Figure 10. Gram stain of a 48 hour culture of Bacillus cereus var. mycoides.

Table 31. Inhibition zones produced by Endothal and TD-47.

Compound	Concentration ppm	Inhibition zones* B. megaterium mm.	B. cereus var. mycoides mm.	Aspergillus niger mm.
Control	----	0	0	0
Endothal	20	0	0	0
	200	0	0	0
	2000	0	0	0
TD-47	10	1	0	0
	100	4	5	1
	1000	9	11	6

* Measured from edge of disc to edge of bacterial or fungal growth.

the particular microorganism.

The comparative inhibition effected by Endothal and TD-47 on these microorganisms is shown in Figures 11 to 13.

Soil Respiration

Continuous Aeration. The results by this method are presented in Tables 32 to 34. In most cases the differences found were small and not considered to be indicative.

Generally after 30 days a slightly greater respiration was obtained from the treatment with Endothal at 20 ppm in Willamette silt loam, Colorado sandy clay loam and Klamath loamy sand. With 200 ppm Endothal slightly lower respirations were obtained in the Willamette and Klamath soils. Treatments of TD-47 at 10 ppm and of

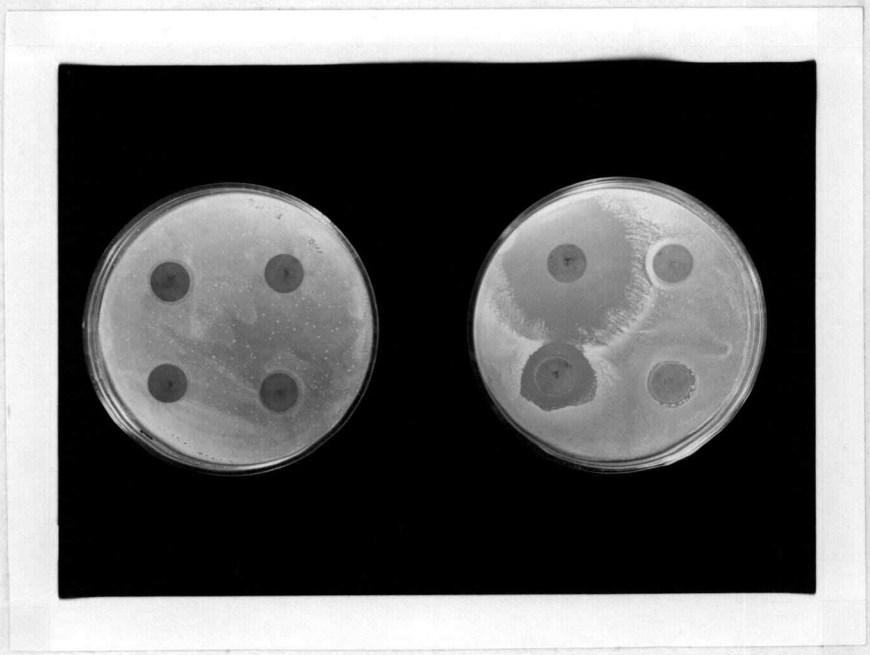

Figure 11. Effect of Endothal and TD-47 on Bacillus megaterium.

Left plate-Endothal: Upper right-control; lower right-20 ppm; lower left-200 ppm; upper left-2000 ppm.
Right plate-TD-47: Upper right-control; lower right-10 ppm; lower left-100 ppm; upper left-1000 ppm.

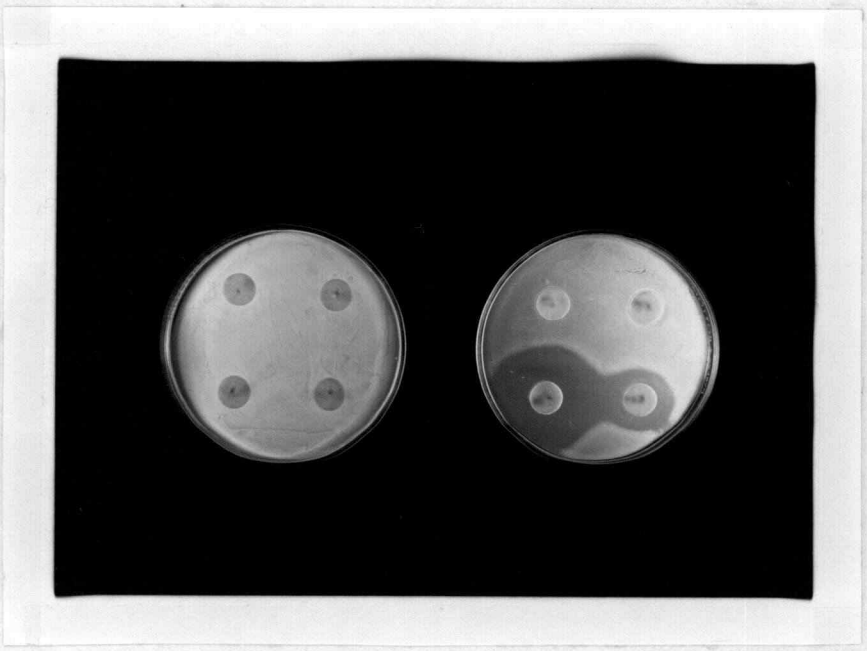

Figure 12. Effect of Endothal and TD-47 on Bacillus cereus var. mycoides.

Left plate-Endothal: Upper left-control; upper right-20 ppm; lower right-200 ppm; lower left-2000 ppm.
Right plate-TD-47: Upper left-control; upper right-10 ppm; lower right-100 ppm; lower left-1000 ppm.

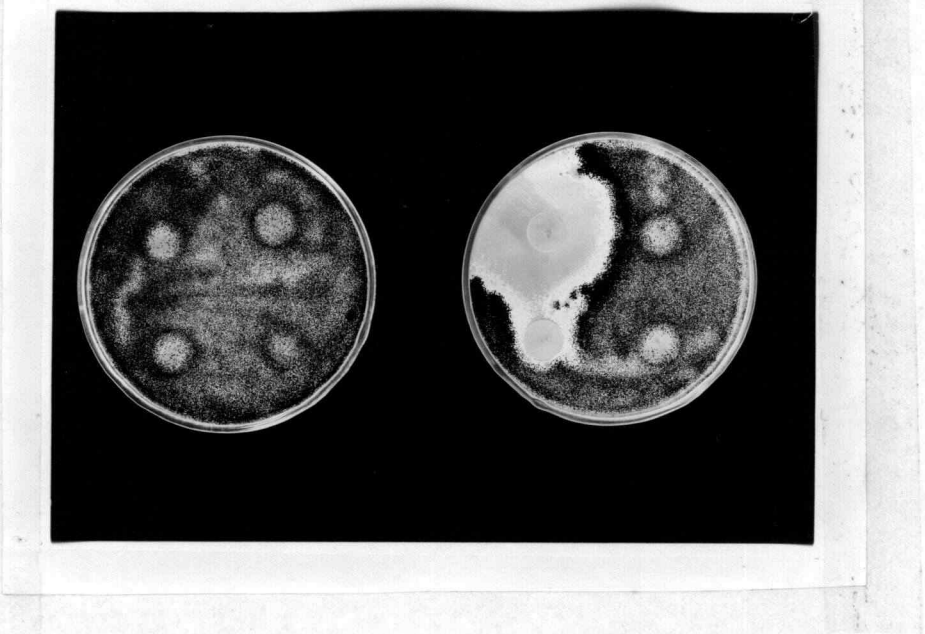

Figure 13. Effect of Endothal and TD-47 on Aspergillus niger van Tiegham.

Left plate-Endothal: Upper right-control; lower right-20 ppm; lower left-200 ppm; upper left-2000 ppm.
Right plate-TD-47: Upper right-control; lower right-10 ppm; lower left-100 ppm; upper left-1000 ppm.

Table 32. Effect of Endothal, Na-PCP and TD-47 on soil respiration of Willamette silt loam as measured by the continuous aeration method.

Treatments				Cumulative C as CO_2 evolved, mg./100 g. soil				
				Days			Increase	
Glucose (as C)	Endothal	Na-PCP	TD-47	1-1/2	12	30		
ppm	ppm	ppm	ppm	mg	mg	mg	mg	%*
0	0	0	0	7.5	20.5	29.1	-	0.12
0	20	0	0	7.5	20.3	29.3	0.2	0.12
0	200	0	0	7.9	19.6	28.3	- 0.8	0.12
0	0	0	10	7.4	18.0	26.2	- 2.9	0.11
0	0	0	100	7.6	21.3	31.5	2.4	0.13
0	0	40	0	6.5	17.7	26.2	- 2.9	0.11
0	20	40	0	7.0	17.9	26.6	- 2.5	0.11
0	200	40	0	7.0	19.5	28.7	- 0.4	0.12
800	0	0	0	41.2	72.1	87.2	58.1	7.26
800	20	0	0	39.2	67.5	80.3	- 6.9	-0.86
800	200	0	0	40.7	71.2	84.7	- 2.5	-0.31
800	0	0	10	39.1	71.0	89.2	2.0	0.25
800	0	0	100	39.5	73.0	90.6	3.4	0.42
800	0	40	0	41.0	72.4	83.8	- 3.4	-0.42
800	20	40	0	36.1	70.3	85.2	- 2.0	-0.25
800	200	40	0	38.6	71.2	82.9	- 4.3	-0.54

* Apparent decomposition assumes that all CO_2 evolved comes from decomposition of soil organic matter, or from organic additions where they are made.

Table 33. Effect of Endothal, Na-PCP and TD-47 on soil respiration of Colorado sandy clay loam as measured by the continuous aeration method.

Treatments				Cumulative C as CO_2 evolved, mg./100 g. soil				
				Days			Increase	
Glucose (as C)	Endothal	Na-PCP	TD-47	1-1/2	12	30		
ppm	ppm	ppm	ppm	mg	mg	mg	mg	%*
0	0	0	0	4.8	12.4	20.8	-	0.27
0	20	0	0	5.2	14.2	23.5	2.7	0.31
0	200	0	0	5.0	18.6	28.4	7.6	0.37
0	0	0	10	5.2	13.8	24.1	3.3	0.32
0	0	0	100	5.5	23.0	37.1	16.3	0.49
0	0	40	0	4.5	12.0	21.6	0.8	0.28
0	20	40	0	4.7	11.9	18.7	- 2.1	0.25
0	200	40	0	4.2	13.0	23.3	2.5	0.31
800	0	0	0	31.4	58.8	75.8	55.0	6.88
800	20	0	0	32.2	61.0	77.6	1.8	0.23
800	200	0	0	31.2	60.3	82.8	7.0	0.88
800	0	0	10	29.3	58.6	76.7	0.9	0.11
800	0	0	100	30.4	62.3	87.4	11.6	1.45
800	0	40	0	31.7	62.4	72.3	- 3.5	-0.44
800	20	40	0	30.3	62.1	73.8	- 2.0	-0.25
800	200	40	0	31.9	63.9	79.9	4.1	0.51

* Apparent decomposition assumes that all CO_2 evolved comes from decomposition of soil organic matter, or from organic additions where they are made.

Table 34. Effect of Endothal, Na-PCP and TD-47 on soil respiration of Klamath loamy sand as measured by the continuous aeration method.

Treatments				Cumulative C as CO_2 evolved, mg./100 g. soil				
				Days			Increase	
Glucose (as C)	Endothal	Na-PCP	TD-47	1-1/2	12	30		
ppm	ppm	ppm	ppm	mg	mg	mg	mg	%*
0	0	0	0	9.6	23.4	36.5	-	0.52
0	20	0	0	8.3	24.1	37.1	0.6	0.53
0	200	0	0	8.5	21.2	31.1	- 5.4	0.44
0	0	0	10	8.8	20.6	30.6	- 5.9	0.44
0	0	0	100	8.6	22.9	35.7	- 0.8	0.51
0	0	40	0	6.7	15.3	23.7	-12.8	0.34
0	20	40	0	7.5	15.2	22.9	-13.6	0.33
0	200	40	0	6.5	16.1	24.6	-11.9	0.35
800	0	0	0	53.0	78.3	94.0	57.5	7.20
800	20	0	0	47.2	74.4	88.1	- 5.9	-0.74
800	200	0	0	49.8	75.4	88.9	- 5.1	-0.64
800	0	0	10	46.1	70.9	83.0	-11.0	-1.38
800	0	0	100	44.8	70.5	86.6	- 7.4	-0.93
800	0	40	0	47.5	68.9	78.9	-15.1	-1.89
800	20	40	0	49.9	72.6	82.1	-11.9	-1.49
800	200	40	0	45.3	69.7	81.7	-12.3	-1.54

* Apparent decomposition assumes that all CO_2 evolved comes from decomposition of soil organic matter, or from organic additions where they are made.

Na-PCP decreased respiration to a small extent in the Willamette silt loam and more so in the Klamath loamy sand. However, an increase resulted from 100 ppm TD-47 in the Colorado soil. This tends to agree with the increase in total molds and bacteria produced by this concentration. The combined treatments of Na-PCP and Endothal at 20 and 200 ppm decreased respiration a little, the greater decrease of 4 percent occurring with the lower Endothal concentration.

Except for a slightly lower respiration with the Klamath soil evolution of carbon dioxide from added glucose was not significantly depressed by the herbicides.

Periodic Aeration. The results with the apparatus of Bartha and Pramer (1965) are presented in Table 35.

Respiration was lower with Na-PCP alone and with Na-PCP combined with Endothal. The pattern of results by this method is similar to that obtained for the Willamette soil under continuous aeration.

Respiration by Warburg Apparatus. The results are presented in Tables 36 to 38.

In Willamette silt loam soil after 96 hours the oxygen uptake was slightly increased by Endothal at 20 and 200 ppm. It was decreased by 17 percent in the Na-PCP treatment and by 13 percent in the combined Na-PCP and 20 ppm Endothal treatment. Treatments

Table 35. Effect of Endothal, Na-PCP and TD-47 on soil respiration of Willamette silt loam as measured by the periodic aeration method.

Treatments				Cumulative C as CO_2 evolved, mg./100 g. soil				
				Days			Increase	
Glucose (as C)	Endothal	Na-PCP	TD-47	1-1/2	10	30		
ppm	ppm	ppm	ppm	mg	mg	mg	mg	%*
0	0	0		9.9	25.3	37.0	-	0.15
0	20	0		9.9	25.0	37.3	0.3	0.16
0	0	40		8.6	22.9	34.0	- 3.0	0.14
0	20	40		8.9	25.0	33.8	- 3.2	0.14
0	0	0		4.9	13.7	20.4	-	0.09
800	0	0		23.5	38.6	47.2	26.8	3.30
800	20	0		23.2	38.5	47.0	- 0.2	-0.03
800	0	40		23.7	40.0	48.8	1.6	0.20
800	20	40		23.1	39.1	47.8	0.6	0.08

* Apparent decomposition assumes that all CO_2 evolved comes from decomposition of soil organic matter or from organic additions which were made.

Table 36. Effect of Endothal, Na-PCP and TD-47 on the oxygen uptake of Willamette silt loam by Warburg respirometers.

Treatments				μl O_2 uptake/g. of soil/hr. Hours				
Glucose (as C) ppm	Endothal ppm	Na-PCP ppm	TD-47 ppm	24 μl	48 μl	72 μl	96 μl	Increase μl
0	0	0	0	219.4	368.1	468.5	543.7	--
0	20	0	0	234.4	393.1	502.3	583.4	39.7
0	200	0	0	220.8	374.4	490.1	555.7	12.0
0	2000	0	0	217.5	372.9	458.0	526.4	- 17.3
0	0	0	10	225.7	385.4	494.5	578.5	34.8
0	0	0	100	226.0	397.0	510.7	598.9	55.2
0	0	40	0	152.0	291.5	386.0	453.8	- 89.9
0	20	40	0	153.2	301.5	398.7	474.4	- 69.3
80	0	0	0	326.7	528.4	654.2	746.7	202.7
80	20	0	0	331.7	535.8	661.9	755.3	8.9
80	200	0	0	270.2	441.4	550.8	629.8	-116.6
80	2000	0	0	260.1	437.0	542.9	618.0	-128.4
80	0	0	10	257.9	431.9	536.8	621.5	-124.9
80	0	0	100	274.0	454.4	570.2	648.2	- 98.2
80	0	40	0	184.6	377.2	487.5	566.1	-180.3
80	20	40	0	205.5	414.4	530.0	613.9	-132.5

Table 37. Effect of Endothal, Na-PCP and TD-47 on the oxygen uptake of Colorado sandy clay loam by Warburg respirometers.

Treatments				μl O_2 uptake/g. of soil/hr. Hours				
Glucose (as C)	Endothal	Na-PCP	TD-47	24	48	72	96	Increase
ppm	ppm	ppm	ppm	μl	μl	μl	μl	μl
0	0	0	0	70.8	110.8	130.1	150.9	--
0	20	0	0	66.9	106.2	125.6	147.1	- 3.8
0	200	0	0	65.0	101.2	120.1	142.3	- 8.6
0	2000	0	0	65.0	104.0	126.4	160.0	9.1
0	0	0	10	70.0	109.0	130.9	155.0	4.1
0	0	0	100	74.9	115.6	140.7	167.6	16.7
0	0	40	0	56.5	89.9	106.9	125.6	-25.3
0	20	40	0	53.3	82.5	98.4	117.5	-33.4
80	0	0	0	123.7	172.9	206.4	234.1	83.2
80	20	0	0	114.3	160.0	192.8	217.4	-16.7
80	200	0	0	103.0	150.1	185.7	211.4	-22.7
80	2000	0	0	129.0	179.5	216.6	249.8	15.7
80	0	0	10	122.6	173.2	208.1	234.1	0
80	0	0	100	124.0	176.5	213.7	244.6	10.5
80	0	40	0	118.3	172.9	205.2	230.3	- 3.8
80	20	40	0	120.0	170.8	203.2	227.7	- 6.4

Table 38. Effect of Endothal, Na-PCP and TD-47 on the oxygen uptake of Klamath loamy sand by Warburg respirometers.

Treatments				μl O_2 uptake/g. of soil/hr.				
				Hours				
Glucose (as C)	Endothal	Na-PCP	TD-47	24	48	72	96	Increase
ppm	ppm	ppm	ppm	μl	μl	μl	μl	μl
0	0	0	0	95.2	151.2	186.3	217.2	--
0	20	0	0	95.9	149.9	184.7	211.4	- 5.8
0	200	0	0	94.0	144.9	174.3	197.4	-19.8
0	2000	0	0	94.0	148.9	180.7	206.6	-10.6
0	0	0	10	91.8	143.0	174.6	199.7	-17.5
0	0	0	100	94.5	147.0	179.8	206.2	-11.0
0	0	40	0	51.9	103.2	133.4	153.3	-63.9
0	20	40	0	51.0	109.8	140.1	162.0	-55.2
80	0	0	0	179.2	251.1	320.9	352.5	135.3
80	20	0	0	173.2	239.8	306.0	335.4	-17.1
80	200	0	0	169.8	231.0	287.9	314.1	-38.4
80	2000	0	0	179.6	242.2	302.4	330.9	-21.6
80	0	0	10	183.9	256.3	330.4	364.7	12.2
80	0	0	100	178.7	246.9	310.5	340.1	-12.4
80	0	40	0	123.3	209.2	266.8	290.3	-62.2
80	20	40	0	128.8	214.8	276.6	301.6	-50.9

with 10 and 100 ppm TD-47 and with Endothal at 200 ppm moderately increased oxygen uptake. With glucose in the Willamette soil all the herbicide treatments, except Endothal at 20 ppm, caused marked decreases in the uptake of oxygen.

In the Klamath loamy sand soil oxygen uptake was decreased by the herbicide treatments, the largest decrease, 30 percent, occurring with Na-PCP. From added glucose the oxygen uptake was generally lessened by all the herbicides.

In the sandy soils a bimodal inhibiting effect on the uptake of oxygen occurred with the Endothal treatments. Endothal at 20 and 200 ppm increased oxygen uptake in Willamette silt loam, while Na-PCP generally decreased oxygen uptake in all three soils. At the field rate, 20 ppm, Endothal did not adversely affect microbial respiration, as measured by each of the three methods. Kratochvil (1951) also observed that Endothal did not affect carbon dioxide evolution but he found that Na-PCP at field rates did decrease respiration.

In analyzing the pattern of the results on the microbial respiration obtained by these three methods, it can be concluded that they give similar results. Yet there are distinct advantages inherent within each method. The primary advantage for the Warburg respirometers is the rapidity in which data can be obtained. However, the demands for concentrated attention, the relatively small number

of treatments limited by the few respirometers available, atmospheric temperature fluctuations, and moisture condensation in the respirometers resulting from this fluctuation, as well as the large initial cost of the equipment, all combine to make this a less practicable method for soil studies.

The continuous aeration method is highly preferred because of the large number of different treatments which can be made with inexpensive apparatus and observed in a single experiment that may be carried on for extensive periods of time. On the other hand, the facility of the periodic aeration method and low initial cost of the equipment make it more preferable than the Warburg respirometers.

Algicidal Properties of Endothal, TD-47, Na-PCP and Other Toxicants on Four Different Algae

The results with eight herbicides on the average growth of four algae are presented in Tables 39 and 40. Figures 14 and 15 show the water bath used and the effects of the herbicides on the growth of Chlamydomonas reinhardti Dangeard. In Table 40 a statistically significant difference is shown only when there is no overlapping of the vertical lines.

Growth of Chlamydomonas reinhardti and Chlorella pyrenoidosa were significantly inhibited by Diuron, TD-47 and Na-PCP. Complete inhibition of Anabaena cylindrica and Nostoc muscorum occurred

Table 39, Comparison of eight herbicides on the growth of four algae.

Herbicide	Rate	Growth Means*			
		Chlamydomonas reinhardti	Chlorella pyrenoidosa	Anabaena cylindrica	Nostoc muscorum
Control	None	84.4	75.5	50.3	14.4
Tordon 22K	Low	82.5	61.0	41.5	11.5
	Medium	76.5	80.5	58.0	12.5
	High	85.5	74.0	54.0	12.0
Amiben	Low	84.5	73.0	39.5	12.0
	Medium	84.0	73.5	53.5	14.5
	High	79.0	76.0	46.0	13.5
Endothal	Low	76.5	71.5	47.0	14.0
	Medium	86.0	75.5	63.5	13.0
	High	86.5	78.5	64.0	14.0
Diuron	Low	0	12.0	0	0
	Medium	0	13.0	0	0
	High	0	13.0	0	0
TD-47	Low	17.0	16.5	13.5	9.5
	Medium	0	13.5	16.5	7.5
	High	0	0	0	0
Na-PCP	Low	63.0	33.5	40.5	12.5
	Medium	7.5	17.0	5.0	8.0
	High	0	0	0	0
Diquat	Low	85.0	80.5	48.5	13.5
	Medium	86.5	71.5	37.5	12.0
	High	88.0	86.5	8.0	11.0
Paraquat	Low	80.5	78.0	50.0	14.5
	Medium	87.0	84.5	51.5	13.0
	High	86.5	81.0	33.0	10.0

* Average diameter of algal growth expressed in millimeters.

Table 40. Duncan Multiple Range Tests on the average diameter of algal growth.*

Organisms	Chlamydomonas reinhardti 85		Chlorella pyrenoidosa 251	
Average growth diameter (m.m.)	Range of herbicide		Average growth diameter (m.m.)	Range of herbicide
0.0	Diuron	(H)+	0.0	Na-PCP (H)
0.0		(M)	0.0	TD-47 (H)
0.0		(L)	12.0	Diuron (L)
0.0	TD-47	(H)	13.0	(M)
0.0		(M)	13.0	(H)
0.0	Na-PCP	(H)	13.5	TD-47 (M)
7.5	Na-PCP	(M)	16.5	TD-47 (L)
17.0	TD-47	(L)	17.0	Na-PCP (M)
63.0	Na-PCP	(L)	33.5	Na-PCP (L)
76.5	Tordon	(M)	61.0	Tordon (L)
76.5	Endothal	(L)		Endothal (L)
79.0	Amiben	(H)	71.5	Diquat (M)
80.5	Paraquat	(L)	73.0	Amiben (L)
82.5	Tordon	(L)	73.5	Amiben (M)
84.0	Amiben	(M)	74.0	Tordon (H)
84.4	Control		75.5	Endothal (M)
84.5	Amiben	(L)	75.5	Control
85.0	Diquat	(L)	76.0	Amiben (H)
85.5	Tordon	(H)	78.0	Paraquat (L)
86.0	Endothal	(M)	78.5	Endothal (H)
86.5	Endothal	(H)	80.5	Diquat (L)
86.5	Diquat	(M)	80.5	Tordon (M)
86.5	Paraquat	(H)	81.0	Paraquat (H)
87.0	Paraquat	(M)	84.5	Paraquat (M)
88.0	Diquat	(H)	86.5	Diquat (H)

Table 40. (continued)

Organisms	Anabaena cylindrica 629		Nostoc muscorum 486	
Average growth diameter (m. m.)	Range of herbicide		Average growth diameter (m. m.)	Range of herbicide
0.0	Diuron	(H)	0.0	Diuron (H)
0.0		(M)	0.0	(M)
0.0		(L)	0.0	(L)
0.0	Tordon	(H)	0.0	TD-47 (H)
0.0	Na-PCP	(H)	0.0	Na-PCP (H)
5.0	Na-PCP	(M)	7.5	TD-47 (M)
8.0	Diquat	(H)	8.0	Na-PCP (M)
13.5	TD-47	(L)	9.5	TD-47 (L)
16.5	TD-47	(M)	10.0	Paraquat (H)
33.0	Paraquat	(H)	11.0	Diquat (H)
37.5	Diquat	(M)	11.5	Tordon (L)
39.5	Amiben	(L)	12.0	Amiben (L)
40.5	Na-PCP	(L)	12.0	Tordon (H)
41.5	Tordon	(L)	12.0	Diquat (M)
46.0	Amiben	(H)	12.5	Tordon (M)
47.0	Endothal	(L)	12.5	Na-PCP (L)
48.5	Diquat	(L)	13.0	Paraquat (M)
50.0	Paraquat	(L)	13.0	Endothal (M)
50.3	Control		13.5	Amiben (H)
51.5	Paraquat	(M)	13.5	Diquat (L)
53.5	Amiben	(M)	14.0	Endothal (L)
54.0	Tordon	(H)	14.0	Endothal (H)
58.0	Tordon	(M)	14.4	Control
63.5	Endothal	(M)	14.5	Amiben (M)
64.0	Endothal	(H)	14.5	Paraquat (L)

* Level of significance at 5%.

+ (H)=High concentration
(M)=Medium concentration
(L)=Low concentration.

Figure 14. Greenhouse water bath for comparison of herbicides as algicides on the growth of four algae.

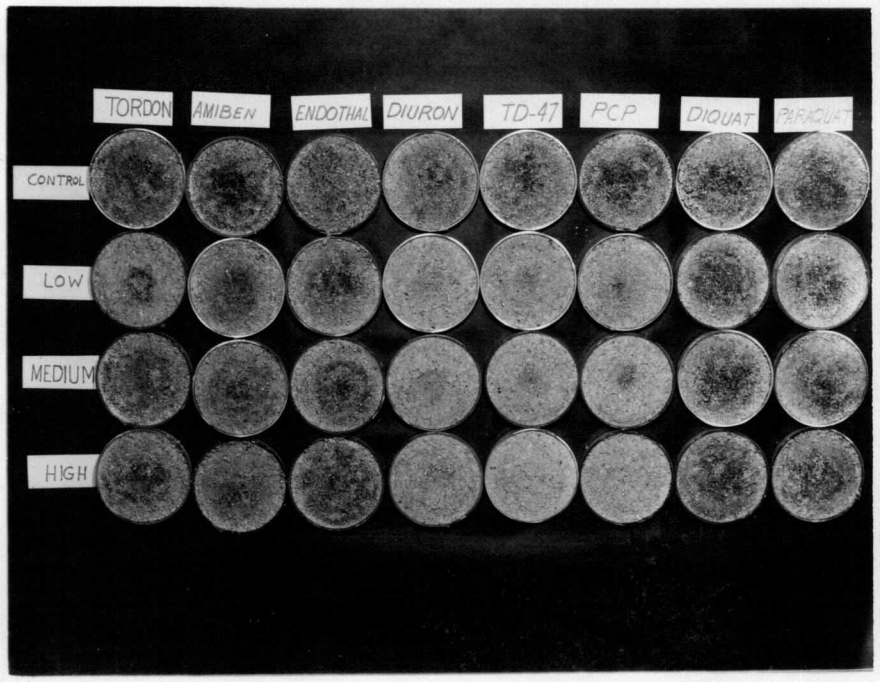

Figure 15. Comparison of herbicides as algicides on Chlamydomonas reinhardti Dangeard.

with all concentrations of Diuron and with the high concentrations of TD-47 and Na-PCP. Significant partial inhibition of Anabaena cylindrica resulted from the high concentrations of Diquat and Paraquat, the medium levels of TD-47 and Na-PCP, and the low levels of TD-47. The partial inhibition of Nostoc muscorum by the medium concentrations of TD-47 was significant. Low concentrations of Na-PCP produced no significant effect on the growth of either of the blue-green algae. It would appear that a direct relationship generally existed between the concentrations of Diuron, TD-47 and Na-PCP and the extent of inhibition; the higher the concentration the more extensive the inhibition. With Endothal a reverse relationship towards growth occurred to some degree for Chlamydomonas reinhardti, Chlorella pyrenoidosa, Anabaena cylindrica and Nostoc muscorum. Because little is known about the mode of action of Endothal, it is difficult to explain this behavior, especially in view of the report of Mann, et al., (1965) who found that Endothal depressed the incorporation of leucine into protein. However, the test plants used by Mann, et al., (1965) were segments of barley seedlings which could yield entirely different results than algae.

Different herbicides have been shown to produce different effects on the same alga. Wells and Chappell (1965) showed that Simazine and Atrazine killed Chlorella pyrenoidosa 7-11-05 at 0.1 ppm while Dichlobenil and 2, 4-D caused no effect at 10 ppm.

In still water Diquat, Paraquat, and TD-47 at 1 ppm were found to give 85 percent inhibition for the submersed weeds Elodea densa and Najas guadalupensis (Blackburn, 1963). It was found that with 3 ppm TD-47 growth recovery of Najas guadalupensis required three months (Blackburn, 1964).

Effectiveness of Diuron in inhibiting algal growth has been reported by Jansen, et al., (1958). Their experiments were conducted in two stages. The inoculum for the first experiment was a nearly pure culture of Chlamydomonas sp. collected from rice pots. The inoculum for the second stage contained predominantly a filamentous Hormiscium and a Protococcus species. They noted that Diuron at 0.25 ppm completely inhibited the growth of Chlamydomonas sp., and that 0.12 ppm inhibited Hormiscium and Protococcus.

Owing perhaps to the much limited use of benzoic acids very little work has been carried out on their mode of action. Minarik, et al., (1951) reported on the activity of about 200 derivatives of benzoic acid and found that only seven were active growth inhibitors. Ready, et al., (1952) observed albinism in the immature leaves of plants after treatment with a number of nitrohydroxy-, methoxy-, and ethoxybenzoic acids. Microscopic examination of the white tissues indicated that the unusually small plastids were devoid of pigments and it was believed that death would result. However, they did not claim that chlorobenzoic acids produced similar effects.

Amiben is a chlorobenzoic acid herbicide, and from these works it is reasonable to infer that Amiben would not significantly inhibit algal growth.

Pentachlorophenol is a typical example of a contact herbicide that is extremely toxic to plant tissue. Its mode of action is similar to dinitrophenols which have been shown to be associated with protein coagulations and the inhibition of the coupling during the oxidation phases of the phosphorylation cycle. At low concentrations usually employed for selective weed control, complete inhibition of respiration of susceptible weeds occurs and death follows (Preston, 1954). Mann, et al., (1965) found with barley seedlings that Na-PCP depressed protein synthesis from leucine as well as the uptake of α-aminobutyric acid. From the data in Table 40 it may be inferred that the mode of action of Na-PCP on algae may be similar to that in higher plants. Na-PCP could well be an effective algicide.

For Tordon 22K the results were similar to those obtained by Hardy (1966), who found that at 1 ppm it did not retard the growth of filamentous and unicellular algae in an aquarium.

A review on the permeability of algae has been presented by Stadelman (1964). In it are discussed the roles of molecular size, permeability constants, and lipid solubilizing ability of chemicals; also, diffusion of herbicides, their influence on permeability, and ecological conditions affecting algal growth. It may be that the

inhibiting action of an herbicide on algal growth may depend upon a combination of these factors as well as upon the mode of action of the herbicide itself.

V. SUMMARY AND CONCLUSION

A number of conclusions may be drawn concerning the influence of Endothal, Na-PCP, and TD-47 on the microbial activity in six different soils.

1. The data on the total microbial count indicate that the addition of only Na-PCP, singly or combined with Endothal, markedly decreased molds, bacteria and Streptomyces in each of the six soils. This influence continued for at least 30 days. Only the addition of Endothal and TD-47 did not adversely influence the microbial population. However, in the sandy soils TD-47 at the highest rate, 100 ppm, appeared to have a stimulatory influence on the molds and bacteria. In these soils, because of their lower adsorptive capacity, more of the herbicide could be available for possible use as a nutrient.
2. In each of the soils the combined treatments of Na-PCP with Endothal at 20 and 200 ppm suppressed ammonification.
3. Nitrification was not adversely decreased by the incorporation of the herbicides, except in the sandy soils where Na-PCP greatly suppressed nitrification of ammonium sulfate.
4. Bacillus megaterium and Bacillus cereus var. mycoides,

isolated from two of the soils, can utilize Endothal and TD-47 as a sole carbon source. In addition to these two bacteria, several other soil isolants were found to utilize Endothal as a carbon source. These were a Penicillium sp., a Streptomyces sp., and Aspergillus niger van Tiegham.

5. Similar patterns of general microbial activity as indicated by soil respiration, using continuous aeration, periodic aeration, and oxygen uptake, showed that field rates of Endothal and TD-47 had no adverse effect. Respiration in the sandy soils was decreased by Na-PCP. Endothal had a bimodal inhibiting effect on oxygen uptake.
6. Using four unialgal species to evaluate eight herbicides as potential algicides, it was found that Na-PCP, TD-47 and Diuron were most effective on Chlamydomonas reinhardti Dangeard, Chlorella pyrenoidosa Chick, Anabaena cylindrica Lemmerman, and Nostoc muscorum Kütz. Endothal was not significantly inhibitive.

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