STUDIES ON THE EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID, CONCENTRATED ACTIVATED DIESEL EMULSION, AND CONCENTRATED ACTIVATED DIESEL EMULSION IN COMBINATION WITH 2,4-DICHLOROPHENOXYACETIC ACID ON THE ACCUMULATION OF NITRATE NITROGEN AND TOTAL PLATE COUNTS IN TWO KANSAS SOILS

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INTRODUCTION

Research during the past three decades has brought increased knowledge and new chemicals for use in the control of weeds on productive as well as nonproductive land. These new chemical weed sprays have grown in popularity and have proven their usefulness in the weed control program.

These herbicides may be placed into 3 groups; namely, (1) contact herbicides, (2) translocated herbicides, and (3) soil sterilants. The studies herein presented involve the use of members of the first 2 groups of herbicides mentioned above. Contact herbicides, a group to which Concentrated Activated Diesel Emulsion (CADE) belongs, manifests its activity by killing only the parts of a plant which are in direct contact with the herbicide.[1] The killing action is believed to be due to the reaction of the toxic constituents of the herbicides with the protoplasm resulting in the death of the plant cells.

Translocated herbicides are exemplified by 2,4-dichlorophenoxyacetic acid, which belongs to a group of organic compounds called plant growth-regulators or plant hormones.

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[1] For a detailed discussion of the herbicide the reader is referred to an article by F. E. Hance entitled "Weed Control on Hawaiian Sugar-Cane Lands - Contact Herbicides", The Hawaiian Planters' Record. Vol. LII (Second Quarter 1948. No. 2), 93-112.

This group of herbicides is conducted into the roots of the plants through the xylem tubes following its application as a spray to the foliage. Death of the entire plant ensues, the rapidity of the action depending on several factors as the type and concentration of the chemical, temperature, sunlight, humidity, etc. 2,4-dichlorophenoxyacetic acid is also widely used as a pre-emergence spray, the main object here being to apply the spray on the soil surface in order to kill the weeds before emergence or as they germinate from seeds. More recently 2,4-dichlorophenoxyacetic acid has been used in combination with other herbicidal sprays, for example Concentrated Activated Diesel Emulsion. This combination results in a greater efficiency of the herbicide as a combination per-emergence and contact spray; the principle of synergism is employed here to great advantage.

In spraying for weed control the soil is unavoidably contaminated or the herbicides may be introduced directly to its surface layer when pre-emergence spraying is employed. In either event it is of interest to investigate the effects of these herbicides on the biological processes in the soil.

The purpose of these studies was to obtain information regarding the effects of 2,4-dichlorophenoxyacetic acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen and the total plate counts in sandy-loam and silt-loam soils.

REVIEW OF LITERATURE

Studies on the persistence of 2,4-dichlorophenoxyacetic acid in the soil have been made by several investigators, including Crafts (6), DeRose (8), Hamner et al. (17), Hanks (18), Mitchell and Marth (31), Taylor (55), and Van Overbeck and Velez (57).

Their findings indicated that the persistence of 2,4-dichlorophenoxyacetic acid in soil varies with the type of soil, the amount of leaching that takes place, the soil moisture content, the soil temperature, and the amount of organic matter present in the soil. The inhibitory effect of 2,4-dichlorophenoxyacetic acid seemed to persist longer in a naturally alkaline, sandy clay-soil and was of a shorter duration in a muck soil. Toxicity to plants might persist for periods ranging from 2 to 8 weeks after treatment in most soils, and it has been shown that 2,4-dichlorophenoxyacetic acid is slowly inactivated when mixed with air-dry soil with indications that toxicity might last even longer than 18 months. There also were indications that 2,4-dichlorophenoxyacetic acid is readily inactivated when mixed with warm, moist soil of high organic matter content. DeRose and Newman (9) found that regardless of the rates used, 2,4-dichlorophenoxyacetic acid persisted in greenhouse conditions for only 67 days. Its persistence varied inversely with the soil temperature and its disappearance was more rapid with increasing moisture levels. They also suggested that the persistence of 2,4-dichlorophenoxyacetic acid is mainly determined by soil microbial activity; autoclaved soil retained the compound longer than soil not autoclaved. Mitchell and Marth (31) and Newman and Norman (34) suggested that the inactivation of 2,4-dichlorophenoxyacetic acid in the absence of leaching is due to the action of microorganisms present in the soil. The compound was found to disappear at a faster rate from soils of high microbial activity than from soils of normal microbial activity. DeRose (8), Hanks (18), and Nutman et al. (37) also found that inactivation due to adsorption or fixation by soil colloids, leaching, and decomposition through the action of microorganisms may be factors concerned in the loss of activity of 2,4-dichlorophenoxyacetic acid in the soil. Kreis (23) found that the persistence of 2,4-dichlorophenoxyacetic acid in soil was prolonged even in the presence of large amounts of organic matter when lime was added. Lucas and Hamner (27) found that activated charcoal readily inactivated preparations of water-soluble 2,4-dichlorophenoxyacetic acid and offered this as a possible explanation for the failure of the acid to kill weed seeds in muck soils.

Carlyle and Thorpe (5) reported that legumes are as a whole more sensitive to different concentrations of 2,4-dichlorophenoxyacetic acid than their corresponding <u>Rhizobium</u>

species. The ordinary field applications of the salts of 2,4-dichlorophenoxyacetic acid would probably be harmful to the legume crops but have very little effect on the rhizobia living free in the soil. Fultz and Payne (11) found that 2,4-dichlorophenoxyacetic acid affected the gram-staining reaction and rod length of the bacterium Rhizobium leguminosarum Frank in the root nodule of common bean. A decrease in bacterial population in nodule smears from common bean plant was also noted. Payne and Fultz (38) noted that applications of 2,4-dichlorophenoxyacetic acid at concentrations of 0.075 pound per acre was sufficient to prevent nodulation of common bean. Lewis and Hamner (24) tried several samples of 2,4-dichlorophenoxyacetic acid and found that Rhizobium leguminosarum was not affected by any of them, even at high concentrations. They concluded tentatively from the available data that under ordinary rates of application for the killing of weeds, the amounts of 2,4-dichlorophenoxyacetic acid reaching the soil would have no important effect on soil microorganisms or on plant pathogens present in the soil. Marth and Mitchell (28) found that the application of 2,4-dichlorophenoxyacetic acid on plants did not greatly inhibit the subsequent development of microorganisms in the tissues after the plants had died. Complete disintegration of plants killed by spray treatments resulted from soil microorganisms.

Dubos (10) observed that 2,4-dichlorophenoxyacetic acid exerted a bacteriostatic effect on the growth of certain microorganisms. With most of the organisms tested the bacteriostatic activity increased as the medium became more acidic. Martin (29) showed that 2,4-dichlorophenoxyacetic acid was slowly destroyed in the soil by the action of microorganisms. Soil bacteria and fungi were not appreciably affected by concentrations of 2,4-dichlorophenoxyacetic acid below 10 ppm. Some microbes were inhibited by large concentrations (100 ppm and above) while others were not. Martin reported that 2,4-dichlorophenoxyacetic acid was more toxic to soil microorganisms under acid than under alkaline conditions. Stevenson and Mitchell (46) observed that 0.02 per cent solution of 2,4-dichlorophenoxyacetic acid or its sodium salt in potato-dextrose agar had a decided retarding effect on the growth of 4 bacteria while it had no effect on the growth of 2 fungi tested. Culler (7) also found that high concentrations of 2,4-dichlorophenoxyacetic acid were necessary to inhibit the growth of many organisms. Some species of *Phytomonas* were inhibited by 1,000 ppm but *Rhizopus nigricans* and certain other fungi required 40,000 ppm for inhibition. Worth and McCabe (58) observed that organisms which require free oxygen for respiration appeared to be "smothered" by 2,4-dichlorophenoxyacetic acid. Anaerobic or faculative anaerobic orgànisms were not affected significantly by the acid.

Smith, Dawson, and Wenzel (43) observed no injurious effects on total plate counts or on the number of actinomyces, fungi and protozoa in either sandy soil of good fertility or silt loam when 2,4-dichlorophenoxyacetic acid was applied at the rates of 0.5 to 100 ppm and even as high as 500 ppm in the sandy soil. The nitrite- and nitrate-forming organisms in both soils were definitely injured by 100 ppm but recovered in 10 to 40 days. The effect was somewhat less in sandy soil. The nitrite-forming bacteria were more sensitive than the nitrate-forming bacteria. When 500 ppm was used the effect was more pronounced and there was only a partial recovery of the nitrate-forming organisms even after 90 days. They found that herbicides vary greatly in their effects on various groups of soil microorganisms; in some cases they are definitely toxic and in others stimulatory. There was no indication that the potency of a herbicide can be tested against any one group of soil microorganisms. Newman (33) and Newman and Norman (34) reported that 2,4-dichlorophenoxyacetic acid had no effects on the numbers of organisms present in the soil or on the nitrification process until the compound was added in great excess. Newman observed that 2,4-dichlorophenoxyacetic acid applied at a rate of 2.5 milligrams per 100 grams of soil appreciably decreased the nitrification of ammonium sulfate. They suggested that some soil microorganisms can probably use 2,4-dichlorophenoxyacetic acid as a source of energy. Jones (21) found that under the conditions of his experiments, rates of 2,4-dichlorophenoxyacetic acid up to 25 pounds per acre, had no unfavorable effects on nitrate formation in a soil to which no nitrogen had been added. However, when nitrogen in the form of urea and sodium nitrate was added, there was an indication that 15 pounds per acre of 2,4-dichlorophenoxyacetic acid was sufficient to inhibit temporarily nitrate formation. The effect was more noticeable when sodium nitrate was added.

The utilization of petroleum, petroleum products, and petroleum hydrocarbons by microorganisms has been studied or noticed by the following investigators: Aiyer (1), Bushnell and Haas (3), Büttner (4), Gainey (12), Gray and Thornton (14), Haag (15), Haas et al. (16), Harper (19), Jensen (20), Kaserer (22), Lipman and Greenberg (25), Matthews (30), Münz (32), Rahn (39), Rogers (40), Söhngen (44, 45), Stone, Fenske, and White (47), Stone, White, and Fenske (48), Störmer (49), Strawinski and Stone (50), Tauson (51, 52), Tauson and Schapiro (53), Tausz and Peter (54), Thaysen (56), and ZoBell (59).

Söhngen (45) reported that gasoline, kerosene, paraffin oil, and paraffin wax could be oxidized to carbon dioxide, water, and traces of organic acids by organisms isolated from garden soil, ditch water, and compost. The organisms belonged principally to the genera Mycobacterium and Pseudomonas.

Tausz and Peter (54) isolated and described three new hydrocarbon-utilizing bacteria from garden soil and assigned the following names to them: Bacterium aliphaticum, Bacterium aliphaticum (liquefaciens), and "Paraffin bacterium".

Matthews (30) observed that an increase in the total counts on soil treated with the following hydrocarbons, benzene, naphthalene, toluene, phenol, xylene, hexane, pseudocumene, mesitylene, cymene, and pinene, was correlated with increases in molecular weights and heat of combustion of the compounds.

Gray and Thornton (14) isolated organisms which were capable of decomposing aromatic hydrocarbons such as naphthalene and toluene and identified them as Micrococcus, Mycobacterium, Bacterium, Bacillus, and Spirillum.

Tauson (52) isolated three species of bacteria, Bacterium naphthalinicus, B. naphthalinicus (liquefaciens), and B. naphthalinicus (non-liquefaciens), which could utilize naphthalene; one species of B. phenanthrenicus which could easily attack phenanthrene, and one species, B. benzoli, which could utilize benzene, toluene, and xylene.

Lipman and Greenberg (25) isolated a coccus, or coccobacillus which possessed the power of completely decomposing petroleum to carbon dioxide.

Harper (19) believed that the observed increase in soil fertility resulting from leaking natural gas mains was due to increased nitrogen content of the soil arising from clostridia in the soil utilizing the gas as a source of energy in the process of fixing nitrogen.

From the water of a kerosene storage tank which had ignited spontaneously, Thaysen (56) isolated organisms capable of decomposing kerosene into methane.

Stone, White, and Fenske (48), by means of a medium containing mineral salts and petroleum, obtained soil microorganisms which were capable of attacking petroleum products. They found that oils high in paraffinic hydrocarbons were more readily assimilated than those containing a high percentage of aromatic compounds.

Bushnell and Haas (3) and Haas, Yantzi, and Bushnell (16) isolated cultures of organisms capable of utilizing petroleum fractions such as "Skelly-solve", gasoline, kerosene, light and heavy mineral oils, and paraffin wax as the sole source of carbon and energy for their metabolism. These organisms were isolated from such sources as oil-bearing soil, sedimentation ponds, and "water bottoms" of various petroleum storage tanks. Hydrocarbon fractions ranging from light petroleum (B.P. 30^{o}-60^{o} C.) to heavy mineral oil and solid paraffin were found subject to bacterial oxidation. They found that Pseudomonas species from different origins were capable of utilizing kerosene; micrococci, corynebacteria and "Culture X" were also found capable of this activity. They established the fact that bacterial utilization of hydrocarbons is a characteristic common to many types of microorganisms and that in nature this process probably occurs to a greater extent than is generally recognized. Molds were also found capable of utilizing such products as mineral oil and wax.

Novelli and ZoBell (36) and Rosenfield (41) observed the anaerobic oxidation of hydrocarbons by sulfate-reducing bacteria. Crude oils, petroleum refinery products, and pure hydrocarbons were oxidized by a considerable number of Desulfovibrio cultures in the absence of other types of utilizable organic matter.

Grant and ZoBell (13) and Novelli (35) demonstrated the presence of petroleum-hydrocarbon-utilizing organisms in marine sediments and sea water. Grant and ZoBell isolated several hydrocarbon-utilizing species of Proactinomyces, Pseudomonas, and Mycobacterium. Long-chain paraffin hydrocarbons were oxidized more readily than those having smaller molecules, and the aliphatic compounds were attacked more readily than aromatic or cyclic hydrocarbons.

ZoBell (60) listed the common hydrocarbons which are attacked by bacteria as follows: methane, petroleum ether, gasoline, kerosene, lubricating oil, paraffin wax, tars, benzene, xylene, anthracene, naphthalene, mineral oil, and cyclohexane. Members of the following genera are mentioned as having the ability to oxidize hydrocarbons: Pseudomonas, Micrococcus, Mycobacterium, Actinomyces, Bacillus, Bacterium, Corynebacterium, Sarcina, Serratia, Spirilla, Aspergillus, Penicillium, Monilia, and Micromonospora.

To the writer's knowledge there has been no publications on the effects of Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen or on the total counts in soil.

MATERIALS AND METHODS

Two laboratory experiments were conducted from June, 1950 to January, 1951 at Kansas State College of Agriculture and Applied Science, Manhattan, Kansas.

Experiment 1. Studies on the Effects of 2,4-Dichlorophenoxyacetic Acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in Combination With 2,4-Dichlorophenoxyacetic Acid on the Accumulation of Nitrate Nitrogen in a Sandy-Loam Soil

This experiment was begun on June 26, 1950 and concluded on October 25, 1950. The soil used in this experiment was a sandy-loam obtained from the vicinity of Manhattan, Kansas and had been under cultivation. Approximately 100 pounds of the surface 6 inches of the soil was secured, brought to the laboratory and air dried for 48 hours. After this period of air drying the soil was screened through a 2 mm mesh sieve. The sieved and thoroughly mixed soil was further air dried for 5 days to constant moisture and stored in canvas bags. The soil had a pH of around 7.2, a moisture holding capacity of 40.6 milliliters per 100 grams of oven-dried soil, and approximately 4.0 ppm of nitrate nitrogen when air-dried.

Samples of the air-dried soil equivalent to 100 grams of oven-dried soil were placed into 350-milliliter wide-mouth bottles. Each bottle containing soil was tapped gently on the surface of the table to allow the soil to settle uniformly but not become too compact. Before adding the soils to the bottles, 1.0 milliliter of an ammonium sulfate solution containing 10 milligrams of nitrogen was added to each 100-gram lot of soil. The soil was spread on a piece of paper and the ammonium sulfate was thoroughly mixed into the soil to assure an even distribution of the chemical in the soil. This was done to prevent the concentration of ammonium sulfate near the top as occurs when it is added to the soil after it is bottled.

The herbicides utilized in these studies were supplied by the Experiment Station of the Hawaiian Sugar Planters' Association, Honolulu, Hawaii. These were received on June 2, 1950 and included the following: (1) H.S.P.A. 2,4-dichlorophenoxyacetic acid magma[1] containing 30 per cent by weight of 2,4-dichlorophenoxyacetic acid which had been converted to the normal sodium salt by treatment with sodium carbonate. This chemical was free from nitrogen and soluble in water. This was dissolved at the rate of 0.25 pound of 2,4-dichlorophenoxyacetic acid per gallon with the resulting pH of about 7.5. The resulting solution was labelled the "blanda" solution and

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[1] Reference is made to the pasty state of the chemical.

was used in making up the combination of Concentrated Activated Diesel Emulsion and 2,4-dichlorophenoxyacetic acid; (2) a Concentrated Activated Diesel Emulsion (CADE) which contained three-fourth of 1.0 per cent of sodium pentachlorophenate. A definite amount of a stock solution of the activator (SSA) was added to the final dilution of 1 part of Concentrated Activated Diesel Emulsion to 16 parts of water before use in these studies. This stock solution of activator contained 6 parts of H.S.P.A. Activator (sodium pentachlorophenate, U.S. patent No. 2,370,349) and 1 part of 2-7-R[2]. The mixture employed was of the following composition: 145 milliliters of distilled water, 5 milliliters of the stock solution of activator, and 10 milliliters of Concentrated Activated Diesel Emulsion; (3) Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid to which was added a definite quantity of the Stock Solution of Activator. The final mixture of these two herbicides included the ingredients in the following proportions: 40 milliliters of "blanda" solution, 445 milliliters of distilled water, 5 milliliters of 2-7-R, 50 milliliters of Stock Solution of Activator and 100 milliliters of Concentrated Activated Diesel Emulsion.

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[2] A water-soluble sulphonated aromatic paste used as a wetting agent.

The sodium salt of 2,4-dichlorophenoxyacetic acid was applied at the rates of 0, 1, 3, 10, 25, and 50 pounds per acre of 2,4-dichlorophenoxyacetic acid on the basis of 2,000,000 pounds of oven-dried soil per acre. This test was installed on June 26, 1950 and concluded on August 22, 1950.

The Concentrated Activated Diesel Emulsion was applied at the rates of 0, 10, 100, 500, and 5,000 gallons per acre. This test was installed on June 29, 1950 and concluded on October 18, 1950.

The mixture of Concentrated Activated Diesel Emulsion and the sodium salt of 2,4-dichlorophenoxyacetic acid was applied at the rates of 0, 3, 30, 100, and 1,000 gallons per acre. This particular test was installed on July 5, 1950 and concluded on October 25, 1950.

The herbicides were applied to the soil in sufficient volumes of water to bring the soil up to 50 per cent of its water-holding capacity. The bottles were stoppered fairly tight with cotton and incubated at room temperature in the dark to reduce to a minimum water loss due to evaporation. The samples were maintained at the desired moisture level by adding distilled water every 6 to 8 days. At intervals of a few days all germinating weed seedlings were either pinched off at the level of the soil surface or pulled out, precautions being taken not to carry off any soil particles with the seedlings. This was done to prevent any measurable loss of nitrate nitrogen due to utilization by the growing plants.

Three samples were extracted from each treatment for nitrate nitrogen at intervals of 0, 2, 4, 8, and 16 weeks using a dilution of 3 parts of distilled water to 1 part of soil. The moisture already present in the soil was taken into consideration. In extracting nitrates from the samples 1.0 gram of calcium oxide (CaO) was added to each bottle before the addition of water. The bottles were stoppered tightly, shaken for 2 minutes on the Kahn shaker and allowed to stand for 20 minutes or longer before filtering the slightly turbid supernatant solution through a filter paper. Preliminary tests had shown that 1.0 gram of calcium oxide was sufficient to clarify the solution provided the filtrates were refiltered twice through the same papers. Duplicates of 10-milliliter aliquots were dried over steam in evaporating dishes, giving a total of 6 replications for each herbicidal concentration. Nitrate nitrogen was determined by the phenoldisulphonic acid method as described by Schreiner and Failyer (42) and modified by Lipman and Sharp (26). The Duboscq colorimeter was utilized for all colorimetric readings.

A preliminary test run to determine whether the herbicides employed in these studies interferred in any way with the phenoldisulphonic acid method indicated that they caused no irregularities in the results.

Experiment 2. Studies on the Effects of 2,4-Dichlorophenoxyacetic Acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in Combination With 2,4-Dichlorophenoxyacetic Acid on the Accumulation of Nitrate Nitrogen and Total Plate Counts in a Silt-Loam Soil

This experiment was installed on August 28, 1950 and concluded on December 29, 1950. A cultivated silt-loam soil obtained from the Kansas State College farm, Manhattan, Kansas was used. The procedures for obtaining and preparing the soil were essentially the same as those described for Experiment 1. The soil had a pH of 6.5, a moisture holding capacity of 47.0 milliliters per 100 grams of oven-dried soil, and 1.0 ppm of nitrate nitrogen when air dried. One-hundred-gram samples were placed in 350-milliliter bottles and 10 milligrams of nitrogen as ammonium sulfate were added as previously described. The original herbicides used in Experiment 1 were utilized. These had been stored in air-tight bottles in the dark. The sodium salt of 2,4-dichlorophenoxyacetic acid was applied at the same rates of 2,4-dichlorophenoxyacetic acid per acre as in Experiment 1. This test was installed on September 7, 1950 and concluded on December 29, 1950.

The Concentrated Activated Diesel Emulsion was applied at the rates of 0, 100, 500, 1000, and 5000 gallons per acre. This test was installed on August 28, 1950 and concluded on December 18, 1950.

The mixture consisting of Concentrated Activated Diesel Emulsion and the sodium salt of 2,4-dichlorophenoxyacetic acid was applied at the rates of 0, 30, 50, 100, and 1000 gallons per acre. This test was installed on September 5, 1950 and concluded on December 26, 1950. Untreated checks receiving no nitrogen as ammonium sulfate were installed alongside the treated bottles to observe whether there would be any appreciable accumulation of nitrate nitrogen in the soil due to the soil's store of organic matter. The herbicides were added to the soil as described in Experiment 1, and the bottles were stoppered and similarly maintained at room temperature. Sterile distilled water was used to maintain the soils at 50 per cent saturation. It was not necessary to remove germinating seedlings with few exceptions, since this particular soil was exceptionally free from viable weed seeds. Therefore, no data were kept for the numbers of germinating weed seeds. Three samples were similarly extracted for nitrate nitrogen from bottles of each treatment, and calcium oxide was added only after 10 milliliter-samples were removed for the determination of total plate counts. Total plate counts were made on the days extractions were made for nitrate-nitrogen determinations. Egg-albumin agar adjusted to pH 7.2 was used. This medium was of the following composition: 1.0 g glucose; 0.25 g $MgSO_4.7H_2O$; 0.50 g K_2HPO_4; trace $Fe_2(SO_4)_3$; 0.50 g yeast extract; 20.0 g agar-agar; 0.25 g egg albumin;

and 1000 ml distilled water. Five plates were poured for each concentration of the herbicides. A dilution of 1 to 100,000 was found most favorable. The plates were incubated for 5 days at room temperature before counts were made. Longer incubation was unwise due to the rapid growth of spreading colonies.

Since the total counts after 2 weeks' incubation of the soils showed a decided increase for the higher concentrations of each herbicide, a test was run to find out whether this was due to a partial-sterilization effect of the herbicides on the soil. Separate 100-gram samples of freshly obtained sandy-loam soil were maintained in 350-milliliter wide-mouth bottles at 50 per cent saturation. The soil was previously sieved through a 2 mm sieve and thoroughly mixed. One sample was maintained as an untreated check and 3 samples were treated with each of the herbicides at the highest concentrations used in this experiment. An aliquot of 10 grams (oven dry basis) was removed from each sample of soil at the end of 3 hours, 6 hours, 24 hours, 7 days, and 14 days. The herbicides were thoroughly mixed into the soil at the time the test was installed, and a thorough mixing of the soil was done at the time of each sampling. The samples were plated out on egg-albumin-agar plates at a dilution of 1 to 1,000,000. Five replications were made and the plates were incubated at room temperature for 5 days before total counts were made.

Determinations of ammonia nitrogen were made along with the nitrate determinations, using the same clarified filtrates. The direct nesslerization method was employed, and was essentially that described by the American Public Health Association and the American Water Works Association (2) using ammonium chloride standards for comparisons. Ammonia-nitrogen determinations were made to test the presence of residual ammonia nitrogen where nitrate-nitrogen accumulation was apparently retarded. This was done to ascertain the fact that the absence of ammonia nitrogen was not the limiting factor for the accumulation of nitrate nitrogen where 100 ppm of ammonia nitrogen had been added as ammonium sulfate.

RESULTS

Experiment 1. Studies on the Effects of 2,4-Dichlorophenoxyacetic Acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in Combination With 2,4-Dichlorophenoxyacetic Acid on the Accumulation of Nitrate Nitrogen in a Sandy-Loam Soil

The results of the first experiment are presented in Tables 1, 2, and 3. According to results in Table 1, the sodium salt of 2,4-dichlorophenoxyacetic acid applied directly to the sandy-loam soil at the ordinary field rate of 3 pounds of 2,4-dichlorophenoxyacetic acid per acre exhibited a slight retarding effect on the rate of accumulation of nitrate nitrogen during the period of 0 to 2 weeks. The amount of nitrate nitrogen which had accumulated during the period of 2 to 4 weeks was only slightly less than that amount which had accumulated in the untreated checks during the same period. Results at the end of 2 weeks indicate that there was a considerable increase in the amount of nitrate nitrogen in the soil as compared to the amount present at the time the test was installed. One pound per acre of 2,4-dichlorophenoxyacetic acid had no noticeable effect on the accumulation of nitrate nitrogen during the periods tested. The effect of 10 pounds per acre was similar to that produced by 3 pounds per acre, except that the result for the 4th week shows that noticeably less nitrate nitrogen was formed during the 2- to 4-week period compared to that amount formed in the 3 pounds per acre treatment during this same interval; i.e., there was a definite retardation, which, however, disappeared completely during the 4- to 8-week period, of the rate of accumulation of nitrate nitrogen. At the rates of 25 to 50 pounds per acre, 2,4-dichlorophenoxyacetic acid definitely retarded the rate of accumulation of nitrate nitrogen; nevertheless even during the period of 0 to 2 weeks considerable nitrate nitrogen had accumulated beyond the amount present at the beginning of the experiment. Determinations at the end of 8 weeks indicated that the final quantities of nitrate nitrogen which had accumulated in samples of soils receiving

Table 1. The effects of 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen in a sandy-loam soil, Manhattan, Kansas, 1950.

Treatments: 2,4-D lbs. per acre	Treatments: Ppm N added*	Ppm NO_3-nitrogen in soil after period of incubation in weeks: 0	2	4	8	Germinated weed seeds**
0	100	4.5	59.7	97.6	98.6	92
1		4.6	57.4	97.6	112.1	48
3		4.5	47.5	80.5	105.3	14
10		4.2	47.0	66.0	101.2	11
25		4.2	35.3	65.1	97.6	1
50		4.4	35.3	58.3	97.6	0

2,4-D lbs. per acre	Ppm N added*	Ppm NO_3-nitrogen formed during intervals between analyses in weeks: 0-2	2-4	4-8
0	100	55.2	37.9	0.9
1		52.8	40.2	14.5
3		43.0	33.0	24.8
10		42.8	19.0	35.2
25		31.1	29.8	32.5
50		30.9	23.0	39.3

* Nitrogen was added as $(NH_4)_2SO_4$.
**Total germinated weed seeds for 8-week period.

different quantities of 2,4-dichlorophenoxyacetic acid were essentially the same as that found in the untreated checks; i.e., there was a complete recovery of the rate of accumulation of nitrate nitorgen in all cases where prolonged retardation was evident sometime during the interval of 4 to 8 weeks. This particular test was discontinued at this stage since nothing would have been gained by carrying it further.

The numbers of germinating weed seeds which were removed during the 16-week period present a clear picture of the increasing toxicity of the herbicide on vegetation with increasing concentrations. At 25 pounds per acre there was almost complete inhibition of germination of weed seeds; 50 pounds per acre of 2,4-dichlorophenoxyacetic acid completely inhibited germination.

Results in Table 2 indicate that Concentrated Activated Diesel Emulsion applied at the usual field rate of 100 gallons per acre or less did not appreciably affect the rate of accumulation of nitrate nitrogen in the soil even during the period of 0 to 2 weeks. At the rate of 500 gallons per acre, the upper limit for field application, there was a significant retardation of the rate of accumulation of nitrate nitrogen during the period of 0 to 2 weeks. The amount of nitrate nitrogen which accumulated during this period was less than one-half that which accumulated in the untreated checks during this same interval. The amount of nitrate nitrogen gradually increased with time, and at the end of 8 weeks approximately equalled that found in the untreated checks. The retarding effect disappeared sometime during the 2-to 4-week period since the rate of accumulation was essentially the same as that in soils treated with the lower concentrations of the herbicide, as shown by the figures for the amount of nitrate nitrogen formed during the intervals between analysis. The

Table 2. The effects of Concentrated Activated Diesel Emulsion on the accumulation of nitrate nitrogen in a sandy-loam soil, Manhattan, Kansas, 1950.

Treatments: CADE gals per acre	Treatments: Ppm N added*	Ppm NO_3-nitrogen in soil after period of incubation in weeks: 0	2	4	8	16	Germinated weed seeds**
0	100	4.3	64.2	97.6	99.4	130.2	73
10		4.5	57.4	97.6	101.2	130.2	95
100		4.1	58.8	97.6	99.4	135.6	84
500		4.5	27.3	67.3	97.6	130.2	37
5000		4.2	7.7	9.5	14.0	23.5	0

CADE gals per acre	Ppm N added	Ppm NO_3-nitrogen formed during intervals between analyses in weeks: 0-2	2-4	4-8	8-16
0	100	59.0	33.4	1.8	30.8
10		52.9	40.2	3.6	29.0
100		54.7	38.8	1.8	36.2
500		22.8	40.0	30.3	32.6
5000		3.5	1.8	4.5	9.5

* Nitrogen was added as $(NH_4)_2SO_4$.
** Total germinated weed seeds for 16-week period.

amount of nitrate nitrogen which accumulated during the period of 4 to 8 weeks was noticeably higher than the amounts in soils treated with other concentrations of the herbicide, probably due to the complete recovery from the retarding effects and to a large amount of available ammonia nitrogen present. At the rate of 5000 gallons per acre Concentrated Activated Diesel Emulsion definitely retarded the rate of accumulation of nitrate nitrogen. Nevertheless the amount of nitrate nitrogen which accumulated during the 0 to 2 week period was in excess to that amount originally present in the soil. There was a gradual accumulation of nitrate nitrogen with time but the rate of increase was considerably less than that observed for the lower rates of application. Results for the 8-to-16 week period show that a marked retardation of the rate of accumulation of nitrate nitrogen was evident at 5000 gallons per acre even after a 16 week incubation period. During this interval the rate of accumulation of nitrate nitrogen was approximately one-third that of soils treated with the lower concentrations of Concentrated Activated Diesel Emulsion.

Weed-seed germination was considerably inhibited at the rates of 500 and 5000 gallons per acre; there was complete inhibition at the higher rate. No significant differences in the effects on weed-seed germination could be observed with the lower concentrations of the herbicide.

As shown in Table 3, Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid applied directly to the soil at the rates of 3 gallons per acre and the usual field applications of 30 gallons per acre had no effect on the rate of accumulation of nitrate nitrogen even during the first 2 weeks. At the rates of 100 and 1000 gallons per acre the rate of accumulation of nitrate nitrogen was definitely retarded, more so at the rate of 1000

Table 3. The effects of Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen in a sandy-loam soil, Manhattan, Kansas, 1950.

Treatments: CADE + 2,4-D gals per acre	Ppm N added*	Ppm of NO_3-nitrogen in soil after period of incubation in weeks: 0	2	4	8	16	Germinated weed seeds**
0	100	3.5	59.7	97.6	108.5	172.7	100
3		3.4	59.7	97.6	108.5	172.7	96
30		3.5	59.7	97.6	108.5	177.2	94
100		3.4	42.5	82.7	108.5	172.7	19
1000		2.7	7.2	20.1	40.7	108.5	0

Treatments: CADE + 2,4-D gals per acre	Ppm N added*	Ppm NO_3-nitrogen formed during intervals between analyses in weeks: 0-2	2-4	4-8	8-16
0	100	56.2	37.9	10.9	64.2
3		56.3	37.9	10.9	64.2
30		56.2	37.9	10.9	68.7
100		39.1	40.2	25.8	64.2
1000		4.5	12.9	20.6	67.8

* Nitrogen was added as $(NH_4)_2SO_4$.
** Total germinated weed seeds for 16-week period.

gallons per acre. At the rate of 100 gallons per acre the rate of accumulation of nitrate nitrogen had completely recovered sometime during the period of 2 to 4 weeks since 40.2 ppm of nitrate nitrogen accumulated during this period as compared to 37.9 ppm during this same period in soil samples treated with the lower concentrations. At the rate of 1000 gallons per acre the rate of accumulation of nitrate nitrogen was considerably retarded during this same period and also during the period of 4 to 8 weeks, although not as noticeably during the latter period. Results for the 16-week period show that the final amount of nitrate nitrogen was considerably less in soil samples treated with the highest concentration of the herbicidal combination than that present in samples treated with the lower concentrations. Nevertheless the factors responsible for the accumulation of nitrate nitrogen had fully recovered from the retarding effects of the herbicidal combination sometime during the 8-to 16-week period, as indicated by the approximately equal accumulation in all samples during this interval. Even at the highest concentration of the herbicidal combination, nitrate nitrogen had accumulated beyond the amount originally present in the soil at the start of the experiment, the rate of accumulation increasing with time.

When the herbicidal combination was applied to the soil at the rates of 0, 3, and 30 gallons per acre, there was no significant difference in the number of germinating weed seeds. At the rate of 100 gallons per acre there was a decided inhibition, and at the rate of 1000 gallons per acre a complete inhibition was recorded.

Experiment 2. Studies on the Effects of 2,4-Dichlorophenoxyacetic Acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in Combination With 2,4-Dichlorophenoxyacetic Acid on the Accumulation of Nitrate Nitrogen and Total Plate Counts in a Silt-Loam Soil

The results of the second experiment to determine the effects of the different herbicides used in these studies on the accumulation of nitrate nitrogen and total plate counts in a silt-loam soil are presented in Tables 4, 5, 6, and 7.

According to the results in Table 4, the sodium salt of 2,4-dichlorophenoxyacetic acid added to the silt-loam soil at the rates of 1 and 3 pounds per acre of 2,4-dichlorophenoxyacetic acid, did not appreciably retard the rate of accumulation of nitrate nitrogen even during the period of 0 to 2 weeks. At the rate of 10 pounds per acre there was a slight retardation which persisted for 4 weeks. During the period of 4 to 8 weeks the rate of accumulation had increased to equal that in soil samples treated with the lower concentrations of the herbicide. At the rates of 25 and 50 pounds per acre the herbicide decidedly retarded the rate of accumulation of nitrate nitrogen, the effect being more pronounced with the 50-pounds-per-acre treatment. The retardation at these higher rates definitely persisted for 8 weeks, and even for 16 weeks in the higher concentration. In all instances where retardation was evident the total amount of nitrate nitrogen present at each analysis was approximately equal to or exceeded that present in the untreated control, hence in no instance was nitrification completely inhibited.

Results for the determinations of the residual ammonia nitrogen are also presented. Ammonia nitrogen was present in all samples to which ammonium sulfate was added and persisted throughout the 16 weeks in soil samples treated with 25 and 50 pounds per acre of 2,4-dichlorophenoxyacetic acid. The amount of residual ammonia nitrogen was greater in soil samples containing the higher concentration of the herbicide.

The data in Table 5 indicate that Concentrated Activated Diesel Emulsion added to the silt-loam soil at the rate of 100 gallons per acre had a slight retarding effect on the rate of accumulation of nitrate nitrogen which definitely persisted for 4 weeks. During the interval of 4 to 8 weeks the total amount of nitrate nitrogen which accumulated was for all practical purposes the same as that in the checks to which ammoniun sulfate was added, and the rate of accumulation of nitrate nitrogen had fully recovered sometime during this period. At the rate of 500 gallons per acre there was a slightly greater retardation in nitrification during the periods of 0 to 2 weeks and 2 to 4 weeks. During the period of 4 to 8 weeks nitrate nitrogen accumulated at a higher rate than in soil samples treated with 100 gallons per acre of the emulsion or in the untreated checks containing added ammonium sulfate. The retarding effect was more noticeable at the rate of 1000

Table 4. The effects of 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen and total counts in a silt-loam soil, Manhattan, Kansas, 1950.

Treatments: 2,4-D: lbs per acre	Ppm N added*	Ppm of nitrogen as NO_3 and NH_3 in soil, and total counts in millions per gram of soil after period of incubation in weeks														
		0			2			4			8			16		
		NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts
0	0	1.9	0.0	5.10	13.6	0.0	4.86	20.3	0.0	5.58	30.7	0.0	5.52	47.0	0.0	2.55
0	100	1.9	+++++	5.10	29.8	++++	6.66	59.7	++++	3.06	101.7	++	5.63	143.7	0.0	2.04
1	↓	1.9	+++++	5.10	28.0	+++++	5.15	59.7	++++	5.10	101.7	++	3.30	143.7	0.0	1.98
3	↓	1.9	+++++	5.10	26.2	+++++	4.62	59.7	++++	6.48	101.7	++	3.30	143.7	0.0	1.32
10	↓	1.9	+++++	5.10	21.7	+++++	7.13	46.1	++++	6.30	89.5	+++	2.22	134.2	0.0	2.16
25	↓	1.9	+++++	5.10	14.5	+++++	12.98	31.6	++++	10.43	61.0	++++	7.50	103.1	++	3.54
50	↓	1.9	+++++	5.10	12.2	+++++	13.35	19.0	+++++	10.98	35.3	+++++	7.80	50.6	++++	1.98

2,4-D lbs per acre	Ppm N added*	Ppm NO_3-nitrogen formed during intervals between analyses in weeks: 0-2	2-4	4-8	8-16
0	0	11.7	6.7	10.4	16.3
0	100	27.9	29.9	42.0	42.0
1	↓	26.1	31.7	42.0	42.0
3	↓	24.3	33.5	42.0	42.0
10	↓	19.8	24.4	43.4	44.7
25	↓	12.6	17.1	29.4	42.1
50	↓	10.3	6.8	16.3	15.3

* Nitrogen was added as $(NH_4)_2SO_4$.

+ = less than 5 ppm NH_3 nitrogen
++ = 6 to 10 ppm NH_3 nitrogen
+++ = 11 to 25 ppm NH_3 nitrogen
++++ = 26 to 50 ppm NH_3 nitrogen
+++++ = more than 51 ppm NH_3 nitrogen

Table 5. The effects of Concentrated Activated Diesel Emulsion on the accumulation of nitrate nitrogen and total counts in a silt-loam soil, Manhattan, Kansas, 1950.

Treatments: CADE gals per acre	Ppm N added*	Ppm of nitrogen as NO_3 and NH_3 in soil, and total counts in millions per gram of soil after period of incubation in weeks: 0			2			4			8			16		
		NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts
0	0	1.9	0.0	5.10	13.6	0.0	7.65	20.3	0.0	6.48	32.5	0.0	6.23	46.1	0.0	5.10
0	100	1.9	+++++	5.10	38.0	++++	3.70	78.7	+++	4.98	107.1	+	4.02	134.2	0.0	3.45
100	↓	1.9	+++++	5.10	34.5	++++	8.60	67.8	++++	12.60	101.7	++	5.85	134.2	0.0	3.96
500	↓	1.9	+++++	5.10	32.5	++++	13.30	54.2	++++	16.28	97.6	+++	10.20	128.8	+	2.76
1000	↓	1.9	+++++	5.10	24.4	+++++	26.70	46.1	++++	25.92	93.6	+++	5.46	112.5	++	3.60
5000	↓	1.9	+++++	5.10	10.8	+++++	30.40	18.5	+++++	29.52	32.5	+++++	11.48	61.5	++++	6.45

Ppm NO_3-nitrogen formed during intervals between analyses in weeks

CADE gals per acre	Ppm N added*	0-2	2-4	4-8	8-16
0	0	11.7	6.7	12.2	13.6
0	100	36.1	40.7	28.4	27.1
100	↓	32.6	33.3	33.9	32.5
500	↓	30.6	21.7	43.4	31.2
1000	↓	22.5	21.7	47.5	18.9
5000	↓	8.9	7.7	14.0	29.0

* Nitrogen was added as $(NH_4)_2SO_4$.

+ = less than 5 ppm NH_3 nitrogen
++ = 6 to 10 ppm NH_3 nitrogen
+++ = 11 to 25 ppm NH_3 nitrogen
++++ = 26 to 50 ppm NH_3 nitrogen
+++++ = more than 51 ppm NH_3 nitrogen

Table 6. The effects of Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen and total counts in a silt-loam soil, Manhattan, Kansas, 1950.

Treatments: CADE + 2,4-D gals per acre	Ppm N added*	Ppm of nitrogen as NO_3 and NH_3 in soil, and total counts in millions per gram of soil after period of incubation in weeks														
		0			2			4			8			16		
		NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts	NO_3 N	NH_3 N	Total counts
0	0	1.9	0.0	5.10	12.7	0.0	5.80	20.3	0.0	7.38	25.3	0.0	4.20	47.0	0.0	2.52
0	100	1.9	+++++	5.10	35.3	++++	3.30	65.1	+++	5.10	86.8	+	3.10	128.8	0.0	1.80
30		1.9	+++++	5.10	33.4	+++++	2.10	59.7	+++	6.42	89.5	++	4.32	134.2	0.0	2.10
50		1.9	+++++	5.10	26.2	+++++	8.10	58.8	++++	5.70	88.1	++	5.10	134.2	0.0	2.70
100		1.9	+++++	5.10	25.8	+++++	8.80	56.0	++++	7.95	89.5	+++	3.96	128.8	0.0	3.24
1000	↓	1.9	+++++	5.10	9.3	+++++	22.55	17.4	+++++	23.16	28.0	+++++	18.90	51.5	++++	15.24

Ppm NO_3-nitrogen formed during intervals between analyses in weeks

CADE + 2,4-D gals per acre	Ppm N added*	0-2	2-4	4-8	8-16
0	0	10.8	7.6	5.0	21.7
0	100	33.4	29.8	21.7	42.0
30		31.5	26.3	29.8	44.7
50		24.3	32.6	29.3	46.1
100		23.9	30.2	33.5	39.3
1000	↓	7.4	8.1	10.6	23.5

* Nitrogen was added as $(NH_4)_2SO_4$.

+ = less than 5 ppm NH_3 nitrogen
++ = 6 to 10 ppm NH_3 nitrogen
+++ = 11 to 25 ppm NH_3 nitrogen
++++ = 26 to 50 ppm NH_3 nitrogen
+++++ = more than 51 ppm NH_3 nitrogen

gallons per acre especially during the 0- to 2-week period and persisted for 4 weeks. During the period of 4 to 8 weeks the retarding effects had fully disappeared. The amount of nitrate nitrogen which was formed between the period of 8 to 16 weeks was for some unknown reason less than that in soils treated with the lower concentrations, although results for the 4- to 8-week period clearly showed that the rate of accumulation of nitrate nitrogen had fully recovered sometime during this period. At the rate of 5000 gallons per acre the retardation of nitrification was more marked than for the lighter applications and definitely persisted for 8 weeks. There are indications, however, that complete recovery in nitrifying activity was attained sometime during the last 2 weeks of incubation, since the total formed during this period approximated that in other samples. At this highest concentration of the herbicide the total nitrate nitrogen accumulating during the first 8-week period was essentially equal to the amount present in the untreated checks, suggesting the possibility that the added ammonium sulfate was not being nitrified. The results from the residual ammonia-nitrogen determinations show that ammonia nitrogen persisted in the soil for 16 weeks in soil samples treated with 500, 1000, and 5000 gallons per acre of Concentrated Activated Diesel Emulsion, the amount remaining in the soil being greater with the higher concentrations of the herbicide.

According to the results presented in Table 6, Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, at the rate of 30 gallons per acre, did not retard the rate of nitrate accumulation even during the periods of 0 to 2 weeks and 2 to 4 weeks, the differences here being insignificant. At the rates of 50 and 100 gallons per acre there was a pronounced retardation during the period of 0 to 2 weeks; the retarding effect completely disappeared during the period of 2 to 4 weeks, as shown by the nitrate nitrogen formed during the intervals between analyses. In all the concentrations of the combination of the herbicides discussed so far; namely, 30, 50, and 100 gallons per acre, the total amount of nitrate nitrogen which accumulated during the 8-week period was essentially the same. On the other hand at the rate of 1000 gallons per acre there was a marked retardation of the rate of accumulation of nitrate nitrogen which persisted for 16 weeks.

A review of the results in Tables 4, 5, and 6 shows that at the highest concentrations of the herbicides; namely, 50 pounds per acre of 2,4-dichlorophenoxyacetic acid, 5000 gallons per acre of Concentrated Activated Diesel Emulsion, and 1000 gallons per acre of Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, the amounts of nitrate nitrogen accumulating during the different intervals were, with one exception, essentially the same as in the untreated checks receiving no ammonium sulfate. Since in the latter the sole source of nitrate nitrogen was the soil's store of organic nitrogen this parallel formation might be interpreted as indicating that in the presence of high concentrations of the herbicides the added ammonia was so altered as to render it nonavailable to the nitrifying organisms. At all lower concentrations, however, there was definite evidence that the nitrogen added in the form of ammonium sulfate was being converted to nitrate nitrogen.

The quantities of ammonia nitrogen recovered at the different analyses definitely indicate that it was residual added ammonia nitrogen that was not converted to nitrate nitrogen, and not ammonia formed from soil's organic nitrogen. The soil samples receiving no ammonium sulfate gave no indication of the accumulation of ammonia nitrogen during the periods tested. In some cases this residual ammonia nitrogen persisted in the soil for the duration of the tests, and the quantities recovered were directly related to the concentration of the herbicides added.

The results for the total plate counts (including bacteria, actinomyces, and fungi which grew on the egg-albumin-agar plates) obtained from soil samples treated with the different herbicides are also presented in Tables 4, 5, and 6. A most striking observation was the many-fold increase in the total plate counts in soils treated with the higher concentrations of the herbicides. There was an evident increase in the total plate counts when 2,4-dichlorophenoxyacetic acid was added to the soil samples at the rates of 25 and 50 pounds per acre, the increases being greatest during the periods of 0 to 2 weeks and 2 to 4 weeks as shown in Table 4. Concentrated Activated Diesel Emulsion added to the soil at the rates of 1000 and 5000 gallons per acre (Table 5) also caused a marked increase in the total plate counts in the soil as shown by the results obtained at the end of 2 and 4 weeks. The lower rate of 500 gallons per acre also appeared to increase the total plate counts during the same intervals. Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid also caused an increase in the total plate counts when added to the soil at the rate of 1000 gallons per acre. This increase was noticeable throughout the periods tested. With the exception of the combination of the herbicides, the differences in total plate counts between the soils treated with high concentrations of the herbicides and the untreated soils seemed to gradually narrow down with time, the increase being most noticeable during the first 2- and 4-week periods; a wide difference in the total plate counts was still evident at the end of 16 weeks in soils treated with the herbicidal combination.

Results of the studies to find out whether the different herbicides behaved as partial sterilants of the soil are presented in Table 7. This test was performed since the increases in total counts mentioned in the preceding paragraph suggested a partial sterilization of the soil with the higher concentrations of the herbicides tested. According to the results, 2,4-dichlorophenoxyacetic acid at the rate of 50 pounds per acre exhibited no semblance of a partial sterilization phenomenon. Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid at the rates of 5000 gallons and 1000 gallons per acre, respectively, effected a typical partial sterilization of the soil under the conditions of this test. With Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid there was a marked reduction in the total counts evident during the first 3 hours of incubation, persisting for a period of 24 hours. The total plate counts in these cases increased approximately four-fold during the interval of 24 hours to 7 days and increased further during the following 7 days. The results with 50 pounds per acre of 2,4-dichlorophenoxyacetic acid in this experiment with sandy-loam soil differ somewhat from previous results with a silt-loam soil in that in the latter soil 50 pounds per acre of 2,4-dichlorophenoxyacetic acid showed approximately a three-fold increase in total counts during the initial 2-week period (Table 4).

Table 7. Total counts in millions per gram of sandy-loam soil after periods of incubation of soil samples as indicated.

Treatments		Hours				Days	
Herbicide	Concentration	0	3	6	24	7	14
Untreated		47.40	43.40	53.00	66.80	75.80	31.80
2,4-D	50 lbs/acre	47.40	69.20	53.25	49.20	69.25	32.66
CADE	5000 gals/acre	47.40	19.50	12.80	48.40	183.40	207.20
CADE+2,4-D	1000 gals/acre	47.40	28.20	28.40	30.40	143.67	172.40

DISCUSSION OF RESULTS

The toxicity to vegetation of the 3 herbicides used in these studies is demonstrated by the marked inhibition of the germination of various weed seeds that were present in the soil; this was especially noticeable at concentrations of the herbicides greater than would normally be employed in a weed control program. For the control of weeds 2,4-dichlorophenoxyacetic acid is usually applied at the rate of 3 pounds per acre. Concentrated Activated Diesel Emulsion is used at the rate of 100 gallons per acre in areas of sparse rainfall and at a rate as high as 500 gallons per acre in areas of plentiful rainfall. The combination of 2,4-dichlorophenoxyacetic acid and Concentrated Activated Diesel Emulsion is usually applied at the rate of 30 gallons per acre.

The toxicity of 2,4-dichlorophenoxyacetic acid to various soil microorganisms has been observed by several investigators and reviewed earlier in this paper. The concentrations of 2,4-dichlorophenoxyacetic acid recorded as toxic to several microorganisms were far above those which are normally used in the control of weeds. Soil conditions were found to exert some influence on the toxicity of the acid to microorganisms. To the writer's knowledge the toxicity of Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid to soil microorganisms has not been investigated to date.

Results obtained from Experiment 1 indicate that 2,4-dichlorophenoxyacetic acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, when applied to a sandy-loam soil under the conditions of these studies, have no significant effects on the rate of accumulation of nitrate nitrogen at the usual concentrations used in field applications for weed control. The retardation of the rate of accumulation of nitrate nitrogen became more marked with increasing concentrations of the herbicides; nevertheless a complete inhibition of nitrate-nitrogen accumulation was not evident with the highest concentrations utilized here. Even with the highest concentration of each herbicide, nitrate nitrogen steadily accumulated beyond the amount present at the beginning of the tests. With the exception of 2,4-dichlorophenoxyacetic acid the total amount of nitrate nitrogen accumulating in soil samples treated with the highest concentrations of the herbicides did not equal that accumulating in samples treated with the lower concentrations of the herbicides during a period of 16 weeks.

Measured in terms of the effects upon the rate of accumulation of nitrate nitrogen, the persistence of the herbicides in the sandy-loam soil depended on the concentrations or rates of application. In each case, with concentrations below the normal field applications, the effects on the rate of accumulation of nitrate nitrogen did not persist longer than 4 weeks. In the case of Concentrated Activated Diesel Emulsion at the highest concentration studied; namely, 5000 gallons per acre, the effects on the rate of accumulation of nitrate nitrogen persisted for the 16-week duration of the test. With 2,4-dichlorophenoxyacetic acid at the rate of 50 pounds per acre, the rate of nitrate-nitrogen accumulation was back to normal sometime during the period of 4 to 8 weeks, while recovery of the rate of accumulation of nitrate nitrogen took place sometime during the period of 8 to 16 weeks in the case of Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid at the rate of 1000 gallons per acre.

Similar results were obtained for the studies on the effects of the different herbicides on the accumulation of nitrate nitrogen in a silt-loam soil (Experiment 2). It has been suggested by several investigators that colloidal matter in the soil might be a factor in the inactivation of 2,4-dichlorophenoxyacetic acid in the soil. Results of these studies indicate that the retarding effects of 2,4-dichlorophenoxyacetic acid on the rate of accumulation of nitrate nitrogen was somewhat longer in the silt-loam soil than in the sandy-loam, although the differences were not very pronounced. For example, the retarding effects of this herbicide on the rate of accumulation of nitrate nitrogen disappeared in the sandy-loam soil during the period of 4 to 8 weeks even with the highest rate of application, whereas it appeared to persist in the silt-loam soil for 8 weeks at the rate of 25 pounds per acre, disappearing during the period of 8 to 16 weeks, and for 16 weeks at the rate of 50 pounds per acre.

There were variations in the results obtained in the two different soils treated with Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid. Concentrated Activated Diesel Emulsion at the rate of 100 gallons per acre showed only a very slight influence on the rate of accumulation of nitrate nitrogen in the sandy-loam soil during the period of 0 to 2 weeks, while the slight retardation at this same rate in the silt-loam soil persisted for a period of 4 weeks, disappearing completely during the 4- to 8-week period. Similarly at the rate of 500 gallons per acre the retardation was apparently more prolonged in the silt-loam soil, being evident during the period of 0 to 4 weeks and disappearing completely sometime during the period of 4 to 8 weeks. At 5000 gallons per acre there was a marked retardation of the rate of accumulation in both soils which definitely persisted for 16 weeks in the sandy-loam soil, while recovery was evident during the period of 8 to 16 weeks in the silt-loam soil, contrary to the behavior of the lower concentrations whose retarding effects seemed to persist longer in the silt-loam soil. The small quantities of nitrate nitrogen which accumulated between the period of 4 to 8 weeks in the sandy-loam soil treated with 0, 10, and 100 gallons per acre of Concentrated Activated Diesel Emulsion (Table 2) seem out of line. Although no ammonia determinations were made here, it may be assumed that almost all of the added ammonia nitrogen had been oxidized during the first 4 weeks, leaving only a small quantity of nitrogen from the soil's organic matter available during the period of 4 to 8 weeks; i.e., the availability of ammonia nitrogen seems to have been the limiting factor on the rate of accumulation of nitrate nitrogen during this period. The increased rate of accumulation during the period of 8 to 16 weeks may have been due to the greater amount of organic nitrogen which became available to the nitrifying organisms as ammonia nitrogen. Perhaps there are some other reasons for this odd behavior which was not evident elsewhere. Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid also appeared to persist longer in the silt-loam soil than in the sandy-loam soil. At the rate of 30 gallons per acre there was apparently no effect on the rate of accumulation of nitrate nitrogen in the sandy-loam and silt-loam soils even during the first 2-week period. At the rate of 100 gallons per acre there apparently was no difference in the persistence of the effects on the rate of accumulation of nitrate nitrogen in the two soils, similarly persisting in each case for the period of 0 to 2 weeks and disappearing sometime during the period of 2 to 4 weeks. At the rate of 1000 gallons per acre the retardation persisted in the silt-loam soil throughout the 16-week period of the experiment, whereas it persisted during the period of 0 to 8 weeks in the sandy-loam soil, disappearing sometime during the 8- to 16-week period.

Results show that the nitrate nitrogen accumulated at a faster rate in the sandy-loam soil during the 0- to 2-week period than in the silt-loam soil during the same period, although the differences in rates of accumulation were not significantly different during the subsequent periods.

The following factors probably affect the rate of inactivation of herbicides in the soil: (1) the type and concentration of the herbicides, (2) the conditions under which the soils are maintained, (3) pH of the soil, (4) composition and type of soil, and (5) the activity of the specific organisms in the soil which are capable of utilizing the herbicides as sources of food. The utilization of 2,4-dichlorophenoxyacetic acid by several microorganisms has been mentioned earlier. Studies on the utilization of Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid by soil microorganisms should provide interesting results. With the presence of a number of microorganisms in the soil capable of utilizing petroleum, petroleum products, and petroleum hydrocarbons, there is the possibility of the presence of a number of microorganisms which are capable of utilizing the herbicides or their components as sources of energy. This may very well explain the gradual disappearance of the retarding effects of these herbicides on the rate of accumulation of nitrate nitrogen in the soil. Nevertheless, the fact that the retardation of the rate of accumulation of nitrate nitrogen persisted in some instances throughout the 16-week duration in soils treated with the highest concentrations would seem to indicate that the utilization of the herbicides was not very rapid. If they were utilized rapidly the rate of nitrate accumulation should be expected to return to normal as soon as the greater part of the herbicides was utilized. This might be interpreted as indicating that the microbial activity was a factor in the slow inactivation of the herbicides in the soils under the conditions of this experiment where leaching was absent.

Although there was a marked increase in the total plate counts in soil samples treated with the higher concentrations of the herbicides, this increase was not accompanied by an increase in the nitrification processes. No attempt was made to study the nitrifying organisms quantitatively, due to a lack of a satisfactory method, but it may be assumed that any marked increase in the counts of nitrifying organisms would be reflected by an increase in the rate of nitrate accumulation in the presence of an abundance of ammonia nitrogen. This being true, no beneficial effect of herbicides upon nitrifying organisms was observed. Apparently the nitrifying organisms are suppressed in their activities by the presence of high concentrations of the herbicides, however, their activity was not totally inhibited even in the presence of the highest concentrations used.

There are probably types of organisms in the soil which are capable of utilizing 2,4-dichlorophenoxyacetic acid and the components of Concentrated Activated Diesel Emulsion as sources of energy. Some of these organisms may be capable of growth on egg-albumin-agar, thus accounting in part for the observed increase in total counts, where the high concentrations of the herbicides were applied to the soil.

According to the results of the partial sterilization studies (Table 7), 2,4-dichlorophenoxyacetic acid did not exhibit typical partial sterilization in the sandy-loam soil. It may be assumed therefore that the soil microorganisms present in this particular soil, at least those that were capable of growth on the egg-albumin-agar, were not sensitive to this chemical compound at the rates used. Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel

Emulsion in combination with 2,4-dichlorophenoxyacetic acid proved to be toxic to some microorganisms in the soil, as evidenced by an initial decrease in the total counts which persisted for a period of 24 hours. It may be assumed that during this period the microorganisms more sensitive to the constituents of the herbicides were destroyed, leaving the more tolerant microorganisms to survive and multiply at a rapid rate during the period of 24 hours to 7 days. Some of these latter microorganisms are probably capable of utilizing the components of the herbicides as sources of energy, in addition to other substances already present in the soil which are made available due to the destruction of some other competitive microorganisms.

Although nitrate determinations were not made in the partial sterilization studies, it is possible that the accumulation of nitrate nitrogen might have been accelerated during the earlier period before a pronounced increase in the total plate counts was evident, in the soil treated with Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid. Where there was a marked increase in the total plate counts, some ammonia or nitrate nitrogen must have been utilized by these heterotrophic microorganisms and, to this extent, would be accompanied by a retardation in the accumulation of nitrate nitrogen. This may, to a small degree, account for the earlier low nitrate accumulation in soil samples treated with the higher concentrations of the herbicides. Nevertheless, the direct effects of the herbicides on the activities of the nitrifying organisms are probably of greatest importance and no doubt account to a great extent for the retardations of the accumulation of nitrate nitrogen.

The partial-sterilization effect exhibited by the combination of Concentrated Activated Diesel Emulsion and 2,4-dichlorophenoxyacetic acid is possibly due to the combined action of the 2 herbicides. The rate of 1000 gallons per acre of this herbicidal combination contained Concentrated Activated Diesel Emulsion and 2,4-dichlorophenoxyacetic acid at the rates of 100 gallons per acre and 100 pounds per acre, respectively. The data in Table 5 suggest that the diesel emulsion applied to the soil at the rate of 100 gallons per acre did not exhibit any partial sterilization properties. Although 2,4-dichlorophenoxyacetic acid was not used at a rate of 100 pounds per acre in the partial sterilization studies, this herbicide might possibly exhibit such properties at this rate. The purpose of combining these 2 herbicides in the weed control program is to take advantage of the synergistic effect produced by the herbicides on each other. For example, at the rate of 30 gallons per acre of the combination, 2,4-dichlorophenoxyacetic acid will be present at the rate of 3 pounds per acre, the normal field application, while Concentrated Activated Diesel Emulsion will be present at the rate of 3 gallons per acre, far short of the normal field rates of 100 to 500 gallons per acre. Nevertheless, this combination when applied at this rate behaves as an effective combination pre-emergence-contact spray. The herbicidal properties of Concentrated Activated Diesel Emulsion as a contact spray are greatly enhanced while 2,4-dichlorophenoxyacetic acid is also benefited by an increased pre-emergence action on weeds and weed seeds.

From all indications synergistic relationships between these 2 herbicides do not increase the retarding effects of this herbicidal combination on the accumulation of nitrate nitrogen. Whatever influence this combination exhibited on the accumulation of nitrate nitrogen could apparently be attributed to the 2,4-dichlorophenoxyacetic acid fraction.

Results recorded in Tables 4, 5, and 6 for the residual ammonia-nitrogen determinations also indicate that the retardation of the accumulation of nitrate nitrogen was not due to the absence or lack of ammonia nitrogen in the soil since ammonia nitrogen could be recovered from soil samples in every case where the retardation was evident for the duration of the tests. The form in which the added ammonia nitrogen persisted in the soil is not known, but it could readily be recovered by the direct nesslerization method.

The results of these studies suggest many avenues for further soil investigations utilizing these herbicides.

SUMMARY

The results of the foregoing studies indicate that under the conditions of these experiments:

1. 2,4-dichlorophenoxyacetic acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid applied to sandy-loam and silt-loam soils at the usual field rates of 3 pounds per acre, 100 and 500 gallons per acre, and 30 gallons per acre, respectively, did not appreciably retard the rate of accumulation of nitrate nitrogen.

2. These herbicides when applied to the soils at rates higher than the normal field applications exhibited definite and varying degrees of retardation of the rate of accumulation of nitrate nitrogen, depending on the rates of application. With the highest application of 5000 gallons per acre, Concentrated Activated Diesel Emulsion markedly retarded nitrification, the retardation persisting for 8 weeks in the silt-loam soil and throughout the 16-week test period in the sandy-loam soil. Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, at the rate of 1000 gallons per acre, retarded nitrification for 8 weeks in the sandy-loam soil and 16 weeks in the silt-loam soil. Retardation by 2,4-dichlorophenoxyacetic acid, at the rate of 50 pounds per acre, persisted in the sandy-loam soil for

4 weeks and 16 weeks in the silt-loam soil.

3. Even at the highest concentrations of the herbicides used in these studies, the accumulation of nitrate nitrogen was not completely inhibited, nitrate nitrogen slowly accumulating with time.

4. The retarding effect on the rate of accumulation of nitrate nitrogen seemed to persist longer in the silt-loam soil than in the sandy-loam soil, especially in the case of 2,4-dichlorophenoxyacetic acid and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid.

5. In every case where poured-plate counts were made, the highest concentrations of the herbicides applied to the soil samples caused a marked increase in the total plate counts, as determined on egg-albumin-agar medium.

6. The highest concentrations of Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, exhibited a typical partial sterilization phenomenon, while 2,4-dichlorophenoxyacetic acid alone did not exhibit a similar phenomenon.

7. From all indications the retardation of the rate of accumulation of nitrate nitrogen was due to the direct effects of the herbicides on the nitrifying organisms and not to the absence of ammonia nitrogen resulting from a detrimental effect upon the heterotrophic organisms.

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STUDIES ON THE EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID, CONCENTRATED ACTIVATED DIESEL EMULSION, AND CONCENTRATED ACTIVATED DIESEL EMULSION IN COMBINATION WITH 2,4-DICHLOROPHENOXYACETIC ACID ON THE ACCUMULATION OF NITRATE NITROGEN AND TOTAL PLATE COUNTS IN TWO KANSAS SOILS

by

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Studies were made on the effects of 2,4-dichlorophenoxyacetic acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid on the accumulation of nitrate nitrogen in sandy-loam and silt-loam soils obtained from the vicinity of Manhattan, Kansas. The effects of these herbicides on the total counts, including bacteria, actinomycetes, and fungi, and persistence of added ammonia nitrogen in the silt-loam soil were also investigated.

These herbicides were applied at varying rates to 100-grams lots of soil which were maintained in cotton-stoppered, 350-ml wide-mouth bottles at room temperature. These were kept at 50 per cent saturation by the addition of water at regular intervals. Nitrate and ammonia nitrogen were determined at intervals of 0, 2, 4, 8, and 16 weeks using the phenoldisulphonic acid and direct nesslerization methods, respectively. Total counts were also made at these intervals utilizing egg-albumin-agar plates. A test was also conducted to see whether the herbicides behaved as partial sterilants of the soil or not, utilizing the highest concentrations of the herbicides tested, freshly obtained sandy-loam soil, and egg-albumin-agar medium for making total counts.

Results of these studies indicated that:

1. 2,4-dichlorophenoxyacetic acid, Concentrated Activated Diesel Emulsion, and Concentrated Activated Diesel Emulsion in

combination with 2,4-dichlorophenoxyacetic acid applied to sandy-loam and silt-loam soils at the usual field rates of 3 pounds per acre, 100 and 500 gallons per acre, and 30 gallons per acre, respectively, did not appreciably retard the rate of accumulation of nitrate nitrogen.

2. These herbicides when applied to the soils at rates higher than the normal field applications exhibited definite and varying degrees of retardation of the rate of accumulation of nitrate nitrogen, depending on the rates of application. With the highest application of 5000 gallons per acre, Concentrated Activated Diesel Emulsion markedly retarded nitrification, the retardation persisting for 8 weeks in the silt-loam soil and throughout the 16-week test period in the sandy-loam soil. Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, at the rate of 1000 gallons per acre, retarded nitrification for 8 weeks in the sandy-loam soil and 16 weeks in the silt-loam soil. Retardation by 2,4-dichlorophenoxyacetic acid, at the rate of 50 pounds per acre, persisted in the sandy-loam soil for 4 weeks and 16 weeks in the silt-loam soil.

3. Even at the highest concentrations of the herbicides used in these studies, the accumulation of nitrate nitrogen was not completely inhibited, nitrate nitrogen slowly accumulating with time.

4. The retarding effect on the rate of accumulation of

nitrate nitrogen seemed to persist longer in the silt-loam soil than in the sandy-loam soil, especially in the case of 2,4-dichlorophenoxyacetic acid and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid.

5. In every case where poured-plate counts were made, the highest concentrations of the herbicides applied to the soil samples caused a marked increase in the total plate counts, as determined on egg-albumin-agar medium.

6. The highest concentrations of Concentrated Activated Diesel Emulsion and Concentrated Activated Diesel Emulsion in combination with 2,4-dichlorophenoxyacetic acid, exhibited a typical partial sterilization phenomenon, while 2,4-dichlorophenoxyacetic acid alone did not exhibit a similar phenomenon.

7. From all indications the retardation of the rate of accumulation of nitrate nitrogen was due to the direct effects of the herbicides on the nitrifying organisms and not to the absence of ammonia nitrogen resulting from a detrimental effect upon the heterotrophic organisms.