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DETERMINATION OF SOIL MICROORGANISM POPULATIONS IN THE EGLIN AFB, FLORIDA, BIODEGRADATION PLOTS*

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by

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*An in-house report assessing the role of microorganisms in the disappearance of herbicide Orange in the biodegradation plots at Eglin AFB, Florida. This report is in support of the AFLC Project on the Disposition of Herbicide Orange.

INTRODUCTION AND LITERATURE REVIEW

The general role of microorganisms in the biodegradation of herbicides has been thoroughly investigated and well documented. Research at universities and by interested industries has dealt with biodegradation of commercial herbicides by fungi, bacteria, and actinomycetes, alone, and in combination. This research has tended to stress the action of total numbers of organisms rather than on the effects of specific species or genera involved in the actual breakdown (1,2,3,4,5,6,7,8,9,10,11,12,13,14,17,18,19,20,21,22,23,24,25,26,32,34,35,36,37,38,39).

That soil biodegradation of pesticide compounds does occur, and that soil microorganisms are at least partially responsible for that degradation has been confirmed by Kearney, et al. in several key papers (21,22,23,24). These workers have reported primarily on the role of total microflora on degradation, and on individual enzymes involved in the breakdown process. Bollag (8,9,10) implicated certain soil fungi, especially Geotrichum candidum, in the alteration of pesticides.

Contrary to some irresponsible reporting in mass media publications, the use of pesticides, especially herbicides, does not cause sterility (i.e., complete elimination of microflora) in soil. Tyagny-Ryadno (39), in determining the effect of herbicides on the microflora and agrochemical properties of soil, concluded that some herbicides actually increase bacterial populations. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) did not, however, enhance microbial growth

in studies by Bollag (8,9,10). He stated that some herbicides promote growth of fungi, but seem to inhibit actinomycetes.

In investigating the action of 2,4-D on Azotobacter spp. in sugarcane soils, Colmer (11) has determined that the population levels of these organisms are not affected if herbicide rates are within those normally recommended for sugarcane. Johnson and Colmer (19,20) studied the effects of 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) on metabolic activities of various bacteria including Bacillus cereus, certain Azotobacter spp., and Pseudomonas flourescens. They concluded that concentrations of 2,4-D at less than 5,000 ppm have little inhibitory effect on Pseudomonas flourescens.

One consequence of the spraying of herbicides has been the potential contamination of sprayed sites with 2,3,7,8-tetrachlorodibenzodioxin (TCDD), a persistent and potent teratogen, synthesized as a contaminant in the production of 2,4,5-T herbicide. Although data is incomplete regarding the microbial degradation of TCDD, the problem of the persistence of TCDD in soil is acute. Morbidity and Mortality Weekly Report, Volume 23, Number 34, reported human and animal deaths and/or serious pathology as a result of spraying TCDD contaminated substances on horse arenas for dust control. TCDD accumulation in natural food chains has been studied and data are presently being generated to elucidate the extent to which microorganisms degrade this potentially hazardous material.

One difficulty in the analysis of field soils for effects of herbicide on soil organisms (and vice versa) is the lack of baseline

data describing microfloral populations of areas available for incorporation of large amounts of herbicide. Two areas with some baseline data completed are Eglin AFB Reservation, Eglin AFB, Florida, and the environs of Dugway Proving Ground, Utah (2,3,15,16,27,28,29, 30,31,32). In a recent technical report, Young, et al., showed that field soil sprayed with massive quantities of military herbicides was identical in microfloral character to adjacent non-sprayed control areas 3 years after the last herbicide application (40). This report made maximum use of baseline data available, but would have been even more significant had a detailed microbial survey been conducted immediately before and at intervals following applications of herbicide. In a summary of this same report, Young, et al. indicated that soil microorganism levels are probably linked to the disappearance and reappearance of vascular plants during and following (respectively) applications of herbicide (41).

METHODS AND MATERIALS

Soil biodegradation plots were established on the western border of Test Area C-52A (TA C-52), Eglin AFB Reservation, Florida. The soils of the area are Lakeland Sandy Loam Association containing a high percentage of sand and low organic matter. Table 1 describes a representative soil profile by 15 cm increments to a depth of 90 cm. The area has a mean annual rainfall of 150 cm and an average temperature at 6 feet above the surface of 19.8°C with the minimum mean average of 14.5°C and maximum mean average of 24.9°C.

TABLE 1. SOIL PROFILE (15-cm INCREMENTS) FOR SOIL BIODEGRADATION PLOTS, EGLIN AFB, FLORIDA

DEPTH/CM	SAND-%	SILT-%	CLAY-%	O.M.-%[a]	C.E.C.[b]
0-15	91.6	4.0	4.4	0.46	1.19
15-30	90.1	4.3	5.6	0.20	0.81
30-45	92.1	4.3	3.6	0.20	0.73
45-60	92.9	3.5	3.6	0.00	0.69
60-75	93.1	2.8	4.1	0.07	0.69
75-90	92.8	3.6	3.6	0.07	0.69

[a]Percent organic matter.

[b]C.E.C. (cation exchange capacity) is the ability of a cation to be displaced or exchanged from the soil by another cation. A soil with a cation exchange capacity of 1 can "bind" or "fix" 10 ppm of a given cation(s).

Ten plots, each 3.1 by 3.1 m in dimension were staked in March 1971. Eight of the plots were rototilled to a depth of 30 cm. Six of the eight plots received 4.5 kg lime, 13.5 kg organic matter (dried hay, partially decomposed) and 1.4 kg fertilizer (12:4:8 for N,P,K respectively). On 2 April 1972, six of the 10 plots received 4,480 kg Herbicide Orange (a 50:50 formulation of the n-butyl esters of 2,4-D and 2,4,5-T). The description of the study is shown in Table 2. The soil amendments were those incorporated in the plots in March 1971. The herbicide was applied in each plot into three 30-cm wide trenches approximately 10-12 cm deep. In the two plots receiving charcoal, a 1 cm thick band of activated coconut charcoal was placed in each trench prior to the application of the herbicide. The trenches were covered after treatment.

Soil samples from each of the biodegradation plots were collected

TABLE 2. DESCRIPTION OF THE EGLIN AFB, FL, BIODEGRADATION STUDY OF HERBICIDE ORANGE. PLOTS ESTABLISHED 2 APRIL 1972.

PLOT NO.	TREATMENT	SOIL CONCENTRATION OF HERBICIDE ON 7 APRIL 1972, PPM[a] DEPTH 0-15 CM	15-30 CM
1,2	Control	N.D.[b]	N.D.
3,4	Soil Amendments[c]	N.D.	N.D.
5,6	4,480 kg herbicide/ha	4,900	300
7,8	4,480 kg herbicide/ha plus soil amendments	5,705	230
9,10	4,480 kg herbicide/ha plus soil amendments and activated coconut charcoal	3,075	135

[a] Concentration is a total of the n-butyl esters and free acids of 2,4-D and 2,4,5-T herbicide.

[b] None detected.

[c] Soil amendments included 4.5 kg lime, 13.5 kg organic matter, and 1.4 kg fertilizer (12:4:8 for N,P,K respectively) uniformily mixed within the top 0-30 cm of soil in the plot.

from the 0-15 and 15-30 cm increments in June 1974, August 1974, and April 1975. Samples were collected using sterile ceramic tools and immediately placed in sterile glass jars. All samples were kept refrigerated, but not frozen, until they were delivered to the laboratory for microbial analysis.

The soil samples were prepared for microbial analyses in the following manner. Thirty (30) grams of each sample were added to 300 ml sterile distilled water. These mixtures were each blended for 1 minute in a Honeywell blender. Dilution series were made from these suspensions using sterile distilled water blanks to achieve dilutions of 10^{-3}, 10^{-4}, and 10^{-5}. Dilutions were dispensed in three

media in sterile Petri plates with three replicates per dilution for each sample. Plated samples were incubated at room temperature (approximately 24°C).

Three media were used to determine microorganism levels. Sabouraud's dextrose agar medium plus Tergitol NPX (100 ppm) and chlorotetracycline (40 ppm) was used for development of soil fungi. Potato dextrose agar plus 150 ppm Actidione was used for development of bacteria. Sodium caseinate medium (DIFCO Actinomycete Isolation Agar) plus 50 ppm Actidione was used for elaboration of actinomycetes. Average soil pH was determined to be 4.5. The pH of all media was adjusted to 5.6. It was felt that greater diversity of organisms could be achieved by raising the pH to this higher value.

Sabouraud dextrose agar plates were examined for fungi after 6 days. Potato dextrose agar plates were examined for bacteria after 4 days, and actinomycete agar plates were examined for actinomycetes after 7 days. Counts were made from each plate and predominant organisms isolated for subsequent identification.

RESULTS AND CONCLUSIONS

Tables 3 and 4 show typical microbial counts per gram of soil for each treatment in the top two soil increments for the April 1975 sampling. Similar data were obtained from the June 1974 and August 1974 soil samples. The following statements summarize the experimental data and explain possible trends:

a. The number of bacterial, fungal, and actinomycete organisms

TABLE 3. AVERAGE NUMBER OF MICROORGANISMS PER GRAM OF SOIL IN THE TOP 0-15 CM INCREMENT FROM HERBICIDE BIODEGRADATION PLOTS, EGLIN AFB, FL, APRIL 1975

PLOT	TREATMENT	BACTERIA	FUNGI	ACTINOMYCETES
1,2	Control	7.9×10^5	5.3×10^4	2.9×10^5
5,6	Herbicide	6.8×10^5	6.0×10^4	3.7×10^5
7,8	Herbicide plus amendments	2.0×10^6	3.2×10^5	9.3×10^5
9,10	Herbicide plus amendments and charcoal	5.0×10^6	5.5×10^5	4.0×10^6

TABLE 4. AVERAGE NUMBER OF MICROORGANISMS PER GRAM OF SOIL IN THE 15-30 CM INCREMENT FROM HERBICIDE BIODEGRADATION PLOTS, EGLIN AFB, FL, APRIL 1975

PLOT	TREATMENT	BACTERIA	FUNGI	ACTINOMYCETES
1,2	Control	4.1×10^5	3.0×10^4	1.7×10^5
5,6	Herbicide	2.0×10^5	2.3×10^4	2.5×10^5
7,8	Herbicide plus amendments	7.1×10^5	5.7×10^4	1.5×10^5
9,10	Herbicide plus amendments and charcoal	7.9×10^4	9.5×10^3	1.9×10^4

in Plots 5 and 6 (herbicide treatment) were not significantly different from those of controls (Plots 1 and 2) in the 0-15 cm region. Microorganism levels in the 15-30 cm region were likewise similar to those of controls. Plot 6 levels in April 1975 were virtually identical to levels determined for Plot 5 in August 1974. No differences were immediately evident in bacterial, fungal, and actinomycete genera found

between the control plots or the herbicide treatment plots.

b. Plots 7 and 8 (herbicide plus soil amendments) had microorganism levels that were not significantly different from those of controls in the 15-30 cm region, but were somewhat higher than controls (by a factor of 9 or 10) in the 0-15 cm region. Plot 7 microorganism levels were slightly lower in April 1975 than in soils taken from Plot 7 for analysis in August 1974. In lowering, the levels were going in the direction of the controls.

c. For Plots 9 and 10 (herbicide, soil amendments and activated charcoal), the microorganism levels were higher in the 0-15 cm region than they were from any of the other plots. Plot 10 levels in the April 1975 sampling were similar to, although slightly lower than, Plot 9 levels determined in August 1974. In being lower, the levels were tending toward the direction of the controls. Plot 10 microorganism levels were lower in the 15-30 cm depth than levels for any other samples in the analysis, although higher in April 1975 than Plot 9 levels from this same depth in August 1974. In being higher, the levels were tending toward the direction of the controls for this depth.

d. The predominant bacterial organisms isolated from the plots were Pseudomonas sp. and Bacillus sp. (possibly B. subtilis). The dominant fungal species isolated was the pink yeast, Rhodotorula mucilaginosa. Other genera of fungi included Aspergillus, Penicillium, Fusarium, Helminthosporium, Mucor, Nigrospora, Pullularia and Curvularia. Streptomyces sp. was the dominant actinomycete with lesser amounts of Nocardia sp. also present. The distribution of the above organisms

seemed uniform throughout all the plots, at the two depths, and for the three sampling dates.

The data have indicated that the incorporation of activated coconut charcoal greatly affected the number of microorganisms present in soil biodegradation plots. The numbers of bacterial and fungal organisms present in the 0-15 cm increment of plots 9 and 10 were consistently higher (for all replications) than those from the control plots or from other treatment plots. However, the numbers of these same organisms were less at the 15-30 cm increment than for all other plots. It should be re-emphasized that the charcoal was applied in a 30 cm band having a thickness of 1 cm and located approximately 10-12 cm beneath the surface. Thus, the sample would represent a mix of a 15 cm by 15 cm slice of soil through the center of the plot. It is probable that the high levels of microorganisms were associated directly with the charcoal which may in effect have been adsorbing growth promoting substances on its surface. An analysis of the charcoal band was not performed.

From these studies of the Eglin AFB, Florida, biodegradation plots and from the studies by Stark, Orr and McBride (32) of Herbicide Orange biodegradation plots on the AFLC Test Range Complex, Utah, it is apparent that application of massive quantities of 2,4-D and 2,4,5-T herbicides not only does not sterilize soil, but stimulates growth of certain microflora. That these bacteria and fungi are proliferating to such an extent indicates that they are probably utilizing the herbicide as a carbon source, and, as such, are contributing to its degradation. Follow-on studies should include laboratory determinations of the

effects of these particular microorganisms on herbicide. The organisms should be tested alone and in combination.

LITERATURE CITED

1. Alexander, M. and M.I.H. Aleem. 1961. Effect of chemical structure on microbial decomposition of aromatic herbicides. J. Agr. Food Chem. 9:44-47.

2. Arvik, J.H. 1969. Soil algae of the Eglin AFB Defoliant Test Range and the response of selected species to military herbicides. AFATL-TR-69-35. AF Arm. Lab., Eglin AFB, Florida. Unclassified.

3. Arvik, J.H. and J.H. Hunter. 1971. Soil algae of a herbicide test area, Eglin AFB, Florida, and the response of selected species to military herbicides. Unpublished dissertation.

4. Audus, L.J. 1950. Biological detoxification of 2,4-dichlorophenoxyacetic acid in soils: isolation of an effective organism. Nature 166:356.

5. Audus, L.J. and K.V. Symonds. 1955. Further studies on the breakdown of 2,4-dichlorophenoxyacetic acid by a soil bacterium. Ann. Appl. Biol. 42:174.

6. Bell, G.R. 1960. Some morphological and biochemical characteristics of a soil bacterium which decomposes 2,4-D. Can. J. Microbiol. 3:821-840.

7. Bell, G.R. 1960. Studies on a soil Achromobacter which degrades 2,4-dichlorophenoxyacetic acid. Can. J. Microbiol. 6:325-337.

8. Bollag, J.M. 1972. Biochemical transformation of pesticides by soil fungi. Crit. Rev. Microbiol. 2(1):35-37.

9. Bollag, J.M., G.G. Briggs, J.E. Dawson, and M. Alexander. 1968. Enzymatic degradation of chlorocatechols. J. Agr. Food Chem. 16(5):829-833.

10. Bollag, J.M., C.S. Helling, and M. Alexander. 1968. Enzymatic hydroxylation of chlorinated phenols. J. Agr. Food Chem. 16(5):826-828.

11. Colmer, A.R. 1953. The action of 2,4-D upon the Azotobacter of some sugarcane soils. Appl. Microbiol. 1:184-187.

12. Fletcher, W.W. 1966. Herbicides and the bioactivity of the soil. Invitation lecture given at Wageningen at meeting organized by the Dutch Working Party for Weed Control. 22 Mar 1966.

13. Fletcher, W.W. and J.E. Smith. Herbicides and soil microbes. New Scient. 24:527-528.

14. Hirsch, P. and M. Alexander. 1960. Microbial decomposition of halogenated propionic and acetic acids. Can. J. Microbiol. 6:241-249.

15. Hunter, J.H. 1970. Soil microorganism study of TA-C52A, Eglin AFB, Florida. Unpublished survey. AF Arm. Lab., Eglin AFB, Florida.

16. Hunter, J.H. and A.L. Young. 1972. Vegetative succession studies on a defoliant equipment test area, Eglin AFB Reservation, Florida. AFATL-TR-72-31. AF Arm. Lab., Eglin AFB, Florida. Unclassified.

17. Jensen, H.L. 1957. Decomposition of chloro-substituted aliphatic acids by soil bacteria. Can. J. Microbiol. 3:151-164.

18. Jensen, H.L. 1957. Decomposition of chloro-organic acids by fungi. Nature 180:1416.

19. Johnson, E.J. and A.R. Colmer. 1955. I. The effect of 2,4-dichlorophenoxyacetic acid on some phases of nitrogen metabolism of Bacillus cereus. Appl. Microbiol. 3:123-126.

20. Johnson, E.J. and A.R. Colmer. 1955. II. The effect of 2,4-dichlorophenoxyacetic acid on some phases of the nitrogen metabolism of Pseudomonas fluorescens and the microorganisms of a soil suspension. Appl. Microbiol. 3:126-128.

21. Kaufman, D.D., P.C. Kearney, and T.J. Sheets. 1963. Simazine: degradation by soil microorganisms. Science 142:405.

22. Kaufman, D.D., P.C. Kearney, and T.J. Sheets. 1965. Microbial degradation of simazine. J. Agr. Food Chem. 13:238.

23. Kearney, P.C. 1965. Purification and properties of an enzyme responsible for hydrolyzing phenylcarbamates. J. Agr. Food Chem. 13:561.

24. Kearney, P.C., D.D. Kaufman, and M.L. Beall. 1964. Enzymatic dehalogenation of 2,2-dichloroproprionate. Biochem. Biophys. Res. Commun. 14:29.

25. Lamartinere, C.A., L.T. Hart, and A.D. Larson. 1969. Delayed lethal effect of 2,4-dichlorophenoxyacetic acid on bacteria. Bull. Environ. Contam. Toxicol. 4(2):113-119.

26. Magee, L.A. and A.R. Colmer. 1955. III. The effect of some herbicides on the respiration of Azotobacter. Appl. Microbiol. 3:288-292.

27. Orr, G.F. 1968. Some fungi isolated with Coccidiodes immitis from soils of endemic areas in California. Bull. Torrey Bot. Club 95:424-431.

28. Orr, G.F. 1969. Keratinophilic fungi isolated from soils by a modified hair bait technique. Sabouraudia 7:129-134.

29. Orr, G.F. and D.S. Thorne. 1969. Mycoflora of west central Utah. Deseret Test Center, Dugway Proving Ground, Dugway, Utah. Tech Rpt DTC TB-26.

30. Orr, G.F., D.S. Thorne, and W.C. Tippets. 1970. Comparison of two techniques for isolation of Coccidioides immitis from soil. Deseret Test Center, Dugway Proving Ground, Dugway, Utah. Tech Rpt TB-39.

31. Orr, G.F. and H.H. Kuehn. 1972. Notes on gymnoascaceae. II. Some gymnoascaceae and keratinophilic fungi from Utah. Mycol. 64:55-72.

32. Stark, H.E., G.F. Orr, and J.K. McBride. 1974. Microbiological and baseline ecology studies of a proposed biodegradation site at Lakeside Range, Hill Air Force Base, Utah and environs. US Army Dugway Proving Ground, Utah. TECOM Project No. 5 CO-213-015.

33. Pate, B.D., R.C. Voight, P.J. Lehn, and J.H. Hunter. 1972. Animal survey studies of test area C-52A, Eglin AFB Reservation, Florida. AFATL-TR-72-72. AF Arm. Lab., Eglin AFB, Florida. Unclassified.

34. Pfister, R.M. 1972. Interactions of halogenated pesticides and microorganisms: a review. Crit. Rev. Microbiol. 2(1):1-34.

35. Rogoff, M.H. and J.J. Reid. 1956. Bacterial decomposition of 2,4-dichlorophenoxyacetic acid. J. Bacteriol. 71:303-307.

36. Smith, N.R., V.T. Dawson, and M.E. Wenzel. 1945. The effect of certain herbicides on soil microorganisms. Soil Sci. Soc. Amer., Proc. 10:197-201.

37. Steenson, T.A., and N. Walker. 1956. Observations on the decomposition of chlorophenoxyacetic acids by soil bacteria. Plant Soil 8:17-32.

38. Thiegs, B.J. Fall 1962. Microbial decomposition of herbicides. Down Earth 18(2):7-10.

39. Tyagny-Ryadno, M.G. 1967. Effect of herbicides on the microflora and agrochemical properties of the soil. Trudy Kamenets-podolsk. sel'-khoz. Inst. 9:43-48.

40. Young, A.L., et al. 1974. Ecological studies on a herbicide-equipment test area (TA C-52A), Eglin AFB Reservation, Florida. AFATL-TR-74-12. AF Arm. Lab., Eglin AFB, Florida. Unclassified.

41. Young, A.L., C.E. Thalken, W.E. Ward, and W.J. Cairney. 1974. The ecological consequences of massive quantities of 2,4-D and 2,4,5-T herbicides. Summary of a five year field study. Presentation to the Weed Science Society of America, 14 Feb 74, Las Vegas, Abstract No. 164.