N62604.AR.000089
NCBC GULFPORT
5090.3a

DETERMINATION OF SOIL MICROORGANISM POPULATIONS IN THE HERBICIDE STORAGE AREA SITE 8 NCBC GULFPORT MS
4/1/1977
U S AIR FORCE

DETERMINATION OF SOIL MICROORGANISM POPULATIONS IN THE HERBICIDE STORAGE AREA OF GULFPORT, MISSISSIPPI*

April 1977

by

Capt William J. Cairney
Department of Chemistry and Biological Sciences
US Air Force Academy, Colorado 80840

*An in-house report assessing the role of microorganisms in the disappearance of herbicide Orange in the Herbicide Storage Area, NCBC, Gulfport, MS. This report is in support of the AFLC Project on the Disposition of Herbicide Orange.

INTRODUCTION

The importance of microorganisms in the biodegradation of military herbicides has been the subject of recent intensive studies of soil biodegradation plots established at Eglin AFB Reservation, Florida, and at Deseret Test Center, Dugway Proving Grounds, Utah. These studies have carefully recorded the populations of organisms in soils containing massive amounts of phenoxy-herbicides and established the roles of certain of these soil organisms in the breakdown of these herbicides into harmless simpler products (1,2,3,6,9,10).

Work to date has been summarized by Cairney (2) in an introduction to a report assessing the role of microorganisms in the disappearance of herbicide Orange in the biodegradation plots at Eglin AFB. Young, et al. (9), have shown that field soil sprayed with massive quantities of military herbicides was identical in microbiological population and composition to adjacent non-sprayed control areas 3 years after the last herbicide application. Although 3 years is the interval for which hard data is available, the "microbiological data" section of the Young report infers that such microbiological "evening-out" of sprayed areas could perhaps have occurred in an even shorter time. The Cairney data on Eglin biodegradation plots supports this inference (2).

In all work involving biodegradation of herbicides, the necessity of having extensive microbial, ecological, and chemical baseline data for test areas has been recognized (1,2,3,4,5,6,7,8,9,10).

MATERIALS AND METHODS

Sixteen soil samples for microbial analysis were received on 28 February 1977 from herbicide storage areas at Gulfport, Mississippi. Soil samples were labelled as follows: "Micro-control Hardpan, CHP-A, CHP-B, CHP-C, and CHP-D" (1 sample of each, total of 4 samples); "Micro-control Ditch, CD-A, CD-B, CD-C, and CD-D" (1 sample of each, total of 4 samples); "Micro-site #5, 5-A, 5-B, 5-C, and 5-D" (1 sample of each, total of 4 samples); and "Micro-site #11, 11-A, 11-B, 11-C, and 11-D" (1 sample of each, total of 4 samples).

Soil samples were collected according to methods utilized by Young, Cairney and Hunter in previous studies (2,3,9) and analyzed for soil microorganisms using procedures described by Young (9) and Cairney (2), with the exception that Sabouraud's Dextrose Agar (as opposed to Potato Dextrose Agar) was used to enumerate soil fungi. Actinonycetes were not enumerated.

RESULTS

The following table subjectively describes the appearance of the samples as they were received in glass collecting jars on 28 February 1977.

Label	Sample	Subjective Appearance/Character
Micro-control Hardpan	CHP-A	Grey-brown sand
Micro-control Hardpan	CHP-B	Hard, grey, sandy, cement-like
Micro-control Hardpan	CHP-C	Grey-brown hard sand, wetter than CHP-A and CHP-B
Micro-control Hardpan	CHP-D	Hard, grey, sandy, cement-like
No herbicide smell noticed in any sample		
Micro-control Ditch	CD-A	Dark sand
Micro-control Ditch	CD-B	Dry, granular, clay-sand
Micro-control Ditch	CD-C	Caked, dry sand
Micro-control Ditch	CD-D	Hard, caked, dry clay
No herbicide smell noticed in any sample		
Micro-site #5 Hardpan	5-A	Clay-sand with small pebbles
Micro-site #5 Hardpan	5-B	Reddish clay with small pebbles
Micro-site #5 Hardpan	5-C	Brown-black sandy clay
Micro-site #5 Hardpan	5-D	Black lumpy clay with some organic matter in the form of small roots
Conspicuous herbicide smell in 5-A and 5-B, some herbicide smell in 5-C and 5-D, but very slight		
Micro-site #11, Old Spill Ditch	11-A	Red sandy clay
Micro-site #11, Old Spill Ditch	11-B	Red sandy clay, drier than 11-A
Micro-site #11, Old Spill Ditch	11-C	Red sandy clay (more sand than 11-A and 11-B)
Micro-site #11, Old	11-D	Grey-brown, fine sand
Conspicuous herbicide smell in all samples		

The following table gives numerical data for bacterial and fungal populations in the 16 Gulfport samples.

Label	Sample	Avg # bacteria per gram of soil	Avg # fungal propagules per gram of soil
Micro-control Hardpan	CHP-A	Variable, but $<1.0 \times 10^4$	8.0×10^4
	CHP-B	6.0×10^6	2.3×10^5
	CHP-C	$>9.9 \times 10^6$	1.0×10^4
	CHP-D	$>9.9 \times 10^6$	1.1×10^4
Micro-control Ditch	CD-A	$>9.9 \times 10^6$	Variable in replicates, but $<1.0 \times 10^4$
	CD-B	1.0×10^6	2.1×10^5
	CD-C	$>9.9 \times 10^6$	1.0×10^6
	CD-D	$>9.9 \times 10^6$	5.0×10^4
Micro-site #5 Hardpan	5-A	$>9.9 \times 10^6$	2.2×10^4
	5-B	Variable, but $<1.0 \times 10^4$	3.0×10^4
	5-C	$>9.9 \times 10^4$	Variable in replicates, but $<1.0 \times 10^4$
	5-D	$>9.9 \times 10^6$	7.0×10^4
Micro-site #11 Old Spill Ditch	11-A	1.0×10^6	Variable in replicates, but $<1.0 \times 10^4$
	11-B	Variable, but $<1.0 \times 10^4$	Variable in replicates, but $<1.0 \times 10^4$
	11-C	Variable, but $<1.0 \times 10^4$	2.0×10^4
	11-D	4.0×10^5	1.0×10^6

Extensive identification work was carried out in determining bacterial and especially fungal genera associated with each sample. The following table lists the three or four predominant genera found in each sample. Although Actinomycetes were not specifically enumerated, they were occasionally detected on bacterial and fungal plates and identified to genus.

Label	Sample	Predominant Bacterial genera	Predominant Fungal genera
Micro-control Hardpan	CHP-A	*Pseudomonas* spp.	*Penicillium* (3+ spp.)
	CHP-B	*Bacillus* spp.	*Aspergillus* (2+ spp.) *Absidia* sp. (probably *A. glauca*)
	CHP-C	*Pseudomonas* spp. *Bacillus* spp. *Aerobacter*	Extensive populations of at least 3 genera of order Mucorales incl. *Rhizopus* sp. (probably *R. nigricans*), *Absidia* sp. and *Mucor* spp.; sample comparatively low in Deuteromycete genera
	CHP-D		*Penicillium* (2+ spp.) *Rhizopus* sp.
Micro-control Ditch	CD-A	*Pseudomonas* spp. *Bacillus* spp.	*Penicillium* (4+ spp.) (other organisms not at all predominant although present)
	CD-B	*Pseudomonas* spp. *Bacillus* spp.	*Pencillium* (5+ spp.) *Aspirgillus* (2+ spp.) *Absidia* sp.
	CD-C	*Pseudomonas* spp. *Bacillus* spp.	*Pencillium* (2+ spp.) *Mucor* sp. (samples also contained large number of propagules of a fluffy white non-sparulating mycelial fungus perhaps a mycelial mutant of *Aspergillus*)
	CD-D	*Pseudomonas* spp. *Bacillus* spp.	*Penicillium* sp.

Label	Sample	Predominant Bacterial genera	Predominant Fungal genera
Micro-site #5 Hardpan	5-A	Pseudomonas spp. Bacillus spp.	White, mycelial, non-sporulating fungus predominated by far--perhaps mycelial mutant(s) of Aspergillus sp(p).
	5-B	Pseudomonas spp. Bacillus spp.	Aspergillus (3+ spp.)
	5-C	Pseudomonas spp. Bacillus spp.	Penicillium (3+ spp.) Rhodotorula (yeast)
	5-D	Pseudomonas spp. Bacillus spp.	White, non-sporulating mycelial fungus predominated by far, also Rhizopus sp., probably R. nigricans
Micro-site #11 Old Spill Ditch	11-A	Pseudomonas spp.	Penicillium (2+ spp.) Aspirgillus (2+ spp.)
	11-B	Pseudomonas spp.	Penicillium (3+ spp.) Several numbers of mucoralis especially Absidia and Mucor; Fluffy white non-sporulating mycelial fungus
	11-C	Pseudomonas spp.	Fluffy white non-sporulating mycelial fungus
	11-D	Pseudomonas spp.	Rhodotorula (yeast)

Other fungal genera isolated from both control and micro-site samples were Fusarium, Cephalosporium, Alternaria, Trichoderma, Cunninghamella, and Helminthosparium. Actinomycetes occasionally isolated from all samples were Streptomyces and Nocardia.

DISCUSSION

Populations of microorganisms associated with the Gulfport soil were highly variable from sample to sample, even within the four major sampling areas. Although numbers of bacterial propagules varied widely, the same genera predominated in all samples. Pseudomonas spp. and Bacillus spp. were present in greatest numbers. Fungal populations were also variable from sample to sample. Control areas were dominated by several different species of Penicillium and Aspergillus. In the CHP-C sample, members of the order Mucorales were well represented, including at least one species each of Rhizopus, Mucor, and Absidia. The "C" sample of the CD control also contained large numbers of Mucor propagules.

There is a natural tendency for certain conidial state fungi to mutate to a form where the organism no longer produces conidia and grows vegetatively as a fluffy white mycelium. This trend can easily be seen in culture, and, in some fungi, the mutation occurs at a predictable rate. Soil fungi such as the Penicillia, Aspergilli, Fusaria, and numerous others are examples of those in which this mutation is easily observed. While the control samples exhibited some white, fluffy, non-sporulating mycelial colonies, the micro-site samples were particularly characterized by this type growth. Samples 5-A, 5-D, 11-B, and 11-C produced great numbers of these colonies. There was also a tendency for the micro-site samples to support the growth of yeasts, particularly Rhodotorula. Actinomycetes were recovered at low levels on plates of all samples. Two genera were seen, Streptomyces and Nocardia.

Micro-site samples in a least two cases (5-D and 11-B) were notably high in Mucorales genera as was one sample (CHP-C) of the controls. Genera of the Mucorales, particularly Absidia have been implicated in the breakdown of 2,4-D and 2,4,5-T herbicides as shown by past studies (9).

Other genera isolated are listed at the end of the tables.

REFERENCES

1. Arnold, E.L., A.L. Young, and A.M. Wachinski. 1976. Three years of field studies on the soil persistence and movement of 2,4-D, 2,4,5-T and TCDD. Presentation to Weed Society of America, 3 Feb 1976, Denver, CO (Abstr. No. 206).

2. Cairney, W.J. 1975. Determination of soil microorganism populations in the Eglin AFB, Florida, biodegradation plots. In-house report in support of AFLC project on the disposition of herbicide orange. USAF Academy, CO.

3. Hunter, J.H. 1970. Soil microorganism study of TA C-52A, Eglin AFB, Florida. Unpublished survey. AF Armament Lab, Eglin AFB, FL.

4. Hunter, J.H. 1972. Vegetative succession studies on a defoliant equipment test area, Eglin AFB Reservation, Florida. AFATL-TR-72-31. AF Armament Lab, Eglin AFB, FL. Unclassified.

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

6. 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, UT. TECOM Project No. 5 CO-213-015.

7. Young, A.L. and J.H. Hunter. 1977. A long-term field study of vegetative succession following repetitive application of phenoxy herbicides. Abstract for the Weed Society of America Meetings, St. Louis, MO, 8-10 Feb 1977. Abstr. No. 18.

8. Young, A.L., C.E. Thalken, E.L. Arnold, J.M. Cupello, and L.G. Cockerham. 1976. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the environment: summary and decontamination recommendations. USAFA-TR-76-18. US Air Force Academy, CO. Unclassified.

9. 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 Armament Lab, Eglin AFB, FL. Unclassified.

10. 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 Society of America, 14 Feb 1974, Las Vegas. Abstr. No. 164.