BIOMEDICAL AND ENVIRONMENTAL SCIENCES 15, 306-314 (2002)

Mineralization of ^{14}C-ring Labelled 2,4-D in Egyptian Soils Under Aerobic and Anaerobic Conditions[1]

S. M. A. D. ZAYED, M. FARGHALY, AND H. TAHA

National Research Center, Dokki, Cairo, Egypt

Objectives To study the mineralization of 2,4-D in clay and clay loam Egyptian soils under subtropical conditions over a period of 90 d. **Methods** Using ^{14}C-ring labelled pesticide, laboratory studies under aerobic and anaerobic conditions were conducted. ^{14}C-activity in solutions was directly determined by liquid scintillation counting. Unextractable soil residues were determined by combustion. The nature of methanolic ^{14}C-residues was determined by thin layer and high performance liquid chromatographic analysis. **Results** Under aerobic conditions 10%-14% of applied dose was mineralized during 90 d irrespective of soil type. The soil extractable pesticide residues decreased with time and the bound residues gradually increased. The highest binding capacity of about 26%-29% was observed in clay soil under aerobic conditions after 90 d. A good balance sheet was obtained and the percentage recovery was generally between 91% and 100%. **Conclusion** The mineralization of 2,4-D in clay soil was higher than that in clay loam soil under anaerobic conditions. Under aerobic conditions, the soil type had no influence on mineralizaion capacity of 2,4-D during 90 d. The soil binding increased with time whereby the extractable ^{14}C-residues simultaneously decreased. Chromatographic analysis of the methanol extractable ^{14}C-residues of soils revealed the presence of 2,4-D as a main product together with 2,4-dichlorophenol.

Key words: ^{14}C-ring labelled; Organochlorine pesticide; Egyptian soil; Radiochemicals

INTRODUCTION

The organochlorine pesticide 2,4-D is a worldwide well-known herbicide and a plant growth regulator. It is commonly used for post-harvest treatment of citrus fruits in countries like Argentina and South Africa[1]. It is also used on cereals as a selective pre-and postemergence herbicide for the control of broad-leaf weeds[2].

The use of 2,4-D for removal of aquatic herbs, e.g. Nymphea plant from River Nile (especially near the origin in Africa) is associated with much concern because of its possible adverse effects related to the contamination of the river water with 2,4-D and/or its degradation products.

2,4-D was reported to be genetoxic in rat bone-marrow[3] as well as in bone-marrow, germ cells of mouse[4,5] and fetotoxic of newborn jung and adult rats[6].

Several studies on mineralzation and degradation of pesticides in soil have been carried out during the last two decades[7-10].

Most of the reports available so far on the persistence and degradation of pesticides in soil originate from temperate regions of the world. Only little information is reported on the

[1] This work was supported by FAO/IAEA-division for its financial support.

Biographical note of the first author: Dr. Zayed is a research professor of organic and environmental chemistry since 1968. He is principle investigator of several national and international research projects.

0895-3988/2002
CN 11-2816

mineralization of pesticides with aromatic rings in tropical and subtropical soils.

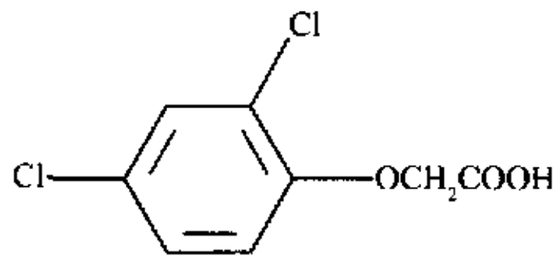
The present work was to study the mineralization rate of 2,4-D in two Egyptian soils under aerobic and anaerobic conditions. For these investigations, radiolabelled laboratory soil dissipation studies were conducted using the pesticides labelled in the aromatic nucleus.

MATERIALS AND METHODS

Radiochemicals

U-ring labelled 2,4-D (2,4-dichlorophenoxy acetic acid, "I") was provided by International Atomic Energy Agency (IAEA). It had a specific activity of 2.14 MBq/mg (473.6 MBq/mmole) and a radiometric purity (by thin layer chromatography)over 97%.

The labelled pesticide was diluted with a pure non-labelled chemical to give a radioactive preparation of the insecticide with specific activity of 0.037 MBq/5mg (1 μCi/5mg) which was used for the mineralization studies.



Ring (UL-^{14}C) –2,4-D

(*I*)

Soils

Two types of local soil were used, namely clay (soil A) and clay loam (soil B). Soils repeatedly treated with pesticides were used alongside with non-treated control soil of the same type. The characteristics of the soils used in this study are shown in the following Table.

Soil Type	pH	Clay	Silt	Sand	Organic
Clay(A)	8.1	67.0	27.3	5.7	1.65
Clay loam(B)	7.7	43.2	23.6	33.2	1.10

Laboratory Mineralization Experiments

The soil capacity to mineralize the aromatic ring of 2,4-D was studied under aerobic and anaerobic conditions. The mineralization studies were investigated in two Egyptian soils. Clay soil was from Middle Delta and clay loam from Giza.

For mineralization studies of 2,4-D under anaerobic conditions, 100 g of soil moistened with water to about 75% of field moisture capacity was used. The soil was transferred to standard biometer flask of 250mL capacity. The side arm of the flask contained 10 mL of 1 mol/L KOH to trap evolved $^{14}CO_2$. The soil was spiked with 0.5 mg of the labelled herbicide and flasks were incubated at about 25℃ in the darkness over twelve weeks. At certain time intervals, a sample of the alkaline solution was determined for its radioactivity and the soil in the biometer flask was analyzed for its extractable and bound residues. For soil analysis

duplicates were used and data were expressed as percentage of applied radioactivity.

Mineralization of 2,4-D under aerobic conditions was conducted for 90 d. Soils were incubated in a closed discontinuously aerated laboratory system described previously[11].

Flasks in triplicates for each type of soil (with about 55% water holding capacity) were treated with the radiolabelled insecticide at a concentration of 5 ppm (5 mg of 2,4-D/kg soil). Flasks were connected to a trapping line containing 1 mol/L KOH, to collect $^{14}CO_2$ at 25°C. The system was air-flushed once a day for a few minutes by a pump to help trapping of liberated $^{14}CO_2$. This $^{14}CO_2$ was monitored at different time intervals over a period of three months. The extent of mineralization was estimated fron the determined cumulative radioactivity of liberated $^{14}CO_2$, and expressed as percentage of applied dose. In both experiments parallel control biometer flasks containing all constituents except the insecticide were used.

Soil Analysis

Soil in biometer flasks was analyzed at specific time intervals (1, 15, 30, 45, 60, and 90 d) for ^{14}C-methanol extractables and bound residues. Extraction was performed with 95% methanol in a soxhlet apparatus for 4-6 h. Unextractable soil residues (bound) were determined by combustion.

The nature of methanolic ^{14}C-residues was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Analysis of soluble residues by HPLC was performed on a Water-Association Model 510 equipped with a Water-Association Model U6K loop injector and a UV-Tunable absorbance detector at 214 nm, (Model 484, France) using 10μC18-column (Water- Association, France) and methanol : water (6:4) as a mobile phase.

From standard curves, the concentration of 2,4-D, 2,4-dichlorophenol and 2,4-dichloroanisole in the extractables was determined, respectively.

The TLC of methanolic extract was conducted using precoated silica-gel plates (Merck, Germany) with chloroform: acetic acid (9:1), and hexane: benzene: acetic acid (10:6:2) as developing systems.

Radioactive Measurement

^{14}C-activity in aliquots (1 mL) of alkaline or methanolic solutions was determined by liquid scintillation counting (LSC) using a dioxane-based scintillation cocktail[12]. Measurements were corrected for background and for quench by using internal standard.

For radioscaning, the TLC plates were scraped at 1cm zones into vials mixed with cocktail and counted by LSC.

The soil-bound (unextractable) residues were determined by combustion of 50-100 mg of extracted soil to $^{14}CO_2$ in a Harvey Biological Oxidizer (OX-600, USA) followed by LSC.

RESULTS

In the present investigations, monitoring of liberated $^{14}CO_2$ from the degradation of ring labeled 2,4-D in Egyptian soils showed that considerable amounts of this chemical was mineralized during three months. Tables 1 and 2 illustrate the cumulative evolution of $^{14}CO_2$ from the radiolabelled 2,4-D under aerobic, anaerobic and subtropical conditions in clay and clay loam Egyptian soils, respectively. The two soils did not show significant variations regarding to their capacity to mineralize 2,4-D under anaerobic conditions. The percentage of mineralization showed a slight, but consistent increase with time and reached, by the end of

the experiment (90 d), under anaerobic conditions, 10% and 14% of applied radioactivity in clay loam and clay soil, respectively (Fig.1).

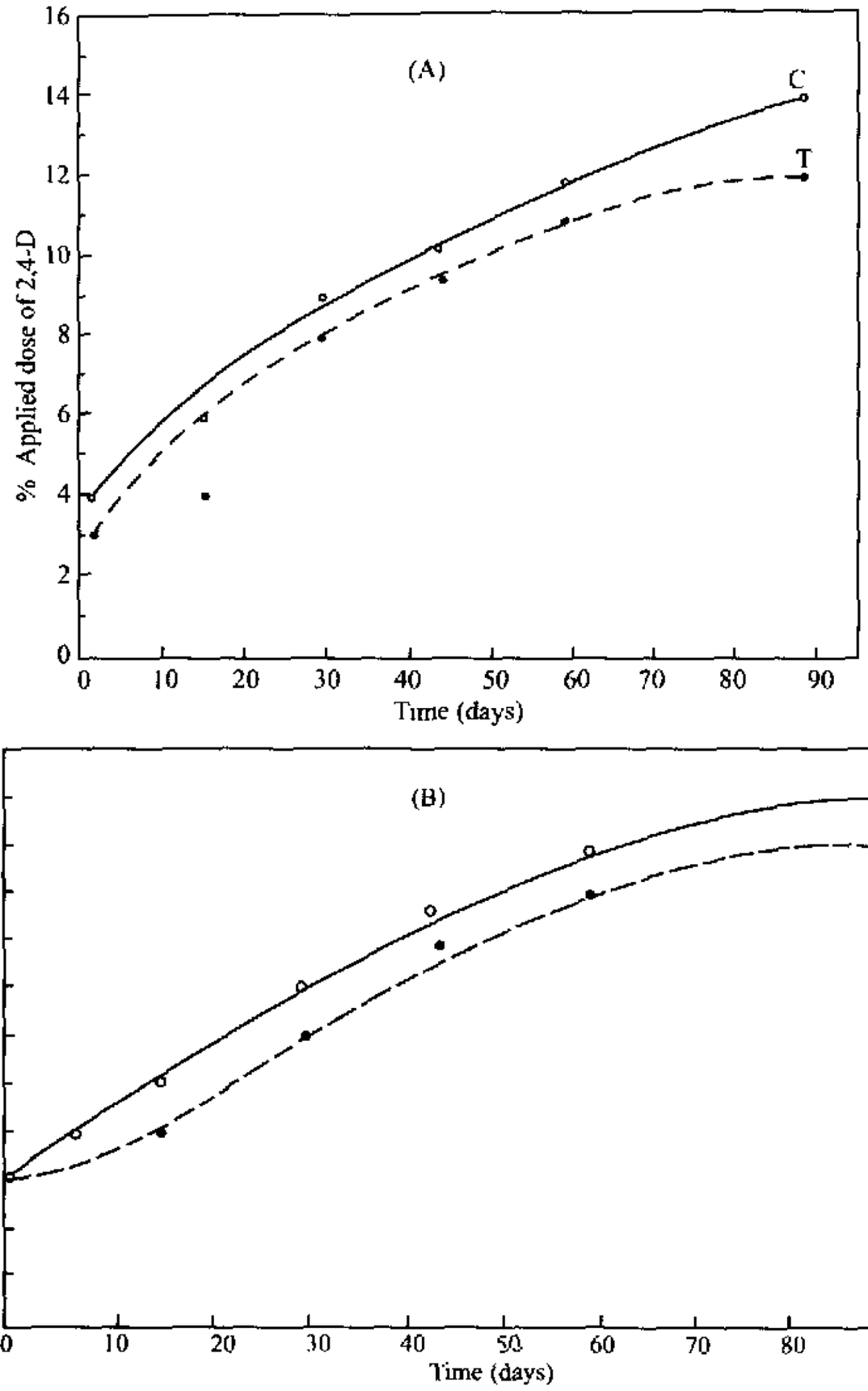


FIG.1. Cumulative $^{14}CO_2$ for clay (A) and clay loam (B) soils spiked with ^{14}C-2,4-D under anaerobic conditions during 12 weeks. C=Control, T=Treated.

Under anaerobic conditions, the amount of extractable residues decreased with time, whereas the percentage of soil bound residues gradually increased (Tables 1 and 2 and Figs. 2 and 3) to reach 30%-35% of the applied dose in 90 d. The binding capacity of 2,4-D residues was slightly lower under aerobic conditions.

In almost all laboratory experiments, a good balance sheet was obtained, and the percentage recovery was generally between 91% and 100%. This suggests that volatilization probably does not represent a significant percentage in the dissipation of 2,4-D from soil under the tested conditions.

TABLE 1

Fate of ^{14}C-2,4-D in Clay Soil Under Laboratory Conditions

Sampling Time (days)	Soil	Anaerobic Conditions				Aerobic Conditions			
		^{14}C-residues in Soil% Applied Dose		$^{14}CO_2$ (%)	Total ^{14}C-recovered (%)	^{14}C-residues in Soil% Applied Dose		$^{14}CO_2$ (%)	Total ^{14}C-recovered (%)
		^{14}C-extractable	^{14}C-bound			^{14}C-extractable	^{14}C-bound		
1	C	88	8	4	100	80	15	5	100
	T	90	7	3	100	88	11	4	103
15	C	76	9	6	91	68	21	7	96
	T	80	8	4	92	76	19	6	101
30	C	67	15	9	91	57	23	18	98
	T	70	14	8	92	65	20	16	101
45	C	64	19	10	93	45	27	25	97
	T	67	17	9	93	50	24	22	96
60	C	60	25	12	97	40	29	30	99
	T	65	22	11	98	47	25	25	97
90	C	52	35	14	101	37	29	34	100
	T	56	31	12	99	44	26	29	99

Note. C=Control soil; T=Treated soil.

TABLE 2

Fate of ^{14}C-2,4-D in Clay Loam Soil Under Laboratory Conditions

Sampling Time (days)	Soil	Anaerobic Conditions				Aerobic Conditions			
		^{14}C-residues in Soil% Applied Dose		$^{14}CO_2$ (%)	Total ^{14}C-recovered (%)	^{14}C-residues in Soil% Applied Dose		$^{14}CO_2$ (%)	Total ^{14}C-recovered (%)
		^{14}C-extractable	^{14}C-bound			^{14}C-extractable	^{14}C-bound		
1	C	89	7	3	99	92	6	1	99
	T	89	7	3	99	95	5	1	101
15	C	84	8	5	97	84	10	3	97
	T	86	7	4	97	88	9	3	100
30	C	72	12	7	91	72	14	9	95
	T	74	11	6	91	82	10	6	98
45	C	70	16	9	95	55	19	14	88
	T	72	18	8	98	76	14	10	100
60	C	64	21	10	95	49	24	18	91
	T	68	20	9	97	61	21	16	98
90	C	54	30	11	95	45	25	22	92
	T	56	29	10	95	57	23	19	99

Note. C=Control soil; T=Treated soil.

The results of thin layer chromatographic analysis of the alcoholic extracts from treated soil are summarized in Table 3, which shows the presence of 2,4-dichloroacetic acid (2,4-D)

and 2,4-dichlorophenol (2,4-DCP) as main degradation products in addition to small amount of 2,4-dichloroanisole (2,4-DCA).

HPLC analysis of the methanol extract from treated soils showed comparable results, and the peaks with retention times (R_t: 2.76, 11.16 and 25.83) for 2,4-D, 2,4-DCP and 2,4-DCA were observed respectively. The relative concentration of these degradation products is shown in Table 3.

TABLE 3

R_f-and R_t-Values of 2,4-D and Its Residues in Egyptian Soils Treated With ^{14}C-2,4-D for 90 D.

Compound	R_f		R_t	Concentration %
	System 1	System 2	System 3	
2,4-D	0.84	0.73	2.76	70-80
2,4-DCP	0.90	0.93	11.16	15-20
2,4-DCA	0.93	0.95	25.83	3-5

Note. TLC: System 1:Chloroform: acetic acid (9:1); System 2: Hexane: benzene: acetic acid (10:6:2); HPLC: System 3: Methanol: water (6:4) mobile phase; UV detection: at 214 nm; Column: 10μ C_{18}.

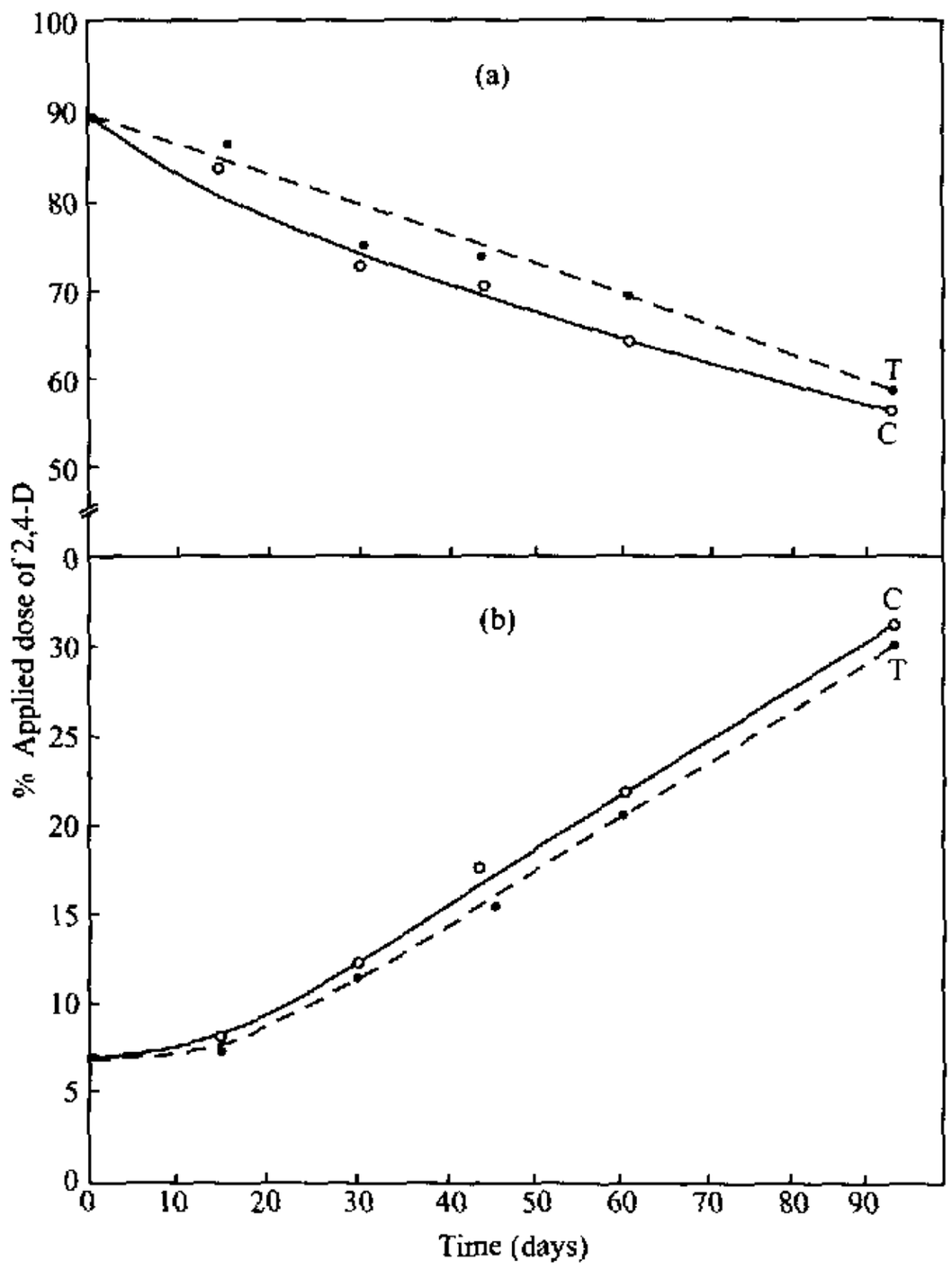


FIG.2. ^{14}C-Methanol extractable (a) and ^{14}C-bound residues (b) in Clay loam soil spiked with ^{14}C-2,4-D under anaerobic Conditions during 12 weeks. C=Control, T=Treated.

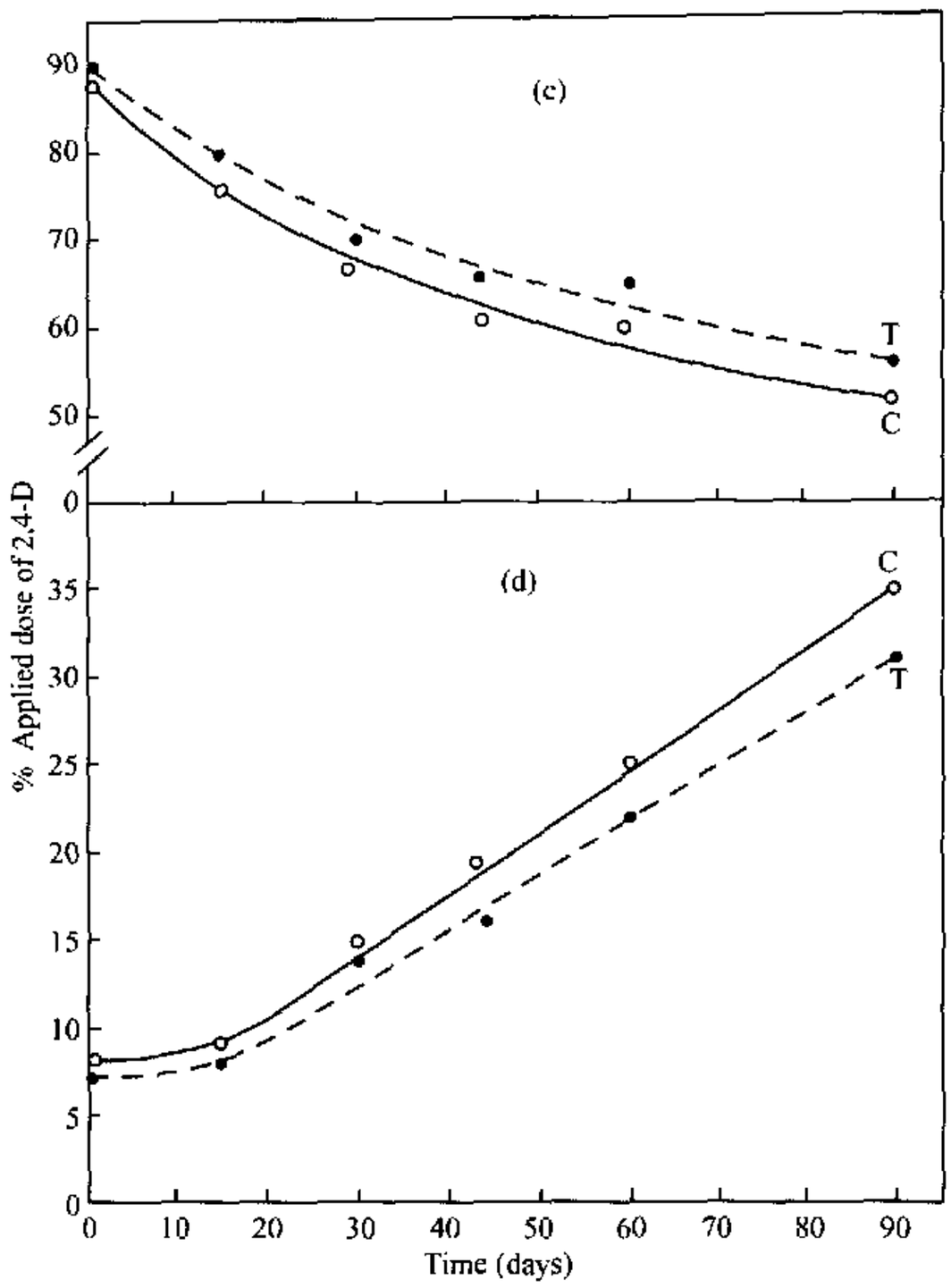


FIG.3. ^{14}C-Methanol extractable (c) and ^{14}C-bound residues (d) in Clay soil spiked with ^{14}C-2,4-D under anaerobic Conditions during 12 weeks. C=Control, T=Treated.

DISCUSSION

The biodegradation of pesticides in soil is a well-documented microbial phenomenon. An understanding of biodegradation of such chemicals is essential for practical applications of microbial bioremediation of pollutants in soil.

Microorganisms play an important role in determining the fate of organic compounds in the environment. In soil, the fate of organic contaminants depends on many factors such as sorption, biodegradation and transportation[13]. Their use for the degradation of unwanted chemicals is now receiving an increasing interest in the field remediation of polluted areas. The degradative activity of soil microorganisms includes mineralization of plants, animals, microbial and organic synthetic compounds, where the formation of carbon dioxide represents the last step of carbon mineralization.

With regard to health implications and epidemiological data pertinent to 2,4-D exposure, the results reported in the literature are equivocal or ambiguous. The EPA Carcinogenicity Peer Review Committee concluded in 1997, that 2,4-D should be rated as a class D

compound, thus indicating insufficient evidence of carcinogenicity to place the compound in a higher category[14].

In general, soils previously treated with pesticides gave a somewhat lower percentage of mineralization than soils without previous exposure to pesticides (control soils). This correlates well with the fact that microbial bioactivity recovers almost completely in several weeks following pesticide application[15].

Under aerobic conditions, 2,4-D was mineralized in clay soil more readily than under anaerobic condition within 90 d. The percentage of mineralization reached 34% and 29% in control and treated soils, respectively (Table 1). Previous work on the aerobic degradation of ^{14}C-ring labelled 2,4-D in soils with no previous herbicide exposure showed that, over a period of 24 d, 25%-31% of the applied ^{14}C was released as carbon dioxide. In soils previously treated with 2,4-D, however, the rate of mineralization was faster with approximately 50% of the applied ^{14}C released as $^{14}CO_2$[16].

The obtained results represent a remarkably high efficiency for Egyptian soils, especially clay soil, in mineralization of 2,4-D under aerobic conditions, as compared with other organochlorine pesticides such as DDT and hexachlorocyclohexane. Both ^{14}C-DDT and ^{14}C-DDE were reported to be only slightly mineralized in Egyptian soils where the mineralized amount didn't exceed 3% of applied dose within 90 d under aerobic conditions[17]. A low percentage of DDT mineralization that amounted to 2.6% in 3 months was also observed in tropical Pakistani soil[18].

The ready mineralization of 2,4-D in clay soil finds analogy with the behavior of ^{14}C-U ring labelled phenyl urea herbicide isoproturon in soil. The latter was reported to be readily mineralized under aerobic conditions, where according to soil type, 14%-23% of initial activity was liberated as $^{14}CO_2$ over 67 d. The authors reported that mineralization rate was generally significantly correlated with soil biomass[11].

In this connection, it is worthy of mentioning that the non-aromatic insecticide hexachlorocyclohexane (HCH) mineralization showed a special pattern in soil. Lindane the γ-isomer of hexachlorocyclohexane as well as α, β and δ-isomers have been known to undergo fairly rapid degradation in anaerobic ecosystem[19,20]. Under aerobic conditions about 10%-12% of the ^{14}C-carbon in α- and γ-HCH was reported to be mineralized during 48 h after inoculation with soil bacterium pseudomanus spp. In this case, lindane is utilized as a source of carbon for proliferation by aerobic γ-HCH-degrading bacterium[21,22].

TLC analysis proved that 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4-dichlorophenol (2,4-DCP) were the primary ^{14}C-degradation compounds found in the alcoholic extract from treated soils together with a small amount of 2,4-dichloroanisole (2,4-DCA). Smith and Aubin[16] reported that 2,4-D was transformed to 2,4-DCP which undergoes biological methylation to 2,4-DCA which possesses a relatively high vapor pressure.

In summary, the mineralization of 2,4-D is higher in clay soil is than in clay loam soil under anaerobic condition. Under aerobic conditions, however, the soil type has no influence on the mineralization capacity of 2,4-D during 90 d. The soil binding increases with time whereby the extractable ^{14}C-residues simultaneously decrease. Chromatographic analysis of alcoholic extracts revealed that 2,4-D and 2,4-DCP are the main degradation products.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the joint FAO/IAEA-division for its financial support of this work.

REFERENCES

1. Anastassiades. M. and Schwack, W. (1998). Analysis of carbendazim, benomyl, thiophenate methyl and 2,4-D in fruits and vegetable after supercritical fluid extraction. 9th. international congress pesticide chem. *The Food Environmental Challenge* **5**E-004.
2. Hayes, W.J. (1982). *Pesticides Studies in Man.* Williams and Wilkins, Baltimore (London).
3. Adhikari, A. and Grover, I.S. (1988). Genotoxic effects of some systemic pesticides In-Vivo chromosomal abbreviations in bone-marrow cells in rats. *Environ. Mol. Mutagen* **12,** 235-242.
4. El-Sherbeny, K.M. (1996). *Assessment of Genotoxicity of the Pesticides Dichlorophenoxy Acetic Acid (2,4-D) and Carbofuran in the Mouse.* Ph.D. thesis. Dept. of Zoology, Fac. of Science, Ain Shams, Univ. Egypt.
5. Amer, S.M. and Aly, F.A.E. (2001). Genotoxic effects of 2,4-dichlorophenoxy acetic acid (2,4-D) and its metabolites 2,4-dichlorophenol. *Mutation Research* **494**,1-12
6. Sulik, M., Pilat-Marcinkiewiez, B., Sulik, A., Barwijuk-Machala, M., Sulkowska, M., Baltaziak, M. and Klepacka, J. (1998). Fetotoxic effect of 2,4-dichlorophenoxy acetic acid (2,4-D) in rats. *Rocz. Akad. Med Bialymst.* **43**, 298-308.
7. Aiglabie, J. and Lioyd-Jones, G. (1995). A review of bacterial degradation of pesticides. *Aust. J. Soil Res.* **33**, 925-942
8. Parker, L.W. and Doxtader, K.G. (1982). Kinetics of microbial decomposition of 2,4-D in soils. *J. Environ. Qual.* **11**(4), 679-684
9. Robertson, B.K. and Alexander, M. (1994). Growth linked and cometablic biodegradation. *Pestic. Sci.* **41**, 311-318.
10. Scow, R.S., Simkins, S., and Alexander, M. (1986). Kinetics of mineralization of organic compound at low concentration in soil. *Applied and Environ. Microbio.* **51**,1028-1035
11. Lehr, S., Scheunert, I., and Beese, F. (1996). Mineralization of free and cell wall bound isoporturon in soils in relation to soil microbial parameters. *Soil Biol. Biochem.* **28**,1-8
12. L, annunziata, M.F. (1979). *Radiotracers in Agricultural Chemistry IAEA*, Chapter 4, pp.96. Academic Press, Vienna, Austria.
13. Estrella, M.R., Brusseau, M.L., Maier, R.S., Pepper, I.L., Wierenga, P.J., and Miller, R.M. (1993). Biodegradation, sorption, and transport of 2,4-dichlorophenoxy acetic acid in saturated and unsaturated soils. *Appl. Environ. Microbiol.* **59** (12), 4266-4273
14. Page, D.L. (1999). National Cancer Institute's 2,4-D studies. *Wheat Alife* **42**,20-23
15. Tu, C.M. (1995). Effect of five insecticides on microbial and enzymatic activities in sandy soil. *J. Environ. Sci. Health* **B30**, 289-306
16. Smith, A.E. and Aubin, A.J. (1991). Metabolism of (^{14}C)-2,4-Dichlorophenoxy acetic acid in Saskatchewan soil. *J. Agric. Food Chem.* **39**, 2019-2021
17. Zayed, S.M.A.D., Mostafa, I.Y., and El-Arab, A.E. (1994). Degradation and fata of ^{14}C-DDT and ^{14}C-DDE in Egyptian soil. *J.Environ.Sci.Health* **B29**, 47-56
18. Hussain, A., Timrazi, S.H., Maqbool, U., Asi, M., and Chaughtai, C.A. (1994). Studies of the effects of temperatures and solar radition on volatlization, mineralizationand binding of ^{14}C-DDT in soil under laboratory conditions *J. Environ. Sci. Health* **B29**, 141-151
19. Sethunathan, N., Rao, V.R., Adhya, T.K., and Raghu, K. (1983). Microbiology of rice soils, CRC. *Critical Rev. Microbio.***10**, 125-172.
20. Straube, G. (1991). Microbial transformation of hexachlorocyclohexane. *Zentralbl. Mikrobiol.***146**, 327-338.
21. Sahu, S.K., Patnaik, K.K., and Sethunathan, N. (1990). Degradation of α-, β- and γ-isomers of hexachlorocyclohexane by Rhizosphere soil from sugarcane. Proc. *Indian Acad .Sci. Plant* **100**, 165-172.
22. Sahu, S.K., Patnaik, K.K., Bhuyan, S., Sreedharan, B., Kurihara, N., Adhya, T.K., and Sethunathan, N. (1995). Mineralization of α, γ and β-isomers of hexachlorocyclohexane by soil bacterium under aerobic conditions. *J. Agric. Food Chem.* **43**, 833-837.

(*Received March* 16, 2002 *Accepted Augusl* 5, 2002)