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Studies on Degradation of ^{14}C-Nitrofen in Soils Under Moist and Flooded Conditions Using a Continuous Flow System in the Laboratory

S. P. Kale, N. B. K. Murthy, K. Raghu

Nuclear Agriculture Division, Bhabha Atomic Research Centre, Trombay,
Mumbai 400 085, India

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The pesticides applied to the soil interact with natural biological systems. The knowledge of the fate of pesticides in soil which is an important part of our environment, is necessary both for agricultural practices and environmental safety. Numerous experimental approaches have been suggested in the literature to study the fate of pesticides in soil and these are excellently reviewed by Guth (1981). We have used a continuous flow system resembling the one developed by Parr and Smith (1969) to study the fate of nitrofen (2,4-dichloro-4’-nitro diphenyl ether) in soil. This herbicide was used in rice cultivation till recently. Since a major part of rice crop is grown under flooded conditions in India, we have incorporated the studies under flooded conditions as well.

MATERIALS AND METHODS

Soil used in these experiments had the following characteristics: Vertisol, pH 7.2, organic carbon 1.26%, nitrogen 0.455%, texture black clay. The metabolism of ^{14}C -nitrofen in moist and flooded soils was studied using a manifold assembly system permitting ^{14}C -mass balance. The Vertisol soil (50g on oven dry weight basis) was maintained under either moist or flooded conditions in 250ml Erlenmeyer flasks. Air was pumped at 5 mL/min into these flasks with the help of a fish pump through a series of three scrubbing flasks. Of these three flasks, the first two contained 10N KOH for the removal of carbon dioxide and the third one contained water for maintaining a saturated humid atmosphere. $^{14}CO_2$ was trapped in 1N NaOH and volatile organic compounds were absorbed in 2-ethoxyethanol (Guth 1981). Polyurethane filters were used for trapping any organic volatile compounds that might have escaped from the 2-ethoxyethanol traps (Kearney and Konston 1976). The ^{14}C-labelled nitrofen (sp activity 0,5254MBq/mg, Rohm and Hass Co.) was mixed with the analytical grade nitrofen (99.9% pure, Indofil Ltd. Bombay) so as to give 40.7KBq/50g in each flask and applied at the field application rate of 2.5ppm in acetone. The soil in control flasks received only equivalent amounts of acetone. Duplicate flasks were used for moist and flooded soil studies. The formation of volatile organic compounds and $^{14}CO_2$ evolution were monitored over a period of 60 days. The ^{14}C- activity in NaOH and ethoxyethanol traps was

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Correspondence to: S. P. Kale

determined by counting 0.2ml aliquots from each in liquid scintillation counter (Packard Tricarb 3255). The samples were counted in cocktail D (naphthalene l00g, PPO 4g, dioxane 900ml). For counting the aliquots from NaOH traps , 0.2% Cab-O-Sil thixotrophic gel powder (Packard Instruments Co., Inc., La Grange, Illinois, USA) was incorporated into the cocktail D (Sissons 1976). The polyurethane foams were extracted with hexane and aliquots were counted for determining the ^{14}C contents.

The soils were extracted at the end of two months incubation period. Before extraction, the excess water from flooded flasks was removed and its radioactive contents were determined by counting 0.5ml aliquots. The soils were extracted twice with 150ml acetone by shaking for 1 hr on a rotary shaker at 100rpm. The extracts were filtered through Whatman filter paper No. 42 using a Buchner funnel. The filtered soils were then subjected to two more extractions with 100ml methanol followed by filtration. Extractable radioactivity was determined by counting of the aliquots. The acetone extracts were partitioned with 25ml petroleum ether using 2% Na_2SO_4 solution and the aliquots were counted.

The petroleum ether extracts were analysed on aluminum oxide TLC plates (0.25mm thickness) using hexane: dichloromethane (1:1) as the developing solvent system alongwith the standards. One set of TLC plates was subjected to autoradiography using INDU X-ray films (Hindustan Photo Films, Ootacamund, Tamilnadu, India). The areas on the TLC plates corresponding to spots on X-ray films were marked. The silica gel powder under these areas was scrapped out and counted in liquid scintillation counter to determine the radioactivity.

Non-extractable or bound residues were determined by combustion of 50mg of solvent extracted soil in a biological material oxidizer (R.J. Harvey Instruments, NJ., USA). The $^{14}CO_2$ evolved was trapped in Oxysolve-C cocktail (Zinsser Analytic UK Ltd.) ad quantitated by liquid scintillation counting. Quench and background corrections were made wherever necessary.

RESULTS AND DISCUSSION

The evolution of $^{14}CO_2$ and other organic volatiles from nitrofen in soil was followed at periodic intervals over a period of sixty days. There was no significant mineralization of nitrofen. The ethoxyethanol traps did not register any detectable ^{14}C -activity indicating that there were no organic volatile products released from nitrofen during the two months incubation period. Similarly, the polyurethane foam plugs did not show any radioactivity indicating that there was no loss of nitrofen due to volatilization. Yukimoto et al (1979) have studied the evaporation of several herbicides from aqueous solutions and found that the rate of volatilization of nitrofen was very low as compared to many other herbicides.

A good ^{14}C-mass balance was obtained for the labelled nitrofen under moist conditions (Table 1). The extractable activity in moist soil accounted for 73.5% while the bound activity for 28.8% of the total applied ^{14}C-activity. In flooded soil

87.60% of the applied activity could be recovered of which 30.80% was in the form of extractable residues. The bound activity in flooded soil was 56.8%.

Table 1. ^{14}C -mass balance of ^{14}C nitrofen in soil using a continuous flow system.

	Moist soil	Flooded soil
	(% of the applied activity)	
Extractable residues	73.50 ±1.69	30.80 ±0.10
Bound residues	28.80 ±1.67	56.80 ±2.58
Total residues	102.30	87.60

These results indicate extensive degradation of nitrofen in flooded soil as compared to moist soil. Significant amount of bound residues was formed in both the soils under different moisture conditions; flooded soil harboring more of these residues. The ^{14}C -mass balance values in flooded soil indicate that ^{14}C -residues have contributed to the formation of bound residues. It has been amply demonstrated that the persistence of pesticides is lowered considerably under flooded conditions (Raghu and MacRae 1966; Sethunathan 1973). That the recalcitrant organochlorine pesticides like HCH and DDT could be degraded effectively is equally true for another chlorinated compound nitrofen. Since the nature of the bound residues is not known it is not clear whether the bound residues are formed as a result of direct binding of the parent compound or initially by formation of metabolites leading to then bound residue formation.

Table 2. TLC and autoradiography of ^{14}C nitrofen in a continuous flow system.

Soil	spot	R_f value	% of applied ^{14}C -activity after 2 months
Moist	Origin	0.0	--
	Nitrofen	0.76	64.76
Flooded	Origin	0.0	9.72
	spot 1	0.08	3.27
	spot 2	0.18	1.67
	spot 3	0.28	1.22
	Amino-nitrofen	0.40	1.95
	spot 4	0.54	1.06
	Nitrofen	0.76	7.40

Autoradiography of the extractable residues showed that in moist soil 64.76% of the ^{14}C-residues was in the form of parent compound as nitrofen. However, under flooded conditions, nitrofen underwent extensive degradation and ^{14}C -residues were seen in different fractions (Table 2). A small amount of amino-nitrofen was formed and also there were four unidentified metabolites of nitrofen under flooded conditions which could not be specified because of want of standards. Lee et al (1980) have identified the formation of amino-nitrofen (4-chloro-4'-amino diphenyl ether) and N-(4',4-chlorophenoxy) phenylacetamide in nitrofen treated Korean soils. There are reports that flooding enhances the degradation of nitrofen (Kale and Raghu 1994; Niki and Kuwatsuka 1976; Oh et al 198 1; Oyamada et al 1988; Quian et al 1982) and the present experiments confirm the same.

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