The Loss of Three Chloronitrobenzene Fungicides From the Soil

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In recent years increasing amounts of fungicides have been used to control soil pathogens. Apart from fungicides used as soil treatments and seed dressings, large quantities of foliar sprays miss their target and fall onto the soil. The fate of these compounds in the soil is of interest as fungicide residues may be toxic to subsequent crops and desirable soil organisms.

PCNB, pentachloronitrobenzene, is used as a seed dressing and as a soil treatment for several pathogens including Rhizoctonia spp and Sclerotinia spp (1a). TCNB, 1,2,4,5-tetrachloronitrobenzene, controls dry rot in stored potatoes and Botrytis spp in certain glasshouse crops (1b). Trichlorodinitrobenzene, an isomeric mixture of 80%

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1,2,4-trichloro-4,6-dinitrobenzene and 20% 1,2,3-trichloro-4, 6-dinitrobenzene, shows promise as a soil applied fungicide for the control of several damping off and a stem and root rotting fungi (1c).

Chacko et al (2) found that eight soil fungi and eight actinomycetes, grown in nutrient media, degraded PCNB. Streptomyces aureofaciens reduced the largest quantity of PCNB, producing pentachloroaniline. Degradation occurred only during the active growth phase of the organism and the fungicide was not utilized as a sole source of carbon.

The purpose of the present study was to follow the disappearance of three fungicides from the soil, considering both biological and non-biological pathways.

PROCEDURE

The soil used in all of the experiments was Yolo fine sandy loam described in Table 1.

TABLE 1

PROPERTIES OF YOLO FINE SANDY LOAM 0-8"

Sand	52%
Silt	25%
Clay	22%
Organic Matter	1.3%
Bulk density	1.5
pH	7.5
CEC	17.5

Five milligrams of technical grade fungicide in 2 ml of hexane were applied to 50 g. of air dry soil contained in a 125 ml Erlenmeyer flask. An equal volume of hexane without fungicide was added to the control soil. The flasks were left unplugged for 6 hours to allow the hexane to evaporate and then the soil was thoroughly mixed and water was added to bring the moisture content to approximately field capacity. The samples were incubated in controlled temperature cabinets at 25°C and a stream of air saturated with water flowed through each flask at approximately 2 ml per minute. In order to distinguish between microbiological and non-microbiological disappearance, one set of flasks for each treatment was sterilized at 120°C for 20 minutes before the addition of the fungicides. As an additional precaution against microbial activity, an aqueous solution of 0.1% mercuric chloride was added in place of water. The flasks were removed at intervals and the fungicide remaining in the soil was determined.

In some experiments the air leaving each flask was passed through a trap containing 30 ml of a mixture of 70% acetone and 30% hexane to determine the loss of fungicide in the air stream. The traps were made up to volume daily and changed weekly.

The influence of soil moisture level on the loss of PCNB was followed in sterilized soil containing 3%, 20% and 50% water by weight, levels approximately equivalent to air dry, field capacity and saturated soil. As the loss of PCNB

increased at the higher water levels, its persistence in water was also investigated. Two milliliters of acetone containing 3.5 mg of PCNB were added to 50 ml of distilled water contained in a 125 ml Erlenmeyer flask set up as described previously.

The fungicides were extracted from the soil by mechanically shaking with 50 ml of technical grade acetone for 20 minutes. The contents of the flasks were filtered through a Buchner funnel fitted with a No. 42 Whatman filter paper and the soil was rinsed with another 50 ml of acetone. The volume of the fungicide solution was reduced in a rotary evaporator to approximately 50 ml and transferred to a 125 ml separatory funnel containing 20 ml of Nanograde hexane. Twenty milliliters of distilled water acidified with one drop of 0.1 N HCl was added to enhance the transfer of the low water soluble fungicides into the hexane phase. The separatory funnel was shaken for 2 minutes and after the phases had separated, the lower acetone/aqueous phase was run into another separatory funnel and extracted with a further 20 ml of hexane. The hexane phases were placed in a 50 ml volumetric flask and made up to volume. In the PCNB loss from water experiment, the contents of the flask were transferred to a 125 ml separatory funnel, acidified with one drop of 0.1 N HCl and partitioned in hexane as described above. The acetone-hexane mixture from the traps was placed in a 125 ml separatory funnel containing 10 ml of Nanograde

hexane and 20 ml of acidified water and the fungicides were extracted as described above.

The fungicides were determined with a Varian Aerograph 204 gas chromatograph fitted with a tritium foil electron capture detector and set up as follows:

Column: PCNB and TCNB - 5 ft stainless steel, 1/8" O.D., packed with 5% SE30 on 60-80 mesh, acid washed, DMCS treated Chromosorb W.
Trichlorodinitrobenzene - 5 ft. stainless steel 1/8" O.D., packed with 4% XE60 on 70-80 mesh, acid washed, DMCS treated Chromosorb G.

Carrier gas: Water pumped nitrogen, flow rate, PCNB and TCNB - 55 ml/min, trichlorodinitrobenzene - 70 ml/min.

Temperature: PCNB and TCNB - inlet 225°C, column 175°C, detector 200°C, trichlorodinitrobenzene, inlet 210°C, column 160°C, detector 200°C.

Elution times: PCNB 3.0
TCNB 1.5 min,
1,2,4-trichloro-2,5-dinitrobenzene isomer 5 mins.
1,2,3-trichloro-4,6-dinitrobenzene isomer 6 mins.

All treatments were replicated in triplicate.

During the determination of the fungicides by electron capture chromatography, several small peaks were observed in addition to the starting material, especially in samples that

had been incubated for long periods. The three replicates of the 10 to 12 month samples of PCNB and TCNB and the 4 to 6 month samples of trichlorodinitrobenzene were each bulked and reduced in volume to 10 ml in a rotary vacuum evaporator, and to 1 ml in a stream of nitrogen. One hundred microliter aliquots of the concentrated sample were determined on a Varian Aerograph 202 gas chromatograph fitted with a thermal conductivity detector set up as follows:

Columns: 5 ft stainless steel 1/4" O.D., packed with 4% Dow 11 on 60/80 mesh acid washed Chromosorb G.

Carrier gas: Helium, flow rate 50 ml/min.

Temperature: Inlet 150°C, column programmed 100°C - 250°C at 4°C per minute.

Detector: 275°C

The compounds corresponding to the peaks on the chart were each collected in capillary tubes from the outlet of the detector. The compounds were dissolved in a small volume of benzene and chlorine determinations were made by injecting 10 ul samples into a Dohrman Microcoulometer set up as follows:

Column: 6 ft. glass 1/4" O.D. packed with 5% XE60 on Chromosorb W.

Carrier gas: Helium, 100 ml/min.

Temperature: Inlet 240°C, column 200°C furnace 825°C.

RESULTS AND DISCUSSION

The influence of water content on the loss of PCNB from

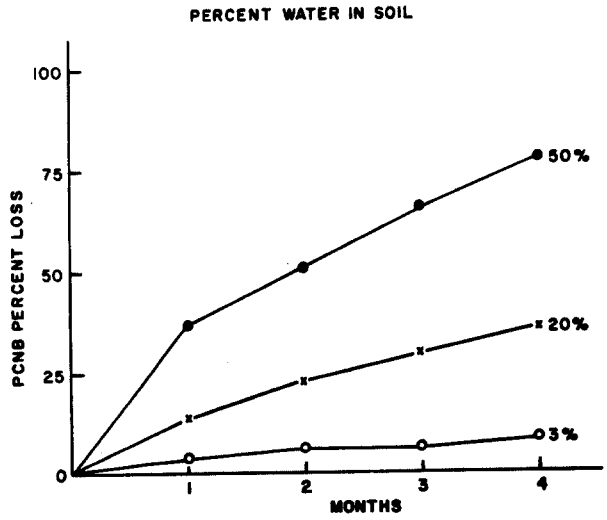


Figure 1

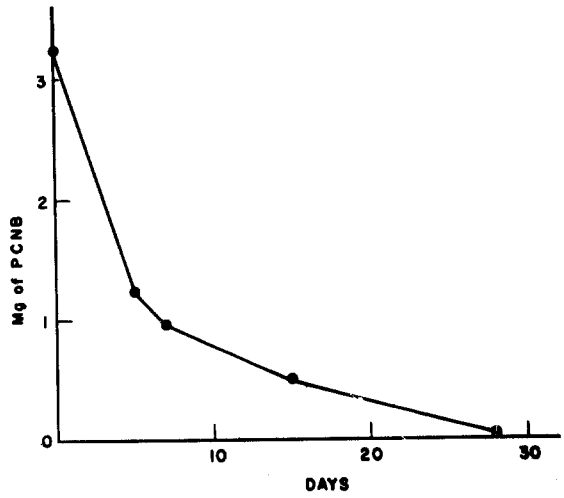


Figure 2

sterilized soil at 25°C is shown in figure 1. Water is a highly polar molecule and can compete effectively with pesticides for adsorption sites. It is probable that in the air dry soil, much of the PCNB is adsorbed and not readily available for removal, while in the field capacity and saturated soils much of the PCNB is unadsorbed.

A similar situation is found with a number of insecticides, including DDT and heptachlor, which are strongly adsorbed by dry fine sandy loam and become increasingly toxic to insects with increasing moisture content (3). Reduced adsorption at the 20% and 50% water levels increases the mobility of PCNB in the soil and facilitates loss by processes such as volatilization.

The rate of loss of PCNB, however, greatly exceeds that accountable for by volatilization as it has a vapor pressure of 11.3×10^{-5} at 25°C (1a). A possible explanation is that PCNB behaves like DDT in the presence of water. Acree et al (4) found that DDT added to water was lost at a rate several times faster than that anticipated by the Rasson-Schultz equation. These workers attributed their results to the great affinity of DDT for the air water interface which facilitates a high co-distillation rate. In the experiments reported here, water saturated air passed over the soil and there was a negligable loss of water from the flasks, so codistillation is not a suitable term to describe the loss of PCNB. However, PCNB

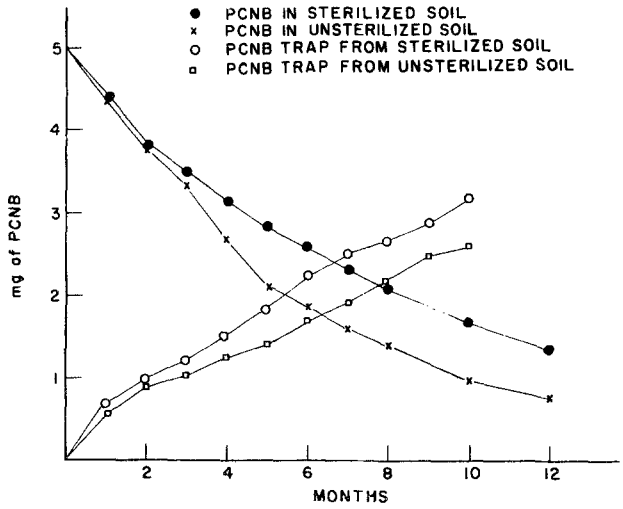


Figure 3

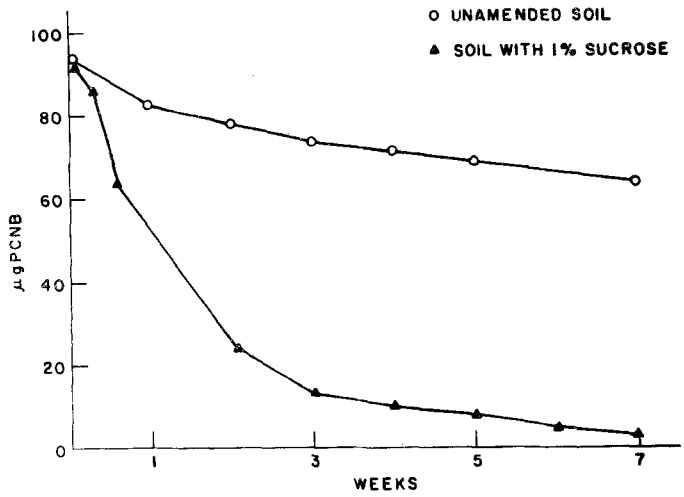


Figure 4

has an affinity for the air water interface and is lost rapidly from distilled water (figure 2) so it would appear that PCNB is removed with the stream of moist air.

The loss of PCNB from sterilized and unsterilized soil is shown in figure 3. After 10 months 80% of the applied PCNB was lost from the soil. Removal in the air stream accounted for 62% of the PCNB and the remaining 18% can be attributed to microbial and chemical degradation. The recoveries from the traps indicate that undegraded PCNB is the major component removed with the air stream. The loss of PCNB from unsterilized soil, between 3 and 5 months, exceeded that from sterilized soil. This may be attributed to microbial action as addition of 1% sucrose increased the breakdown of PCNB (figure 4). As organic matter stimulates microbial growth and increases the degradation of the fungicides, a possible field treatment for excessive quantities of fungicide would be to incorporate plant residues in the soil.

The loss of TCNB from the soil is much more rapid than that of PCNB (figure 5), over 50% disappearing within 2 months and all but 2% lost in 10 months. This difference in rate of loss is due mainly to physical processes as the rate of biological degradation is about the same as for PCNB. TCNB is four times as volatile as PCNB and this probably accounts for the increased rate of loss. Like PCNB, TCNB is insoluble in water and tends to form an oily film at the air-water

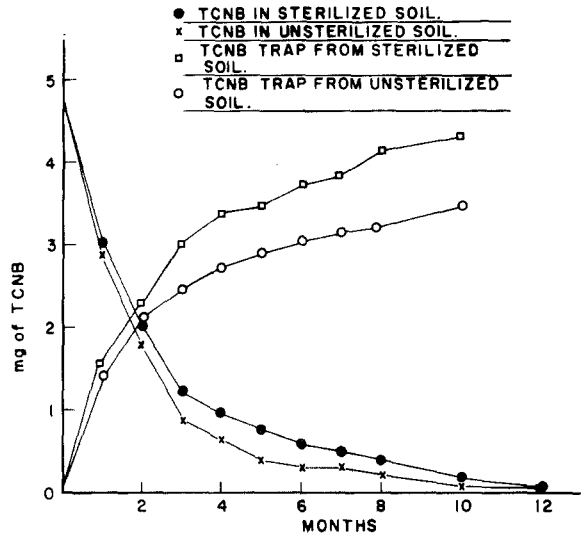


Figure 5

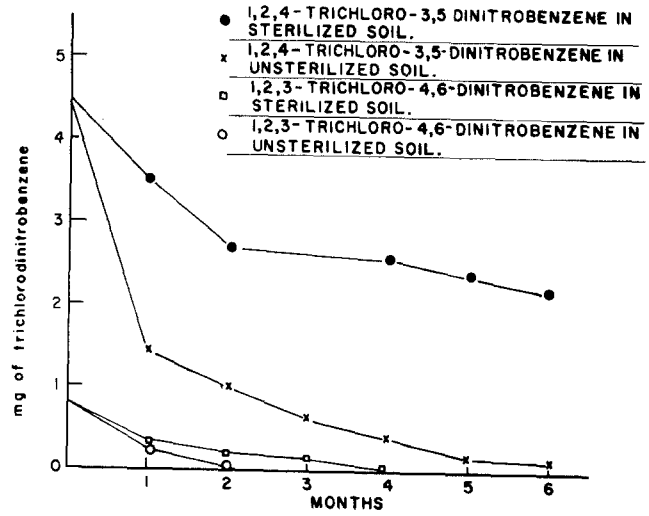


Figure 6

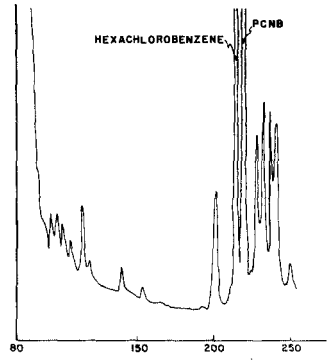
interface, which optimizes the chance of loss in the air stream.

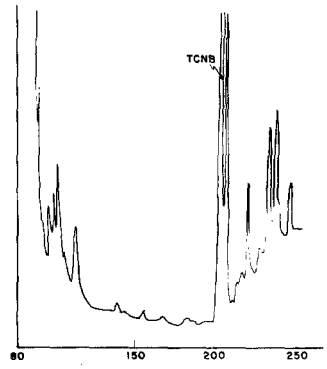
Degradation by microbial and chemical processes in the soil was most significant with the isomers of trichlorodinitrobenzene (figure 6). After 6 months 98% of the fungicide was lost from the soil and microbiological plus chemical processes accounted for over 40% of this loss. That the isomers of trichlorodinitrobenzene were degradated more rapidly than either PCNB or TCNB may be due to the lower number of chlorines on the benzene ring.

This is in general agreement with the work of MacRae and Alexander (6) who concluded that the number of chlorines on the aromatic ring determines the susceptibility of the benzoates to microbial degradation.

Figure 7 shows the gas chromatograms of the three fungicides and their soil microbiological and chemical degradation products. The microcoulometric determinations revealed that the compounds producing the peaks contain chlorine, which indicates that they are degradation products of the starting materials. The three fungicides bear a close resemblance structurally to one another and the breakdown products of each compound have closely corresponding retention times.

Chacko et al (2) found Streptomyces aureofaciens grown in nutrient media reduced PCNB to pentachloroanaline. In the soil, under the conditions of this experiment, several peaks containing chlorine have been found for each fungicide. More


HEXACHLOROBENZENE
PCNB
80
150
200
250


TCNB
80
150
200
250

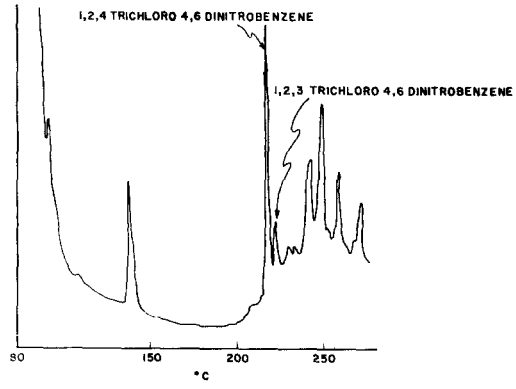

1,2,4 TRICHLORO 4,6 DINITROBENZENE
1,2,3 TRICHLORO 4,6 DINITROBENZENE
80
150
200
250
°C

Figure 7

degradation products would be expected in the soil as the starting material is exposed to a large number of organisms and chemical processes and the initial breakdown products may be susceptible to further degradation.

Fungicides are widely used in intensively cropped areas where irrigation is employed, e.g. in the San Joaquin Valley 400,000 lbs of PCNB were applied to cotton in 1963 (6). The results in this paper indicate that under these conditions considerable quantities of fungicide would be lost to the atmosphere. The loss of chloronitrobenzene fungicides from the soil under field conditions and their fate in the atmosphere deserves further study as these chemicals appear to be potential environmental contaminants.

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