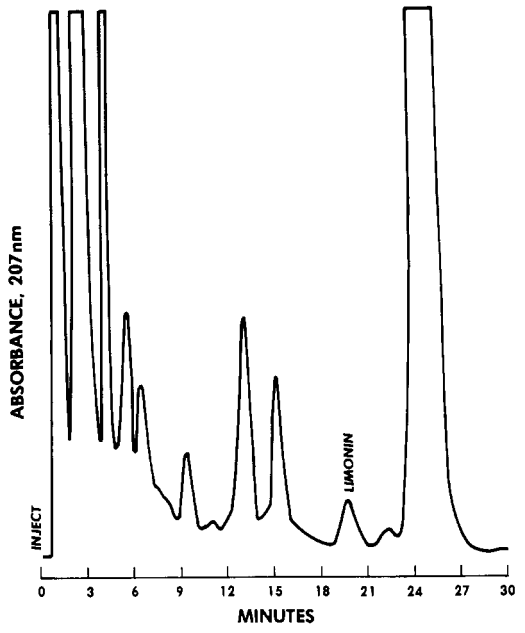

Figure 3. High-pressure liquid chromatogram of limonin in the chloroform–acetonitrile extract from single-strength grapefruit juice.

debittered to 285 μg/mL naringin at 45 °C at a rate of 130 mL min^{-1} (M^2 of HF surface area)$^{-1}$.

Limonin Content of Juice. The limonin contribution to the bitterness in this grapefruit juice was checked by comparing the limonin concentrations of samples of grapefruit juice taken before and after HF naringinase reactor debittering.

Figure 3 shows a typical chromatograph of the chloroform–acetonitrile extract without added limonin. No corrections for unresolved interfering limonoids were made; therefore, the results should be maximum values. The plot of detector response vs. amount of limonin added produced a straight line which was extrapolated back to zero detector response; the limonin content of both untreated and treated juice was 4 μg/mL. This level of limonin was not considered high enough to be a significant contributor to grapefruit juice bitterness. In addition, the results show that limonin levels were not changed by the process procedure.

Sensory Evaluation. Grapefruit juice tasting panels may be divided into three subgroups: A, those who liked grapefruit juice no matter how bitter; B, those whose ratings of taste increased as the bitterness was reduced; C, those who disliked grapefruit juice.

Approximately 50% of the panel of 42 persons were in group B (there were none in group C). This group of panelists assigned an average score of 4.8 ("neither like nor dislike") to the untreated juice containing 885 μg/mL naringin and an average score of 7.3 ("like moderately") to juice partially debittered with the HF naringinase reactor to a level of 275 μg/mL naringin. This preference for debittered juice was highly significant, with a confidence of $P < 0.01$. In addition, those panelists who like bitter juice, group A, did not dislike debittered juice and rated the untreated juice at 6.1 and the HF naringinase treated juice at 6.7.

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Microbiological Release of Unextracted (Bound) Residues from an Organic Soil Treated with Prometryn

Soil-bound ^{14}C-labeled residues were released by microbes from an organic soil treated with ^{14}C-ring-labeled prometryn [2-(methylthio)-4,6-bis(isopropylamino)-*s*-triazine]. The compounds that were extractable from the soil after incubation (27% of the total ^{14}C) were identified as prometryn and small amounts of hydroxypropazine [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] and deisopropylprometryn [2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine].

In recent years there has been a growing concern about the release of bound pesticide residues from soil. A number of studies have demonstrated the potential availability of the bound residues to plants (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Fuhr and Mittelstaedt, 1980; Khan, 1980) and earthworms (Fuhremann and Lichtenstein, 1978). Thus, soil-bound pesticide residues are not excluded from environmental interactions (Fuhremann and Lichtenstein, 1978).

Previously we reported that a considerable portion of the bound ^{14}C-labeled residues in an incubated soil treated with ^{14}C-ring-labeled prometryn [2-(methylthio)-4,6-bis-

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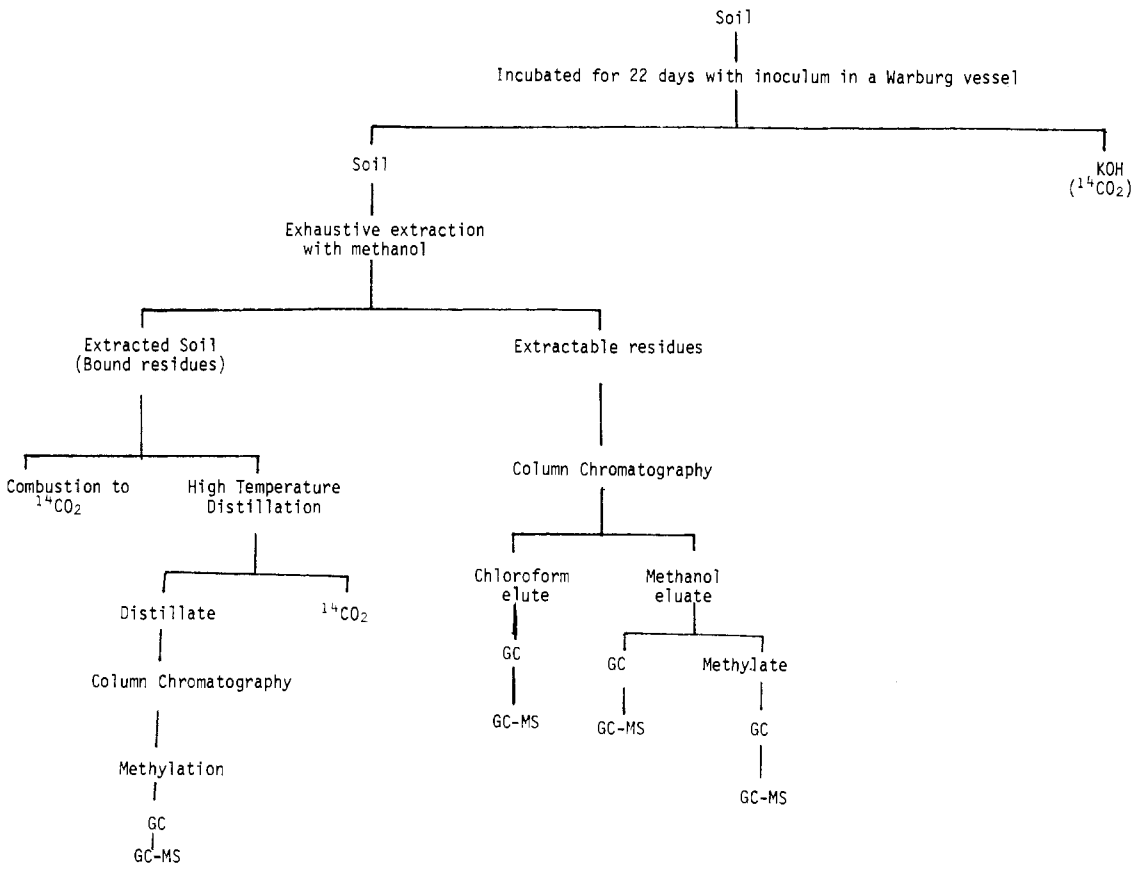

Figure 1. Schematic diagram for the analysis of extractable and bound residues in soil incubated with inoculum.

(isopropylamino)-*s*-triazine] was present in the form of the parent compound, whereas the remainder constituted some unidentifiable products (Khan and Hamilton, 1980). It became of interest to determine whether these bound residues (parent compound and/or the metabolites) were susceptible to biodegradation by soil microbes. Thus, the purpose of this paper is to describe our studies to determine if and to what extent bound ^{14}C-labeled residues are released and degraded to other products by soil microorganisms.

EXPERIMENTAL SECTION

Soil. Bound soil residues were produced as described in an earlier study (Khan and Hamilton, 1980). Briefly, the ^{14}C-ring-labeled prometryn treated moist soil samples (0.047 μCi/g) were incubated for 1 year at ~23 °C in the dark and exhaustively extracted with solvents, and the extracts were dried and pooled together. The soil was again moistened with water, incubated, and extracted with methanol (Khan and Hamilton, 1980). Residual methanol in the extracted soil was allowed to evaporate by air-drying the sample. The soil was then exhaustively extracted with water and again air-dried. The total bound ^{14}C-labeled residues were determined by combustion of the air-dried soil to $^{14}CO_2$.

Warburg Experiment. A Warburg apparatus was used to evaluate the ability of soil microbes to degrade or release bound residues. Ten grams (oven-dried basis) of air-dried soil containing ^{14}C-labeled bound residues was placed in a Warburg vessel. A liquid inoculum was added to bring the moisture to 70% of field capacity. The inoculum was prepared as follows: 500 mL of distilled water was added to 25 g of moist untreated and unextracted control soil, and the mixture was shaken for 30 min and allowed to stand overnight. The resultant supernatant solution was used as the inoculum. The control consisted of untreated soil exhaustively extracted with the same solvents as those used for producing bound residues. The control sample was also placed in a Warburg vessel and inoculum added. KOH (20%) was used in the center wells of the vessels to trap $^{14}CO_2$ and the temperature maintained at 20 °C. All systems were duplicated. Differences between duplicate Warburg runs were in all instances less than 1%, which agrees with observations of (Chase and Gray, 1957) that one of the outstanding features of the Warburg method for studying the decomposition of soil organic matter is its reproducibility. Oxygen uptake was measured daily for a period of 22 days. At the end of the experiment, KOH from the center well was removed and the radioactivity determined. The soil was removed from the Warburg vessel and processed as shown in Figure 1 to determine extractable and bound residues (Khan and Hamilton, 1980).

Determination of Radioactivity. Combustion of the dried soil was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The radioactivity of aliquots of extracts or KOH was determined by liquid scintillation counting (Khan and Hamilton, 1980).

Gas Chromatography (GC). The capillary column gas chromatograph and its operating conditions were similar to those described earlier (Khan, 1980). The identity of the compounds was confirmed by comparing the retention times with those of authentic samples, cochromatography, and finally gas chromatography–mass spectrometry (GC–MS).

RESULTS AND DISCUSSION

Following a soil incubation period of 1 year, the soil contained 57.4% bound radioactivity of the total applied ^{14}C. Analysis of soil samples by the high temperature distillation (HTD) technique (Khan and Hamilton, 1980) revealed that more than half (54%) of the total bound

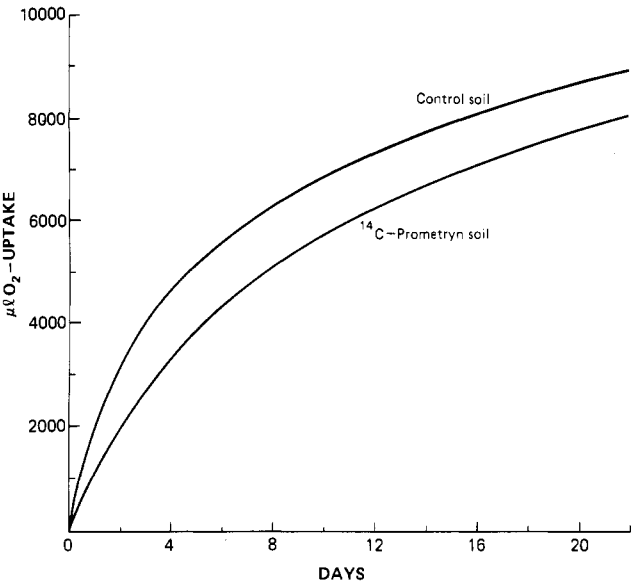

Figure 2. O_2 uptake of soil incubated with inoculum for 22 days.

residue in soil was in the form of prometryn or its dealkylated metabolites. The remainder constituted hydroxypropazine [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] (8%), and unidentifiable methanol-soluble material (18%), and 20% was thermally decomposed to $^{14}CO_2$ during distillation.

The respiration rate of the inoculated soil containing ^{14}C-labeled bound residues was lower than that of the control (Figure 2). It appeared that the presence of bound residues had a inhibitory effect on the respiratory activity of microbes in the soil. The loss of radiocarbon due to evolution of $^{14}CO_2$ during the incubation period of 22 days was negligible from the soil containing ^{14}C-labeled bound residues. This was indicated by the presence of only trace amounts of radioactivity ($\simeq$0.01% of the bound) in the KOH used in the center of Warburg vessel.

The extractable and unextractable (bound) radioactivity in the soil after incubation with an inoculum for 22 days amounted to 27.0% and 71.6%, respectively, of the total ^{14}C-labeled bound residues. This indicates that microbes released part of the ^{14}C-labeled bound residues from soil. In a preliminary experiment, an aliquot of the soil containing bound residues was mixed with sterilized distilled water (field capacity), allowed to stand at $\simeq$20 °C for $\sim$3 weeks, and then extracted with methanol. Analysis of the extracted material revealed negligible amounts of radioactivity (<1% of the total), indicating that residues were still bound to the soil when no inoculum was added.

GC examination of the extractable residues from the soil incubated with inoculum for 22 days (Figure 1) indicated the presence of 14% prometryn, 9% hydroxypropazine, and a small amount (2%) of the partially N-dealkylated compound [2-(methylthio)-4-amino-6-(isopropylamino)-*s*-triazine] of the initially bound prometryn residues. It appears that the microbes initially released the bound prometryn by breaking the bonds between the herbicide molecule and the soil matrix. This was followed by the degradation of prometryn in the incubated soil to products by hydrolysis and dealkylation. Liberation of substantial amounts of $^{14}CO_2$ from ^{14}C-ring-labeled prometryn was not observed at the end of the incubation period. However, liberation of $^{14}CO_2$ from the ring-labeled *s*-triazine treated soils has been observed in other studies (Esser et al., 1975).

The HTD technique (Khan and Hamilton, 1980) was used to release the unextractable (bound) ^{14}C-labeled residues from the extracted soil after the incubation experiment. The released ^{14}C was collected in suitable solvents, purified, and analyzed by GC and GC–MS. About 49% of the total initially bound radioactivity was present in the HTD distillates while 19% was found in the hyamine hydroxide trap (Khan and Hamilton, 1980), indicating thermal decomposition to $^{14}CO_2$ during distillation. Analyses of the HTD distillate indicated the presence of 31% prometryn, 7% hydroxypropazine, and small amounts of mono-N-dealkylated prometryn (<4%) of the initially bound prometryn residues. It is possible that some of the metabolites formed during the incubation period may have become a part of the bound portion of the residue in the soil.

The earlier findings that pesticide-bound residues can be released from soil and translocated into plants (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Fuhr and Mittelstaedt, 1980; Khan, 1980) and our observation that soil microbes can also potentially release and degrade bound residues suggest that a hazard of plant assimilation of contaminants may exist from a previously unseen source of residues. Further work is required to determine whether such a possible hazard is in fact a reality under natural field conditions.

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