Effects of Soil Microorganisms on the Release of Bound ^{14}C Residues from Soils Previously Treated with [^{14}C]Parathion

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Experiments were conducted to investigate the importance and significance of soil microorganisms relative to the release of unextractable, bound ^{14}C residues from soils, previously treated with [^{14}C]parathion. Results obtained indicate that soil-bound ^{14}C residues can be released, metabolized, and picked up by oat plants grown in these soils. It became evident that with an increase in soil microorganism populations a concomitant increase in the release of soil-bound ^{14}C residues had occurred. Addition of biologically active substances (glucose, parathion, chloramphenicol, and captafol), known to increase or decrease microbiological activities, to soil-bound ^{14}C residues also resulted in an increase or decrease of the release of soil-bound radiocarbon and in particular in the evolution and release of $^{14}CO_2$. Addition of cow manure to soil-bound ^{14}C residues increased soil microorganism populations and resulted in a decrease of initially soil-bound ^{14}C residues, a decrease in the amounts of total ^{14}C soil residues remaining, a dramatic increase in the evolution of $^{14}CO_2$ and, in comparison to controls, quantitatively different pickup patterns of ^{14}C residues by oats which were grown in these soils. It is obvious, therefore, that soil-bound residues should not be regarded as unavailable for further degradation.

Problems pertaining to unextractable, bound pesticide residues in soils have been investigated in the late 1960's by a number of authors (Getzin, 1967; Probst et al., 1967; Bartha, 1971) and a special symposium dealing with "Bound and Conjugated Pesticide Residues" was held in June of 1975 by the Pesticide Division of the American Chemical Society (Kaufman et al., 1976). Information pertaining to soil-bound residues of parathion, methyl parathion, fonofos, and *p,p'*-DDT was reported later (Katan et al., 1976; Lichtenstein et al., 1977; Katan and Lichtenstein, 1977). Since then concern has also focused on the possible release and further metabolism of these residues. Hsu and Bartha (1974) found that small amounts of soil-bound [^{14}C]3,4-dichloroaniline residues were mineralized to $^{14}CO_2$ upon incubation of soil containing bound residues with fresh soil. This mineralization was decreased with sterilization or anerobic incubation. Roberts and Standen (1981) reported that between 25 and 40% of soil-bound [^{14}C]cypermethrin residues were mineralized to $^{14}CO_2$ after a 26 week incubation of soil containing bound residues to which fresh soil had been added. Khan and Ivarson (1981) studied the potential release of soil-bound [^{14}C]prometryn residues. They found no release of bound residues from a soil which contained bound [^{14}C]-prometryn residues but had been sterilized. However, after incubating soil containing bound residues to which a fresh soil inoculum had been added, 27% of ^{14}C which had been initially unextractable could now be extracted. Examination of the extractable material indicated the presence of [^{14}C]prometryn and several metabolites. In a further study, Khan and Ivarson (1982) found that various physiological groups of soil microorganisms differed in their ability to release soil-bound prometryn residues. You and Bartha (1982) evaluated the "Bleidner distillation process for recovery of the herbicide residue 3,4-dichloroaniline (DCA) from its humic complexes".

Several researchers have reported on the ability of plants grown in soils containing bound residues to take up a portion of these residues. Süss and Grampp (1973) reported that mustard plants took up small amounts of [^{14}C]monolinuron from soils which contained bound [^{14}C]monolinuron residues. Similiarly, Khan (1980) found that oat plants grown in soil containing bound [^{14}C]prometryn residues took up 0.53% of initially bound ^{14}C, some of it being present in oat shoots as [^{14}C]prometryn. Führ and Mittelstaedt (1980) reported that corn plants could take up ^{14}C residues from soil which contained bound [^{14}C]methabenzthiazuron, with a portion of ^{14}C residues in corn leaves comprised of [^{14}C]methabenzthiazuron. Plants have been found to take up bound residues from soil containing bound residues of [^{14}C]trifluralin (Helling and Krivonak, 1978), [^{3}H]trifluralin (Mostafa et al., 1982), [*methyl*-^{14}C]parathion (Fuhremann and Lichtenstein, 1978), [^{14}C]cypermethrin (Roberts and Standen, 1981), and [^{14}C]hydroxymonolinuron (Haque, et al., 1982). However, these latter studies have not characterized the ^{14}C material which was found to be present in these plants. The nature, fate, and analytical recovery of humus-bound 3,4-dichloroaniline (DCA) were explored by Bartha et al. (1983). They reported that due to mirobial turnover of humus, DCA bound to humus was liberated in intact form and gave rise to low-level crop contamination.

Earthworms which have been added to soil containing bound pesticide residues have been found to take up unidentified ^{14}C compounds into their tissues. This was found to be the case with soil-bound [*methyl*-^{14}C]parathion residues (Fuhremann and Lichtenstein, 1978) and with soil-bound [^{14}C]hydroxymonolinuron residues (Haque et al., 1982).

As a continuation of our own studies, experiments were conducted to investigate the importance and significance of soil microorganisms relative to the release of unextractable, bound ^{14}C residues from soils, which previously had been treated with [^{14}C]parathion.

MATERIALS AND METHODS

Chemicals. [*2,6-phenyl*-^{14}C]Parathion (*O,O*-diethyl *O*-(*p*-nitro[*2,6-phenyl*-^{14}C]phenyl) phosphorothionate) was purchased from Amersham/Searle, Arlington Heights, IL, while nonradioactive parathion and several of its potential metabolites were supplied by courtesy of Bayer Pflanzenschutz., Leverkusen, West Germany.

Fungicides. Captafol [*cis*-*N*-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide] was obtained by courtesy of Ortho Chevron Chemical Co, Richmond, CA, and the bactericide chloramphenicol [D(−)-*threo*-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide] was purchased from Sigma Chemical Co.,

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St. Louis, MO. Solvents used were redistilled acetone, benzene, dioxane, hexane, and methyl cellusolve, and analytical grade chloroform, ethanolamine, methanol, and toluene. All other reagents were of analytical grade.

Cow manure was obtained from the University of Wisconsin dairy barn and handled as described by Lichtenstein et al. (1982). Before use, dry cow manure was uniformly mixed with soil at the rate of 100 metric tons/ha, as described by Doyle et al. (1978).

A Plano silt loam soil (4.7% organic matter, 5% sand, 71% silt, and 24% clay with a pH of 6.0) was collected at the University of Wisconsin Experimental Farm near Madison and handled as described by Lichtenstein et al. (1982).

Production of Soil-Bound Residues. Soil treated with [^{14}C]parathion was incubated, then exhaustively extracted, and analyzed by combustion to $^{14}CO_2$ as described (Fuhremann and Lichtestein, 1978). The amounts of unextractable soil-bound radiocarbon determined are referred to as "initially bound" later in this manuscript.

Oats. Oat seeds (*Avena sativa*, Lodi cultivar) were germinated between moist paper towels for 48 h and planted in soil or soil mixtures also containing bound [^{14}C]parathion residues.

Extraction and Analyses. At the end of the various experiments, soils which initially contained only bound ^{14}C residues and oat leaves grown therein were extracted with a mixture of acetone–methanol–benzene (1:1:1) as described by Fuhremann and Lichtenstein (1978). Oat roots were washed with tap water, dried, and combusted to $^{14}CO_2$ for analyses by LSC as described by Fuhremann and Lichtenstein (1978). Whenever possible, soils and oat leaves were analyzed by LSC, thin-layer chromatography (TLC), autoradiography, and gas-liquid chromatography (GLC) as described by Fuhremann and Lichtenstein (1980). Results obtained were finally expressed as amounts of radiocarbon recovered in percent of the initially soil-bound radiocarbon.

Soil Microbial Counts. Populations of soil microorganisms were estimated by standard plate count techniques, utilizing M_{32} agar medium for aerobic bacteria (Ridge and Rovira, 1971) and rose bengal medium for fungi (Martin, 1950).

EXPERIMENTAL PROCEDURES

Release of Bound ^{14}C Residues from Extracted Soil Previously Treated with [^{14}C]Parathion. To study the potential release of soil-bound ^{14}C residues, 1000 g of soil were treated with [^{14}C]parathion at 10 ppm, incubated for 7 weeks, and extracted as described. Unextractable radiocarbon determined at that time amounted to 26.7% of the originally applied [^{14}C]parathion. Three additional extractions of a 100-g soil aliquot resulted in the removal of only 0.9% of the soil-bound radiocarbon determined earlier. One hundred and fifty grams of extracted soil containing 0.91 μCi of ^{14}C were then placed into each of 2 plastic bag-lined cartons (8.5 × 8.7 cm diameter). To restore soil texture and microbial populations (Hsu and Bartha, 1974; Roberts and Standen, 1981), 150 g of insecticide-free, fresh soil were added to each of the extracted soils. After thorough soil mixing, each of the 2 containers was placed in a closed system as described by Ferris and Lichtenstein (1980) and incubated for 4 additional weeks at 28 ± 1 °C and on a 16-h photoperiod. Air was continuously passed through each system and $^{14}CO_2$ released was measured periodically by determining the amounts of radiocarbon in the attached 0.1 N KOH traps. Verification of the presence of $^{14}CO_2$ in the KOH traps was performed as describedby Walter-Echols and Lichtenstein (1978). Water was added as necessary to replace the amount lost by evaporation. At the end of the 4-week incubation period soils in each of the 2 containers were again thoroughly mixed before extraction and analyses of 100-g aliquots.

Effects of Soil Microorganisms on the Release of Soil-Bound ^{14}C Residues. To study potential effects of soil microorganisms on the release and metabolism of soil bound ^{14}C residues, extracted soil containing only bound radiocarbon was either sterilized or inoculated with soil microorganisms. Production of $^{14}CO_2$ was then determined during a 2-week incubation period. To prepare bound ^{14}C residues, 400 g of soil were treated with [^{14}C]parathion at 10 ppm, incubated for 6 weeks at 27.3 ± 1.5 °C, and then extracted as previously described. Bound residues remaining amounted to 29.3% of [^{14}C]parathion applied 6 weeks earlier.

Fifty gram portions of extracted soil containing 0.33 μCi of bound ^{14}C residues were then placed into each of 4 glass jars (13 × 5 cm diameter) which had previously been sterilized by autoclaving. Extracted soils within 2 containers were sterilized by γ-irradiation as described by Katan et al. (1976) and their sterility was confirmed as described by the same authors. Moisture was maintained by periodically adding autoclaved water. Extracted soils in the remaining 2 containers were kept moist by adding periodically a fresh soil–water inoculum, thus adding soil microorganisms. This inoculum was prepared each time by shaking 10 g of fresh soil in 90 mL of water. All 4 soils were then incubated for 2 additional weeks at 28 ± 1 °C in a closed system as described by Ferris and Lichtenstein (1980) while air was passed periodically for 2 h through each of the 4 systems to collect $^{14}CO_2$ within the attached 0.1 N KOH traps. Cumulative amounts of $^{14}CO_2$ determined were finally expressed in percent of the amounts of bound ^{14}C residues determined 2 weeks earlier.

Effects of Biologically Active Substances on the Release of Soil-Bound ^{14}C Residues. To further investigate the importance of soil microorganisms relative to the release and metabolism of soil-bound ^{14}C residues, substances known to either increase or decrease microbiological activities were added to fresh soil and then mixed with extracted soil that contained bound ^{14}C residues. After a specific incubation time, analyses were performed as described. The biologically active substances used were glucose, shown to stimulate soil microorganism activity relative to insecticide degradation (Lichtenstein and Schulz, 1964), chloramphenicol, a bactericide inhibiting bacterial protein synthesis (Stecher, 1968), captafol, a potent inhibitor of soil fungi (Thomas et al. 1962), and parathion, found to stimulate growth and activity of parathion-degrading soil miroorganisms (Barik et al., 1979; Ferris and Lichtenstein, 1980).

To conduct these experiments, soils with bound ^{14}C residues were prepared by treating 1000 g of soil with [^{14}C]parathion at 9.88 ppm followed by a 28-day incubation period. After exhaustive extraction, this soil still contained 34.6% of the originally applied [^{14}C]parathion in the form of bound ^{14}C residues. In addition, 200-g portions of fresh soil were mixed with water solutions of either glucose (2000 ppm) or chloramphenicol (100 ppm) or with acetone solutions of captafol (100 ppm) or parathion (10 ppm). To obtain an effect of these chemicals on the soil microflora, these treated soils were incubated for 4 days at 28 ± 1 °C before they were mixed with extracted soil containing bound ^{14}C residues.

Finally, 50 g of extracted soil containing bound ^{14}C residues (0.57 μCi) were placed into each of 10 glass jars (12 cm × 5 cm diameter). Soils in 2 jars each were then

mixed with either 50 g of glucose-treated soil, 50 g of chloramphenicol-treated soil, 50 g of captafol-treated soil, 50 g of parathion-treated soil, or with 50 g untreated soils (controls). These soil mixtures were then incubated within closed systems as described by Ferris and Lichtenstein (1980) for 4 weeks in the dark and at 24 ± 1 °C. Air was continuously passed through each system with its attached 0.1 N KOH trap. The KOH was periodically changed and analyses were performed as described. After 2 weeks of incubation, soil surface applications of the biologically active chemicals were performed at one half of their original application rate, to enhance their effects on soil microorganisms. After 2 additional weeks all ten units were dismantled and each 100-g soil sample was extracted and analyzed as described.

Effects of Cow Manure on Populations of Soil Microorganisms and on the Release of Soil-Bound ^{14}C Residues and Their Translocation into Oat Plants. The importance of fertilizers and other agricultural chemicals on the fate of pesticides in soil has in recent years been investigated by a number of researchers. Doyle et al. (1978) reported that "altered rates of pesticide degradation were observed in sludge and/or manure amended soils for a number of structurally unrelated pesticides. ^{14}C product distribution varied with soil amendments". The pesticides investigated in Doyle's study were primarily herbicides. In experiments with [^{14}C]parathion-treated soil Lichtenstein et al. (1982) found that the addition of cow manure resulted in an increase of soil-bound ^{14}C residues and in an inhibition of the production and release of $^{14}CO_2$.

Since investigations described in this study dealt with the effects of cow manure on the *release* of soil-bound ^{14}C residues, potential effects of cow manure on populations of soil microorganisms were determined first. Thus, 600 g of insecticide-free soil were extracted as described and 150-g portions of this extracted soil were placed into each of 2 cardboard cartons (8.5 × 8.7 cm diameter). Soil in one carton was thoroughly mixed with 150 g of fresh soil (control) and in the other carton with 150 g of fresh soil to which 13.2 g of air-dried cow manure had been added. To determine bacterial and fungal populations immediately following soil mixing, 5-g aliquots were removed from each of the 2 soil mixtures and subjected in triplicate to standard plate count techniques as described. The remaining soils were then incubated for 6 days at 23 ± 2 °C, at which time bacterial and fungal counts were repeated as described.

In the next series of experiments, potential effects of cow manure on the release and plant uptake of soil-bound radiocarbon were studied as depicted in the attached flow sheet (Figure 1). Initially (day 0), 2000 g of soil were treated with [^{14}C]parathion at 10.7 ppm and incubated for 35 days in the dark at 25 ± 2 °C. After exhaustive extraction, soil-bound ^{14}C residues determined amounted to 15.5% of the radiocarbon applied 35 days earlier. One hundred and fifty grams of extracted soil, containing 0.85 μCi of bound radiocarbon, were then placed into each of 8 plastic-bag lined cartons (8.5 × 8.7 cm diameter). Each soil in 4 of these cartons was thoroughly mixed with 150 g of fresh soil (No. 1–4, day 35c, Figure 1), and soils in the 4 remaining cartons with 150 g of fresh soil and 13.2 g of dried cow manure (No. 5–8, day 35c, Figure 1). Twenty-five oat seedlings were then planted in each soil within the 8 cartons and incubated under Gro-Lux lamps for 4 weeks (day 35–63, Figure 1) on a 16-h photoperiod and at 24 ± 1 °C. As shown in Figure 1, two soil plant systems of each variable (control or addition of cow manure) were grown in open systems and 2 of each variable in closed systems

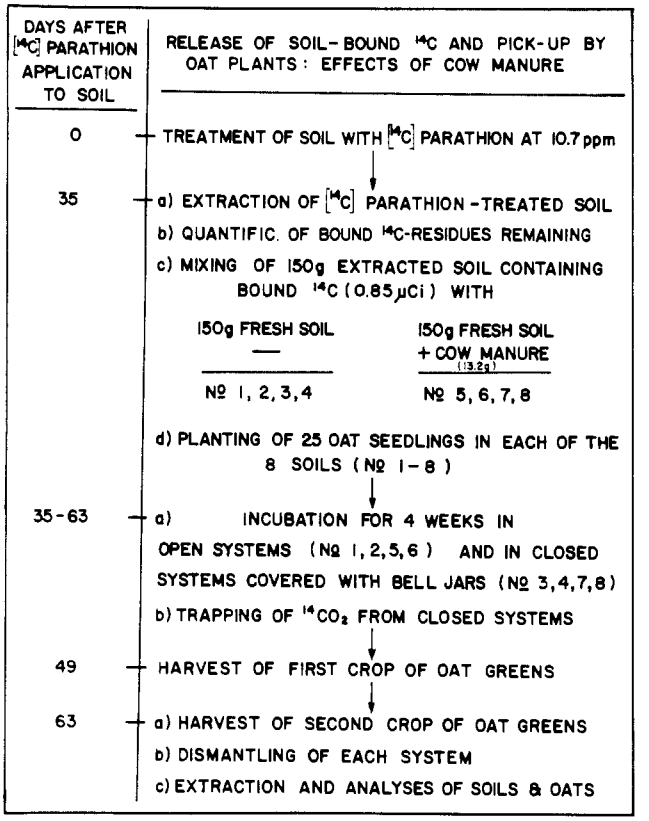

Figure 1. Flow sheet depicting experimental procedures.

Table I. Release of Bound ^{14}C Residues from Extracted Soil Previously Treated with [^{14}C]Parathion[a]

	^{14}C recovered in 1% of initially bound radiocarbon[b]
$^{14}CO_2$[c]	25.95 ± 0.11
soil	
benzene[d]-^{14}C	3.90 ± 0.05
benzene–PS[d]	3.26 ± 0.04
water[d]	0.56 ± 0.03
bound	72.48 ± 0.90
total	102.89 ± 1.10

[a] Soil was treated with [^{14}C]parathion at 10 ppm and incubated for 7 weeks, followed by exhaustive extraction. Bound residues remaining were 26.7% of applied, referred to as "initially bound". [b] In duplicate, 150 g of extracted soil (dry wt) containing bound [^{14}C]parathion residues (0.91 μCi) were mixed with 150 g (dry wt) fresh soil and incubated in closed systems for 4 additional weeks. [c] $^{14}CO_2$ trapped in 0.1 N KOH during the 4-week incubation period. [d] Benzene and water extraction phases of soil. PS = parathion, determined by TLC and GLC.

under bell jars as described by Anderegg and Lichtenstein (1981). To monitor $^{14}CO_2$ evolution, air was continuously passed through these closed systems with their attached 0.1 N KOH traps. To replace water lost through evaporation and transpiration, soils were watered on a daily basis. Since oats grew rather rapidly, oat leaves were harvested after a 2-week growing time and again 2 weeks later (days 49 and 63, respectivey, Figure 1). Both leaf crops were extracted and analyzed as described. On day 63, oat roots and soils were separated, extracted and analyzed as described.

RESULTS AND DISCUSSION

Release of Bound ^{14}C Residues from Extracted Soil Previously Treated with [^{14}C]Parathion. Soil treated with [^{14}C]parathion, contained after 7 weeks of incubation, 26.7% of the applied radiocarbon in the form of unextractable, bound residues. This extracted soil was then

Table II. Effects of Biologically Active Substances on the Release of Bound ^{14}C Residues from Extracted Soil Previously Treated with [^{14}C]Parathion[a]

	extracted soil containing bound ^{14}C plus fresh soil treated with[b]				
	none	glucose	chloramphenicol	captafol	parathion
	^{14}C recovered in percent of initially bound after 4 weeks incubation				
$^{14}CO_2$[c]	22.74 ± 0.15	27.84 ± 0.54[f]	14.91 ± 0.23[e]	20.73 ± 0.15[f]	25.06 ± 0.35[g]
$^{14}CO_2$ in % of control	100	122	65.6	91.2	110.2
soil					
benzene[d]-^{14}C	5.69 ± 0.11	5.36 ± 0.09[g]	5.31 ± 0.06[g]	5.54 ± 0.20	5.24 ± 0.05[g]
benzene–PS[d]	4.68 ± 0.31	4.73 ± 0.06	4.77 ± 0.07	4.95 ± 0.14	4.61 ± 0.03
water[d]	0.75 ± 0.01	0.33 ± 0.02[f]	1.01 ± 0.01[f]	0.87 ± 0.01[f]	0.55 ± 0.02[f]
bound	74.44 ± 0.26	70.09 ± 1.18[g]	79.51 ± 0.17[f]	74.41 ± 0.28	72.58 ± 0.15[g]
total soil	80.88 ± 0.36	75.78 ± 1.11[g]	85.83 ± 0.24[f]	80.82 ± 0.08	78.37 ± 0.11[g]
total	103.62 ± 0.51	103.61 ± 0.57	100.74 ± 0.01	101.57 ± 0.23	103.43 ± 0.24

[a] Soil was treated with [*ring*-^{14}C]parathion at 9.88 ppm and incubated for 4 weeks followed by exhaustive extraction. Bound ^{14}C residues remaining amounted to 34.6% of applied, referred to as "initially bound". [b] Fifty grams of soil (dry wt) which had been treated and incubated for 4 days with none (control), glucose (2000 ppm), chloramphenicol (100 ppm), captafol (100 ppm), or parathion (10 ppm) was mixed with 50 g (dry wt) of soil containing bound [^{14}C]parathion residues (0.57 μCi). Two weeks later, surface applications of the biologically active compounds were performed at $^1/_2$ of their original application rate. Results obtained are averages of duplicate tests. [c] $^{14}CO_2$ trapped in 0.1 N KOH over the 4-week incubation period. [d] Benzene and water extraction phases of soil. PS = parathion as determined by TLC and GLC. [e–g] Results are significantly different from controls (None) at the 0.1% (*e*), 1% (*f*), and 5% (*g*) level (student's *t* test).

mixed with fresh soil and incubated for an additional 4 weeks. Analytical results obtained at that time (Table I) showed that still 72.5% of the unextractable radiocarbon determined initially remained in the form of bound ^{14}C residues, while 26% of the previously unextractable radiocarbon had been released in the form of $^{14}CO_2$, and 4.5% had become extractable, most of it as organic-soluble radiocarbon. TLC, autoradiography, and GLC surprisingly indicated that most of this organic-soluble radiocarbon (3.3% of initially bound, Table I) was identified as parathion. Since previous data (Katan and Lichtenstein, 1977) had shown that the reduction of parathion to aminoparathion was a prerequisite for soil binding, it appears that bound aminoparathion was probably oxidized to parathion before it again became extractable. In addition to [^{14}C]parathion, the benzene extraction phase contained also small amounts of [^{14}C]paraoxon, [^{14}C]*p*-nitrophenol, [^{14}C]aminoparathion, and [^{14}C]aminoparaoxon. The release of $^{14}CO_2$ from soil-bound ^{14}C residues has also been reported by other authors (Khan and Ivarson, 1981; Hsu and Bartha, 1974; Roberts and Standen, 1981). Data, therefore, indicate that soil-bound residues should not be regarded as unavailable for further degradation.

Effects of Soil Microorganisms on the Release of Soil-Bound ^{14}C Residues. Extracted soil, previously treated with [^{14}C]parathion, was either sterilized or inoculated periodically with soil microorganisms as described. Cumulative amounts of $^{14}CO_2$ released during the 2-week incubation period are presented in Figure 2. It is obvious, that practically no $^{14}CO_2$ was released from sterilized soils, while the periodic addition of soil microorganisms to extracted, but not sterilized soil, resulted within 14 days in the release of $^{14}CO_2$, amounting to a total of 12.53 ± 0.28% of the initially bound radiocarbon. The curve depicting the cumulative evolution of $^{14}CO_2$ resembles in part a typical growth curve of microorganisms. As shown by Khan and Ivarson (1981) with [^{14}C]prometrin residues, our data also indicate that soil microorganisms played an important part in the release and metabolism of previously soil-bound ^{14}C residues.

Effects of Biologically Active Substances on the Release of Soil-Bound ^{14}C Residues. The addition of soil containing glucose, chloramphenicol, captafol, or parathion to previously extracted soil-containing bound ^{14}C residues did indeed increase or decrease the release of formerly soil-bound ^{14} residues. As shown in Table II ("none"), 23% of the initially bound radiocarbon had been released from control soils as $^{14}CO_2$. However, significantly

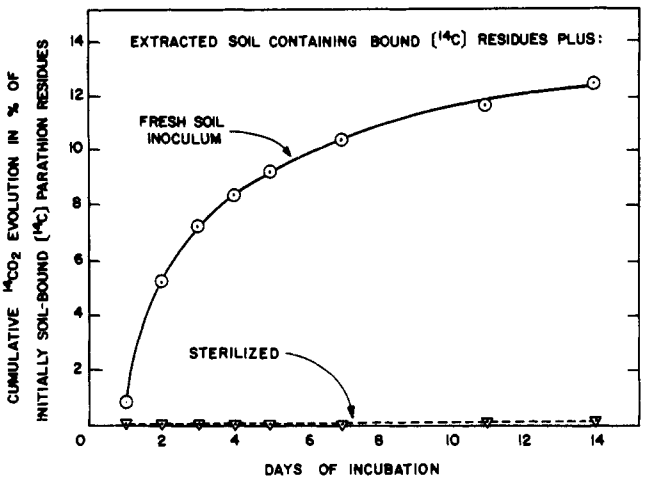

Figure 2. Effects of soil microorganisms on the evolution of $^{14}CO_2$ from soil-bound ^{14}C residues during a 2-week incubation period. Results are averages of duplicate experiments.

more $^{14}CO_2$ was released from soils containing also glucose or parathion, two compounds previously shown to increase microbiological activities. Conversely, addition of the bactericide chloramphenicol or the fungicide captafol to soil-bound ^{14}C residues significantly decreased the evolution of $^{14}CO_2$. Comparing the amounts of $^{14}CO_2$ evolved and the amounts of remaining soil-bound ^{14}C residues to those determined with control soils ($^{14}CO_2$ in percent of control, Table II), differences in results become even more evident. Thus, addition of glucose or parathion resulted in a 22% or 10% increase in $^{14}CO_2$ evolution, respectively, while the addition of chloramphenicol or captafol decreased $^{14}CO_2$ evolution by 35% and 9%, respectively. It is also evident that with an increase or decrease in the amounts of $^{14}CO_2$ produced, a simultaneous decrease or increase in the amounts of the remaining soil-bound radiocarbon had occurred. Amounts of bound ^{14}C residues remaining the captafol-treated soils, however, were similar to those observed with control soils.

Amounts of extractable radiocarbon at the end of the experiment ranged from 5.7% to 6.4% of the initially bound ^{14}C residues. As shown in Table II the major portions of the extractable residues were benzene soluble and contained primarily [^{14}C]parathion (4.61–4.95% of initially bound ^{14}C), but small amounts of [^{14}C]paraoxon, [^{14}C]aminoparathion, [^{14}C]aminoparaoxon, and [^{14}C]*p*-nitrophenol were also detected. Amounts of water-soluble

Table III. Effects of the Addition of Fresh Soil or Fresh Soil and Cow Manure to Extracted Soil on Populations of Aerobic Bacteria and Fungi

soil incubation, days	extracted insecticide-free soil plus[a]	
	fresh soil (control)	fresh soil and cow manure
aerobic bacteria[b] per g dry wt soil (10^6)		
0	7.3 ± 1.0	24.5 ± 1.3[c]
6	31.9 ± 2.8	149.0 ± 10.0[c]
fungi[b] per g dry wt soil (10^4)		
0	3.6 ± 0.1	8.7 ± 3.0[e]
6	3.4 ± 0.3	245.2 ± 54.1[d]

[a] One-hundred fifty-gram (dry wt) portions of extracted soil were mixed with 150 g (dry wt) of fresh soil or with 150 g (dry wt) of fresh soil and 13.2 g of air-dried cow manure. [b] Determined by dilution plate techniques. Results are means ± SD of triplicate plate counts. [c-e] Results are significantly different from controls (fresh soil) at the 0.1% (*c*) 1% (*d*) or 5% (*e*) level (student's *t* test).

radiocarbon were, in comparison to controls, significantly smaller in glucose and parathion-treated soils and significantly larger in the presence of chloramphenicol and captafol.

Data, therefore, indicate that the presence of biologically active substances in soils indeed did affect the release and metabolism of soil-bound [^{14}C]parathion residues, probably via an effect on soil microorganisms.

Effects of Cow Manure on Populations of Soil Microorganisms and on the Release of Soil-Bound ^{14}C Residues and Their Translocation into Oat Plants. Experimental results obtained from studies relative the effect of cow manure on populations of soil microorganisms are summarized in Table III. In control soil a 4.4-fold increase of aerobic bacteria had occurred during the 6-day incubation period, while the number of fungi remained the same. In the presence of cow manure, however, 6.1- and 28.2-fold increases in the populations of aerobic bacteria and fungi, respectively, had taken place.

In the second series of experiments the potential effects of cow manure on the release of soil bound ^{14}C residues and their pickup by oat plants were studied as described in Figure 1. To evaluate these effects, data pertaining to the release of soil-bound ^{14}C residues, the evolution of $^{14}CO_2$, and the pickup of radiocarbon by oats will be primarily discussed. Results obtained with open and closed systems are summarized in Table IV. It is evident that under all conditions the amounts of soil-bound ^{14}C residues remaining were only 63.7–75.8% of those bound at the start of the experiment (day 35, Figure 1). The presence of cow manure resulted, in comparison to controls, in smaller amounts of soil-bound residues remaining, thus indicating an increased release of formerly bound radiocarbon. This increase is also indicated by the recovery of 27.8% of formerly bound radiocarbon in the form of $^{14}CO_2$ as opposed to only 16.6% in controls. As shown previously (Table III), addition of cow manure to soil resulted in significant increases of both bacterial and fungal populations which in turn probably affected the release of soil-bound ^{14}C residues.

Oat leaves contained ^{14}C residues which originated from the originally soil-bound radiocarbon. Since the amounts of leaves produced in each of the 8 containers were not identical, it is difficult to compare amounts of ^{14}C recovered from the total leaf material harvested from each of the 8 soils. It is for this reason that, for comparison purposes, data were also expressed on a per gram leaf basis. Thus (total/g, Table IV) the presence of cow manure in soil resulted in a significant reduction of ^{14}C in oat leaves. This probably was related to the fact that significantly smaller amounts of soluble radiocarbon were recovered from cow manure amended soils than from control soil, therefore, making less radiocarbon available for plant translocation. In addition, due to the enhanced production

Table IV. Effects of Cow Manure in Soil on the Release of Soil-Bound [^{14}C]Parathion Residues and Their Uptake by Oat Plants Grown in Open Systems or in Closed Systems under Bell Jars

	extracted soil containing bound residues[a] in			
	open systems plus[b]		closed systems plus[b]	
	fresh soil (control)	fresh soil + cow manure	fresh soil (control)	fresh soil + cow manuer
	^{14}C recovered[c] in percent of initially bound radiocarbon			
$^{14}CO_2$[d]			16.64 ± 1.06	27.80 ± 1.11[j]
oat plants				
leaves (L)[e]				
benzene[f]	<0.01	<0.01	0.24 ± 0.02	0.22 ± 0.01
water[f]	0.05 ± 0.00	0.04 ± 0.00[i]	0.21 ± 0.01	0.22 ± 0.02
bound[f]	0.07 ± 0.00	0.04 ± 0.00[k]	0.91 ± 0.03	0.90 ± 0.04
total (L)	0.12 ± 0.00	0.08 ± 0.00[i]	1.36 ± 0.06	1.34 ± 0.07
total g[g]	0.02 ± 0.00	0.01 ± 0.00[j]	0.25 ± 0.01	0.18 ± 0.00[i]
roots (R)[h]	0.14 ± 0.01	0.13 ± 0.00	0.12 ± 0.01	0.13 ± 0.00
total (L + R)	0.26 ± 0.01	0.21 ± 0.00[i]	1.48 ± 0.07	1.47 ± 0.08
soil (S)				
benzene[f]-^{14}C	2.34 ± 0.19	1.74 ± 0.02[i]	2.53 ± 0.08	2.08 ± 0.03[i]
benzene–PS[i]	2.05 ± 0.15	1.33 ± 0.03[i]	2.21 ± 0.01	1.51 ± 0.01[j]
water[f]	0.28 ± 0.02	0.20 ± 0.01[i]	0.30 ± 0.05	0.34 ± 0.02
bound[f]	70.83 ± 0.34	63.72 ± 0.56[k]	75.83 ± 0.14	66.40 ± 0.63[k]
total (S)	73.45 ± 0.13	65.67 ± 0.53[k]	78.66 ± 0.16	68.81 ± 0.61[k]
total	73.71 ± 0.14	65.88 ± 0.53[k]	96.78 ± 1.15	98.08 ± 0.87

[a] Soil was treated with [^{14}C]parathion at 10.7 ppm and incubated for 5 weeks, followed by exhaustive extraction. Bound residues remaining were 15.5% (1.66 ppm) of applied, referred to as "initially bound". [b] One-hundred fifty grams of extracted soil (dry wt) containing bound [^{14}C]parathion residues (0.85 μCi) were mixed in duplicate with 150 g (dry wt) of fresh soil or with 150 g (dry wt) of fresh soil and 13.2 g of air-dried cow manure. [c] Results obtained after an additional 4 week incubation (day 35 to 63, Figure 1) are averages of duplicate tests. [d] $^{14}CO_2$ trapped in 0.1 N KOH. Polyurethane traps contained no significant radioactivity. [e] Leaves were harvested after 2 weeks of growth and again after an additional 2 weeks of regrowth. Data represents both crops. [f] Benzene and water extraction phases of leaves or soil. Bound = unextractable ^{14}C residues. [g] Total/g = Total per g weight of leaves. [h] Determined by combustion procedure. [i] PS = parathion determined by TLC and GLC. [j-l] Results are significantly different from controls at the 0.1% (*j*), 1% (*k*), or 5% (*l*) level (student's *t* test).

of $^{14}CO_2$ in the presence of cow manure, less was available for plant translocation.

It is also evident that, on a per gram basis, leaves grown in closed systems contained 12–18 times more radiocarbon than those grown in open systems. In this respect it should be realized, however, that data obtained with open and closed systems are not necessarily expected to be similar. Previous experiments (Anderegg and Lichtenstein, 1981) indicated that both corn and oat greens grown in closed systems transpired considerably less water and took up significantly smaller amounts of [^{14}C]phorate residues than plants grown in open systems, indicating that the insecticide was translocated via the transpiration stream. The authors also demonstrated that the amounts of $^{14}CO_2$ evolved in closed systems in the absence of plants were nearly double of those determined in systems with oats. Later studies (Lichtenstein et al., 1985) conducted with [^{14}C]fonofos- or [^{14}C]carbofuran-treated soils showed that $^{14}CO_2$ released into the air was to some extent picked up by the aerial parts of plants grown in untreated soil adjacent to insecticide-treated soil or directly within insecticide-treated soil. Since the major portion of radiocarbon in these plant greens was water-soluble, it appears that the $^{14}CO_2$ picked up from the air was photosynthesized into water-soluble products.

Total recoveries of radiocarbon from plants plus soils in open systems amounted to 73.7% of initially bound but only to 65.9% in the presence of cow manure. This, however, was related to the increased production of $^{14}CO_2$ in the presence of cow manure as shown with closed systems. Data, therefore, indicate that the presence of cow manure resulted not only in an increased release of soil-bound ^{14}C residues but also in their mineralization to $^{14}CO_2$. Part of this $^{14}CO_2$ could under the experimental conditions, particularly in closed systems, have been picked up by oat leaves.

In summary, data indicate that soil-bound ^{14}C residues can be released, metabolized, and picked up by oat plants grown in these soils. It was shown, that with an increase in populations of soil microorganisms a concomitant increase in the release of soil-bound ^{14}C residues had occurred. Addition of biologically active substances, known to increase or decrease microbiological activities, to soil-bound ^{14}C residues also resulted in an increase or decrease of the release of soil-bound radiocarbon and in particular in the evolution and release of $^{14}CO_2$. Addition of cow manure to soil-bound ^{14}C residues also resulted in larger populations of soil microorganisms, a decrease of initially soil-bound ^{14}C residues, a decrease in the amounts of total ^{14}C soil residues remaining, a dramatic increase in the evolution of $^{14}CO_2$ and, in comparison to controls, quantitatively different pickup patterns by oats which were grown in these soils.

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Registry No. Parathion, 56-38-2; captafol, 2425-06-1; chloramphenicol, 56-75-7; glucose, 50-99-7.

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