FORMATION AND BIORELEASE OF BOUND RESIDUES OF PESTICIDES IN TWO BRAZILIAN SOILS.[1]

I. [^{14}C]-LINDANE

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ABSTRACT - Soil contamination after pesticide applications is often frequent, and the use of radiolabelled molecules had proved the existence of non extractable or bound residues which remain firmly bound to the soil matrix. The extent of bound residues formation varies according to the applied compound, but it may constitute a significant part of residues remaining in the soil. The extractable and bound residues formation of ^{14}C-lindane were studied immediately and three months after the application. Metabolism, biorelease, and possible bioavailability of bound residues to bean plants were investigated by the use of biometer flasks. The results show that the formation and biorelease of lindane bound residues varied according to the soil type and with the aging time of the compound in the soil. There is a gradual binding because bound residues formed immediately after the application were released easier than the bound residues formed after a time of interaction of lindane with the soils. However, although some biorelease had occurred mainly by the microflora action, some bound residues remained as still bound even after the microflora and rhizosphere action, being thus, inactivated.

Index terms: pesticide, applied compound, metabolism, bioavailability, bean plants, microflora, rhizosphere.

FORMAÇÃO E BIOLIBERAÇÃO DE RESÍDUOS-LIGADOS DE PESTICIDAS EM DOIS SOLOS BRASILEIROS

I. [^{14}C]-LINDANE

RESUMO - A contaminação do solo após aplicação de pesticidas é freqüente, e o uso de moléculas radiomarcadas provou a existência de resíduos não extraíveis ou ligados, que permanecem firmemente ligados à matriz do solo. A magnitude de formação de resíduos-ligados varia de acordo com o composto aplicado, mas pode constituir uma parte significativa dos resíduos que permanecem no solo. A formação de resíduos extraíveis e ligados a partir de ^{14}C-lindano foi estudada imediatamente e três meses após a aplicação do pesticida. O metabolismo, a bioliberação e a possível biodisponibilidade dos resíduos-ligados no que tange ao feijoeiro, foram investigados através do uso de frascos biométricos. Os resultados mostram que a formação e a bioliberação de resíduos-ligados de lindano variaram de acordo com o tipo de solo e com o tempo de envelhecimento do composto no solo. Ocorre uma ligação gradual, porque os resíduos-ligados formados imediatamente após a aplicação foram liberados mais facilmente do que os resíduos-ligados produzidos após um tempo de interação do lindano com os solos. Porém, embora alguma bioliberação tenha ocorrido, principalmente por ação da microflora, uma parte dos resíduos-ligados permanecem como resíduos ainda ligados mesmo após a ação da microflora e da rizosfera, sendo, então, inativada.

Termos para indexação: pesticida, composto aplicado, metabolismo, biodispo, feijoeiro, microflora, rizosfera.

INTRODUCTION

Pesticides applied on crops or directly to soils may cause build up of undesirable residues including the formation of bound residue. The nature and/or identity of these bound residues is not known, and very little is known about their bioavailability,

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toxicity, and cumulative nature. Conventional analytical procedures normally used in residues laboratory do not detect them, thereby underestimating the total soil residues concentration (Khan, 1982). Thus, the persistence and bioavailability of bound residues may constitute a potential environmental problem (Khan, 1982; Klein & Scheunert, 1982) as the amount of bound residues can exceed considerably the amount of extractable residues (Scheunert et al., 1985).

Conditions affecting microbial growth in soil may influence the formation and degradation of bound residues (Roberts, 1984; Scheunert et al., 1987; Golovleva et al., 1990). Furthermore, several works have shown that microorganisms are also associated with the release of bound residues from soils (Khan & Ivarson, 1982; Khan, 1982; Khan, 1984; Racke & Lichtenstein, 1985; Khan & Behki, 1990). Thus, it appears that bound residues are associated mainly with microbial activity although their presence in sterilized soils has also been reported (Roberts, 1984).

Although the binding capacity of different classes of pesticides, such as organophosphorus, carbamates, pyrethroids and triazines varies considerably (from 18% to 80%), the organochlorinated pesticides are known to form smaller amounts (1% to 9%) of these residues (Khan & Dupont, 1987; Calderbank, 1989). But, it has been shown that more than 29% of bound residues were produced from application of lindane to soils (Raghu & Ferreira, 1984).

Thus, factors influencing the formation, persistence and biorelease of pesticide soil bound residues include: chemical characteristics of the pesticide, microbial process, chemical changes into the environment or changes in agricultural practices that could release the soil bound residue to the soil solution (Calderbank, 1989). In this way, a system which includes most of these factors was used in order to provide information about persistence of lindane bound residues in two types of Brazilian soils.

MATERIALS AND METHODS

Insecticide

Lindane (gamma isomer of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane) technical grade was obtained from the Chemical Section of Instituto Biológico (92.4% of chemical purity determined by gas-chromatography). The corresponding radiolabelled [U-^{14}C] benzene hexachloride, was purchased from Amersham International, Amersham, England, with specific activity of 2.33 GBq/mmol (63 mCi/mmol) and 98% of radiochemical purity as determined by thin-layer chromatography (TLC).

Soils

Samples of the two soils used in this study were collected at 0-30 cm depth, air dried and passed through 2.0 mm sieve for homogeneization before they were used in the experiments. The silty clay soil was obtained from the Amazon State and contains: 10.9% of sand, 36.9% silt, 52.8% clay, 4.7% of organic matter and pH of 4.7. The sandy loam soil was collected in the coastal region of São Paulo State and contains: 61.0% of sand, 14.0% of silt, 25.0% of clay, 3.3% organic matter and pH of 4.5.

Plant

Seeds of bean, *Phaseolus vulgaris* (L.), were pre-germinated in wet cotton and after one week transferred to Erlenmeyers flasks containing water. The plants were grown in these flasks until to have the first three leaves.

Soil treatment and incubation

The 750 g soil samples were brought up to moisture content of 70% of maximum holding capacity, which was mantained for one week. They were then treated with a 10 ml hexane solution containing ^{14}C-lindane (10μg cold lindane plus 0.04 μCi ^{14}C-lindane/g of soil). The treated soil samples were mixed thoroughly and transferred to plastic bags.

An aliquot (310 g) of each soil was removed after the treatment to determine the residue concentration. The weight of the plastic bag containing the remaining soil was recorded and it was placed into a plastic pot to incubate for three months in a glasshouse. The soil was weighted biweekly and water was added to mantain the initial moisture content.

The amount of ^{14}C in the soil samples (6 x 0.5g) collected immediately after the treatment and at the end of a three month-aging period was determined by combustion (Fig. 1) in a Biological Oxidizer (Harvey OX-400). The ^{14}C-extractable residues were determined by Soxhlet extraction of immediately treated and aged soil samples (6 x 50g) with 150 ml methanol for 24 hours (Fig. 1).

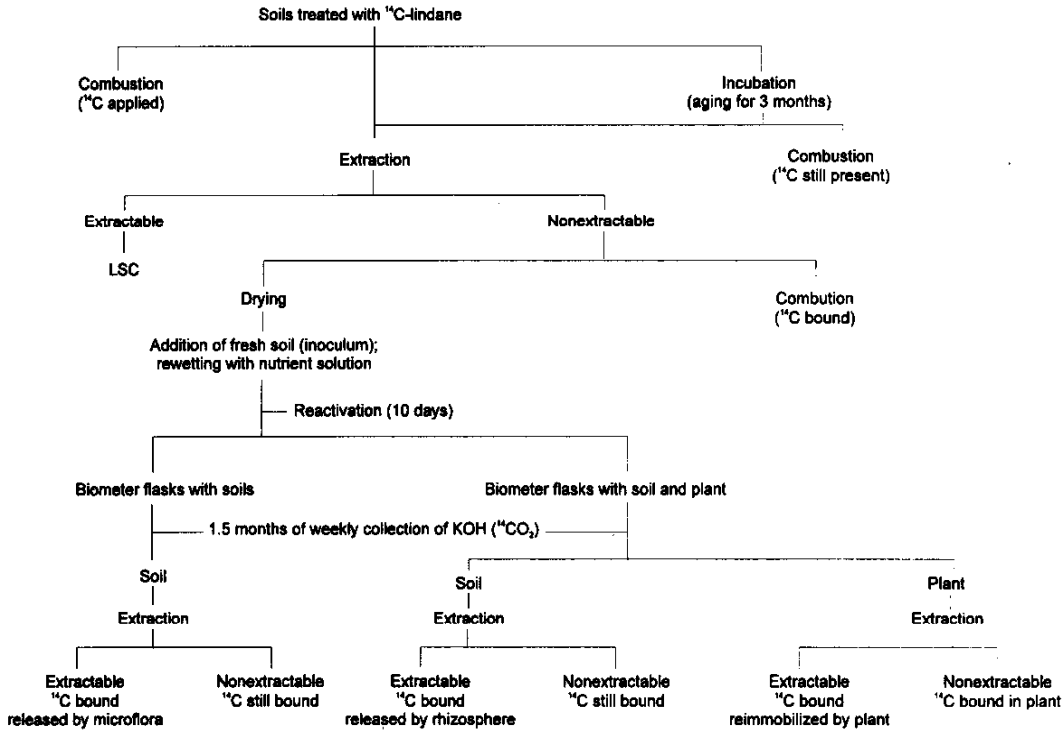

FIG. 1 Schematic diagram for the analysis of ^{14}C in soils and plants.

The extracted soil samples were air dried, placed in biometer flasks together with 1% (dry weight) of untreated fresh soil as inoculum (Hsu & Bartha, 1979; Racke & Lichtenstein, 1985; Khan & Dupont, 1987), and rewetted to the maximum water hold capacity with Hoagland's nutrient solution (Hoagland & Arnon, 1950). After a ten--day period in the dark, the amount of ^{14}C present after the reactivation of the soil microflora (Fuhremann & Lichtenstein, 1978) was again determined by combustion (Fig. 1), and the biotests were set up. At least three biometer flasks were adapted to contain plants (Fig. 2), according to Bartha & Pramer (1965) and Hsu & Bartha (1979), and water was added through the syringe whenever the soil appeared dry. The biometer flasks were tightly closed and put into black bags to prevent photosynthetic reabsorption by the plant of the evolved $^{14}CO_2$ from the soil (Hsu & Bartha, 1979; Klein & Scheunert, 1982).

The ^{14}C mineralized materials were determined weekly during 1.5 month (Fig. 1) by changing the 10 ml of KOH in the side arm of the biometer flasks. Two 1 ml aliquots of KOH from each flask were counted by liquid scintillation (LSC).

After the 1.5-month growing period the systems were opened, the plants were carefully removed from the soils, and roots were washed with tap water to remove soil particles which could represent surface contamination. The plants were weighed and maintained at -5 °C until they were extracted by maceration with small portions of hexane (until 0.6g: 4ml and from 0.6 to 2g: 6ml) in a glass homogeneizer. The hexane was combined and washed with equal volume of water to separate the water soluble compounds. Aliquots of the two phases were quantified by LSC. Thereby, the ^{14}C released from the soil and reimmobilized by the plants was verified. The extracted plant tissues were dried and samples were combusted to account for the ^{14}C bound in the plant (Fig. 1).

The soils were exhaustively Soxhlet re-extracted as described before, to verify and compare the amount of ^{14}C which became extractable by the action of microflora (biometer flasks with soil) and/or rhizosphere (biometer

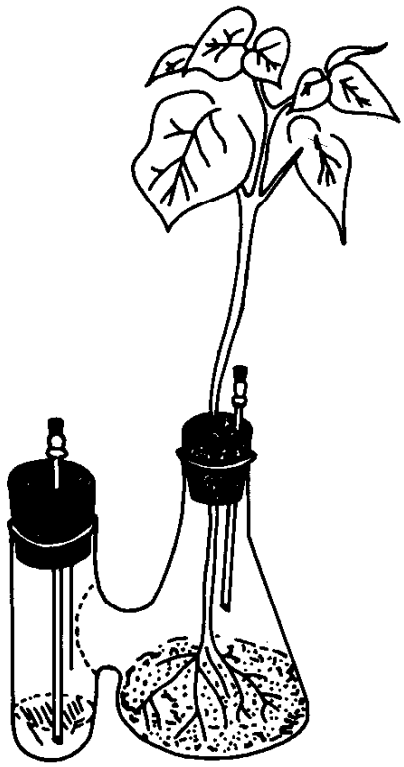

FIG. 2. Adapted biometer flask.
Fonte: Adapted from Bartha & Pramer (1965) and Hsu & Bartha (1979).

flasks with soil and plant). Samples of the re-extracted soils were combusted to account for the ^{14}C still bound in the soils (Fig. 1).

Thin-layer chromatography and Radioscanner

Aliquots (20 ml) of the methanol from soil extraction were extracted with (3x) 10 ml hexane. All the hexane extracts were combined, total volume recorded, and 1 ml aliquots of each phase were quantified by LSC.

Aliquots (10 ml) of the hexane soil extracts and the remaining hexane extract from plants were evaporated to dryness, the residues were re-suspended in small volume portions of hexane, and applied on TLC silica gel-60 F_{254} plates together with the ^{14}C-lindane standard. The plates were developed in hexane:acetone (4:1).

All the extracts and the ^{14}C-lindane were qualitatively examined in the TLC Scanner II LB 2723 (Berthold) using the following conditions: 1 sec. integration time, 1 K cpm sensibility and 300 mm/h speed run. Under these experimental conditions the R_f values for ^{14}C-lindane was 0.50.

Liquid Scintillation Counting (LSC)

All the extraction and combustion samples were quantified in a Beckman LS-5801 after addition of the Mesquita & Ruegg's (1984) scintillation mixture for the extracts and the same cocktail mixed with methanol (7:3) for combustion samples.

RESULTS AND DISCUSSION

From the ^{14}C-lindane applied to the silty clay soil from Amazon (1.29 kBq or 0.035 μCi/g, Table 1) and immediately extracted, approximately 66% were extractable and 45% were detected by combustion. After evaporation of the solvent and reactivation of the soil with fresh inoculum plus nutrient solution for 10 days, the ^{14}C present was about 32%. Thus, during the reactivation, some ^{14}C-volatiles were formed by the reactivated microflora, and released to the atmosphere, as found by others (Khan & Ivarson, 1981; Kloskowski et al., 1986; Raghu & Drego, 1986).

The investigation of the biorelease of the ^{14}C-bound by the biotests with and without plant

TABLE 1. Radiocarbon recovery from silty clay soil immediately after treatment with ^{14}C- lindane.

Applied amount	1.29 kBq/g (or 0.035 μCi/g)	
	---------- % ----------	
Extracted	66.01 ± 8.15	
Bound	45.35 ± 4.72	
Present after reactivation	32.29 ± 9.87	
Biotest	**With plant**	**Without plant**
$^{14}CO_2$	0.65 ± 0.33	0.85 ± 0.56
Released and extracted	31.06 ± 5.43	29.35 ± 10.44
Uptaken by plant	0.22 ± 0.11	- - -
Bound in the plant	0.07 ± 0.03	- - -
Still bound in soil	3.07 ± 0.94	2.47±0.34

(Table 1) revealed that only 0.6% and 0.8% respectively, were metabolized to $^{14}CO_2$. But, 31% and 29% were released in the extractable form. Very little was absorbed and bound in the plants (0.2% and 0.07%, respectively, Table 1), and about 3% remained as still bound in the soil.

After three-month incubation of ^{14}C-lindane in the same soil (Table 2), the amount of ^{14}C still present for the tests were the same applied, i.e., 1.29 kBq/g, thus indicating that volatilization did not take place during the aging time under natural conditions.

However, near 99% were detected as extractable and less than 2% as bound residues. After reactivation, about 1.1% remained for the biotests (Table 2).

The distribution of radiocarbon in the biotests showed insignificant amounts of $^{14}CO_2$, ^{14}C released and extractable and ^{14}C uptook by the plants (Table 2). The sum of all these values reached maximum of 0.35%. But, the amounts of ^{14}C still bound in the soil were near the 1% present after reactivation. Thereby, the aging time was sufficient for the small amounts of bound residues detected, demonstrating that the natural microflora could metabolize the immediately formed bound residues, and then, very little remained as bound after the time of pesticide residence in this soil.

In the sandy loam soil, from the 1.37 kBq/g (0.037 μCi/g) applied, more than 90% were extracted, about 11% were immediately bound, and near 8.7% were present after reactivation (Table 3).

The biotests with and without plants to release these bound residues showed that most of the radiocarbon was released and again extracted after action of the rhizosphere or the microflora (4.8% and 9.4%, respectively, Table 3). Small amounts were detected as $^{14}CO_2$, but about 1% more in the biotest without plant. Very small amount was uptaken by the plants, and only about 1% was detected as ^{14}C still bound in the soil after the biotests (Table 3).

Nevertheless, after aging of the ^{14}C-lindane applied to this soil, less was found as radiocarbon still present (0.99 kBq or 0.027 μCi/g, Table 4), showing that the natural microflora of this soil was active in metabolizing the ^{14}C-lindane and/or its ^{14}C-metabolites to volatile products which scaped to the atmosphere during the three months. The ^{14}C-lindane itself could be volatilized as favoured by the low organic matter and sand contents (Glotfelty et al., 1984; Chessels et al., 1988).

The reactivation of the extracted soil changed very little the initially ^{14}C-bound (about 18%, Table 4), so that, near 16% were still present for the biotests (Table 4). Also in this case, little was biomineralized, but slightly more $^{14}CO_2$ was detected in the biotest without plant (Table 4). Less than 1.5% of the ^{14}C-lindane bound residues were released and re-extracted, independently of the biotest (Table 4). In-

TABLE 2. Radiocarbon recovery from silty clay soil three months after treatment with ^{14}C-lindane.

Amount still present	1.29 kBq/g (or 0.035 μCi/g)	
	%	
Extracted	98.94 ± 3.98	
Bound	1.36 ± 0.42	
Present after reactivation	1.11 ± 0.25	
Biotest	With plant	Without plant
$^{14}CO_2$	0.25 ± 0.14	0.09 ± 0.07
Released and extracted	0.06[a]	0
Uptaken by plant	0.04 ± 0.04	- - -
Bound in the plant	0	- - -
Still bound in soil	0.77 ± 0.29	0.88 ± 0.32

[a] only 1 sample presented radioactivity.

TABLE 3. Radiocarbon recovery from sandy loam soil immediately after treatment with ^{14}C-lindane.

Applied amount	1.37 kBq/g (or 0.037 μCi/g)	
	%	
Extracted	90.75 ± 4.37	
Bound	10.75 ± 4.15	
Present after reactivation	8.68 ± 4.33	
Biotest	With plant	Without plant
$^{14}CO_2$	0.39 ± 0.19	1.39 ± 0.07
Released and extracted	4.85 ± 2.68	9.44 ± 0.48
Uptaken by plant	0.06 ± 0.06	- - -
Bound in the plant	0.02[a]	- - -
Still bound in soil	0.93 ± 0.48	1.25 ± 0.17

[a] only 2 samples presented radioactivity.

TABLE 4. Radiocarbon recovery from sandy loam soil three months after treatment with ^{14}C-lindane.

Amount still present	0.99 kBq/g (or 0.027 μCi/g)	
	---------- % ----------	
Extracted	85.03 ± 1.55	
Bound	17.95 ± 1.99	
Present after reactivation	16.22 ± 1.6	
Biotest	With plant	Without plant
$^{14}CO_2$	0.90 ± 0.34	1.53 ± 0.37
Released and extracted	1.45 ± 0.39	1.24 ± 0.35
Uptaken by plant	0.02[a]	---
Bound in the plant	0.03 ± 0.01	---
Still bound in soil	12.38 ± 0.93	12.35 ± 0.80

[a] only 2 samples presented radioactivity.

significant amounts were detected in the plants, either as extractable (0.02%) or as plant bound residues (0.03%). The highest detected amount was as ^{14}C-still bound in the soil (higher than 12%, Table 4), proving that the residues really bound, i.e., bound residues formed after interaction of the pesticide with this type of soil, remained as still bound residues after action either of the microflora, or the rhizosphere.

Therefore, the aging of the ^{14}C-lindane applied in the soil favoured the small amount of bound residues formed in soil with the characteristics of the silty clay here studied (Table 2). But it favoured the formation of bound residues not susceptible either to the microflora or to the rhizosphere, because once the residue became bound, it was almost not released (Table 2). Nevertheless, the ^{14}C-lindane bound residues formed immediately after its application but released and re-extracted, represented the highest amount of residues present in this soil (approximately 30%, Table 1). Thus, the microflora and/or rhizosphere had little influence on the release of the bound residues formed after the aging time. As stated by Khan (1991), some metabolites produced during the incubation time may become part of the bound residues reservoir.

The bioreleased and extractable residues immediately after the pesticide application in the sandy loam soil also studied, represented the highest proportion of detected residues after the biotests (Table 3). But, the aging time process decreased the amount of bound residues susceptible to the microflora action because the amount of still bound residues was very similar to that of residues present after reactivation (Table 4), as occurred for the silty clay soil (Table 2).

Any way the biomineralization and bioavailability to plants were ever very small in both soils (Tables 1, 2, 3 and 4). But, the biorelease of the bound residues as extractable residues was easier from bound residues formed immediately after the application (Tables 1 and 3), proving that bound residues formed immediately after lindane application were weaker bound than after an aging time, as found by others (Khan & Rauthan, 1985; Calderbank, 1989). Thus, once the ^{14}C-lindane really bound residues were formed they were little susceptible for bioreleasing by the microflora independently of the soil type. Hsu & Bartha (1976), Katan et al. (1976), Lichtenstein (1980), and Khan (1991) suggested that the binding process of pesticides or their metabolites occurs in two steps, the first involving the microflora in the releasing of residues weakly bound, and the second involving the physico-chemical bounding of them into the organic matter.

TLC of soil and plant extracts showed that degradation to apolar compounds was insignificant in the silty clay soil independently of the interaction time of the ^{14}C-lindane with the soil, because very little was detected out of the pesticide R_f (Table 5). Nevertheless, in the sandy loam soil, radioactivity other than lindane was evident in the extracts

TABLE 5. Chromatographic profile of ^{14}C-lindane from the silty clay soil.

R_f	0 - 0.13	0.20-0.40	0.50 (^{14}C-lindane)	0.60-0.73
	---------- Immediately after the treatment ----------			
Extracted	-	-	xxx	-
Released and extracted				
- with plant	x	-	xxx	-
- without plant	-	-	xxx	-
Uptaken by plant	x	-	xxx	-
	--------- Three months after the treatment ----------			
Extracted	-	x	xx	-
Released and extracted				
- with plant	-	-	-	-
- without plant	-	-	-	-

- no radioactivity; x some radioactivity; xx evident radioactivity; xxx maximum radioactivity.

immediately after the ^{14}C-lindane application (Table 6). But, in both soils, the highest detected spots were as the ^{14}C-lindane itself.

Water soluble compounds detected in the methanol after extraction with hexane are presented in Fig. 3. The ^{14}C-lindane degraded rather more to water soluble compounds in both soils. The aging time of the residues was the only cause for production of water soluble compounds in the silty clay, and the main cause in the sandy loam soil. But, in the last, microflora also contributed with some 20% of water solubles arised (Fig. 3).

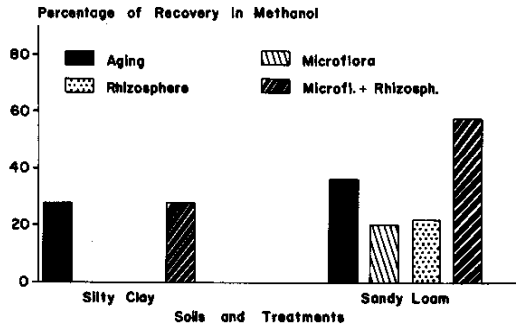

FIG. 3. Degradation of 14C-lindane to water soluble compounds in soil extracts.

TABLE 6. Chromatographic profile of 14C-lindane from the sandy loam soil.

Rf	0 - 0.13	0.20 - 0.40	0.50	0.60 - 0.73
	(^{14}C-lindane)			
	------ Immediately after the treament ------			
Extracted	-	-	xxx	-
Released and extracted				
- with plant	x	-	xxx	xx
- without plant	-	-	xxx	-
Uptaken by plant	x	-	xxx	xx
	------ Three months after the treatment ------			
Extracted	-	-	xxx	-
Released and extracted				
- with plant	-	-	xxx	-
- without plant	-	-	-	-

- no radioactivity; x some radioactivity; xx evident radioactivity; xxx maximum radioactivity.

CONCLUSIONS

The results presented in this study demonstrated that the aging process involving interaction of ^{14}C-lindane with the two Brazilian studied soils was the main cause of recalcitrance or inactivation of its bound residues. There was little difference between the action of the rhizosphere and microflora, but indicating that microflora was the prevalent agent and rhizosphere did not increase any effect. In terms of environmental pollution, it was observed that in the silty clay soil more bound residues were immediately formed, but the bounding was weak and most was again released, generating a problem of future contamination. On the other hand, in soil with the sandy loam characteristics the aging produced slightly more bound residues but they were inactivated because neither the microflora nor the rhizosphere were able to release most of them. Thus, results show that the concept of persistence of pesticides must take into account the bound residues which can be bioreleased by the microflora.

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