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Adaptation of the Biobed Composition for Chlorpyrifos Degradation to Southern Europe Conditions

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Biobeds developed in Sweden bind and degrade pesticides from point sources. The objective of this work was to adapt the biobed to Italian operating conditions, for example, to identify organic materials as effective as those in the original Swedish composition. The capacity of urban and garden composts alone or mixed with citrus peel or straw to degrade chlorpyrifos and its metabolite TCP was compared to the typical Swedish biomix consisting of straw, peat, and soil. A tendency for higher ^{14}C-chlorpyrifos mineralization and lower TCP levels was observed in the biomixes with garden compost alone or amended with straw. In a second trial, a high correlation of lower TCP with increasing levels of straw in typical Swedish biomixes was observed. Straw stimulates production of lignin-degrading enzymes such as manganese peroxidase (MnP), and further trials with pure MnP showed that this enzyme degrades TCP. Materials with an active lignin-degrading microflora are a prerequisite for effective dissipation of chlorpyrifos and non-accumulation of TCP. Thus, lignocellulosic materials as straw and garden composts should be present in biomixes to be used under Italian conditions.

KEYWORDS: Bioprophylaxis; biobed; biomassbed; pesticides; chlorpyrifos; TCP; compost; straw; MnP

INTRODUCTION

Potential sources of surface and groundwater contamination include inappropriate procedures at pesticide mixing and handling sites and inappropriate disposal of pesticide sprayer rinse water (*1*). A low-cost bioprophylaxis system known as the biobeds has been developed to prevent such water contamination (*2*). Biobeds rely on a mixture of organic matter and soil as biofilters for retaining and biodegrading pesticides employed in agricultural crop protection (*2*−*4*) and are effective in reducing point source contamination (*5*). In its original design, the biobed consists of a 60 cm deep pit in the ground with a clay layer in the bottom (10 cm) and the remaining volume filled with a biomix. The typical Swedish biomix consists of straw, topsoil, and peat (50−25−25% v/v). The straw stimulates the growth of lignin-degrading fungi and the activity of ligninolytic enzymes (such as manganese and lignin peroxidases), which can degrade many different pesticides (*6*−*9*). The soil provides sorption capacity and other degrading microorganisms, and the peat contributes to high sorption capacity and also regulates the humidity of the system. A grass layer covering the biobed helps to keep the correct humidity and can be used as an indicator to reveal pesticide spills. To adapt this biological system to Mediterranean operating conditions, there is a need to identify organic materials that can act in the same effective way as those in the original Swedish composition. The type of organic material present in the biobed is crucial for the amount, activity, and genotypic and phenotypic versatility of microorganisms responsible for degradation of pesticides and their metabolites.

A modified Italian biobed system, still under development, is the biomassbed, which utilizes waste byproducts originating from agricultural and agro-industrial activities in biofilters through which pesticide-contaminated water is circulated and decontaminated (*10*). The organic materials used to date have been urban and garden composts, vine branches, and citrus peel because of their high availability and cheapness.

Peat is not easily found in Italy and is expensive, so there is a need to identify a substitute organic material that can give sorption capacity and maintain good humidity in the biomassbed. It would also be advantageous if this peat substitute were a source of microorganisms with pesticide catabolic activity. High-quality compost made from garden residues or municipal waste contains numerous microorganisms with differing activity and has demonstrated a good retention capacity for pesticides (*11*).

The broad-spectrum insecticide chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is widely used in Italian agriculture. Its persistence in some soils is of concern because of its toxicity against many nontarget organisms such as mammals (*12*), amphibia (*13*), and microorganisms (*14*). The

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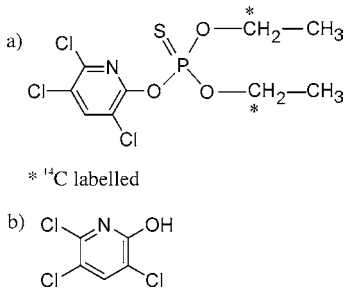

Figure 1. Chemical structures of (**a**) chlorpyrifos and (**b**) TCP.

hydrophobic nature of chlorpyrifos (Log $K_{ow} = 4.7$) (*15*) gives a high adsorption in soil, and reported half-life varies from 10 to 120 days (*16*). Chlorpyrifos degradation is reduced by fumigation of soil samples (*17*) but is not enhanced by repeated applications. Degradative chemical hydrolysis and microbial processes complement one another depending on different environmental factors such as soil pH, temperature, and moisture content (*18*−*21*). The degradation pathway leads to the formation of chlorpyrifos oxon [*O*,*O*-ethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] and desethyl chlorpyrifos [*O*-diethyl *O*-(3,5,6-trichloro-2-pyridiyl) phosphate]. The parent molecule and the metabolites mentioned are characterized by a P−O−C linkage, the hydroxylation of which leads to the formation of a major metabolite that has been identified as 3,5,6-trichloro-2-pyridinol (TCP), which in turn can be degraded to TMP (3,5,6-trichloro-2-methoxy pyridine) (*22*).

Chlorpyrifos does not undergo enhanced biodegradation (*23*), and it has been suggested that the accumulation of TCP, a compound known for its antimicrobial activity, can inhibit the proliferation of chlorpyrifos-degrading microorganisms (*20*, *24*).

The aim of this study was to design an organic biomix for effective dissipation of chlorpyrifos and its metabolite TCP. Therefore, we compared the capacity of the organic materials (urban and garden composts alone or mixed with citrus peel or straw) so far used in the Italian biomassbed system to the typical Swedish biobed mixture consisting of straw, peat, and soil. We also assessed the involvement of the lignin-degrading system in the dissipation of chlorpyrifos and TCP.

MATERIALS AND METHODS

Chemicals. Unlabeled chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] and TCP (3,5,6-trichloro-2-pyridinol) were supplied by Sigma-Aldrich, Sweden AB. Both chemicals had >99% purity. Labeled ^{14}C-chlorpyrifos (**Figure 1**) with a purity of >97% was supplied by the Institute of Isotopes Co., Budapest. Insta-Gel Plus was provided by Perkin-Elmer, USA. All other chemicals used in this work were supplied by VWR International AB.

Enzyme. Manganese peroxidase enzyme ≥20 U g^{-1} was provided by BioChemika, Fluka, Germany.

Substrates. The organic materials and mixtures tested are shown in **Table 1**. The urban compost came from the CONSMARI plant in Tolentino, Macerata, Italy, and the garden compost from the SEGENU installations at Pietramelina, Perugia, Italy. The straw (vine branches) came from the experimental field of Università Politecnica delle Marche, Ancona, Italy, and the citrus peel samples from a farm in Sicily, Italy.

The soil was agricultural topsoil containing 14% clay and 1.0% organic carbon, pH 6.6, collected in Uppsala, Sweden, sieved (2 mm) and stored at 4 °C until use. The peat mould (*Sphagnum*) came from Econova Garden AB, Sweden.

All of the materials were passed through a 2 mm sieve, and the mixtures were prepared in volumetric proportions (**Table 1**). The carbon and nitrogen content, C/N ratio, pH, soluble carbon (*25*), and lignin content (*26*) of each material and their mixtures are presented in **Table 1**.

Chlorpyrifos Dissipation in Urban and Garden Composts Alone or Mixed with Citrus Peel or Straw. The dissipation of chlorpyrifos was monitored by measuring the mineralization of ^{14}C-chlorpyrifos over time and the chlorpyrifos concentration at the beginning and at the end of the incubation period. The TCP concentration was measured at the end of the incubation. The microbial activity was measured as CO_2 production.

The materials used were: (a) urban compost (U), (b) garden compost (G), (c) urban compost plus citrus peel (U+C), (d) garden compost plus citrus peel (G+C), (e) urban compost plus straw (U+S), and (f) garden compost plus straw (G+S). The citrus peel was added in a proportion of 12.5% v/v and the straw at 50% v/v (**Table 1**). All of the materials were passed through a 2 mm sieve and thoroughly mixed.

Triplicates of each biomassbed sample (2 g) were weighed into 100 mL plastic jars. The samples were spiked with cold chlorpyrifos (100 mg g^{-1}) and ^{14}C-chlorpyrifos (78 000 dpm) in an acetone solution according to the method of Brinch et al. (*27*) by treating a sub-sample (25%) with the insecticide, mixing thoroughly, allowing the solvent to evaporate, and finally mixing again with the remainder of the sample. The water content was kept at 60% of the water holding capacity.

The plastic jars were installed into airtight glass jars together with two scintillation vials containing NaOH (0.2 M; 4 mL) to trap carbon dioxide. The glass jars were incubated in the dark at 20 °C, water was added when necessary to maintain the designated moisture, the NaOH solution was periodically removed, and mineralization and respiration were measured.

The $^{14}CO_2$ arising from the mineralization of chlorpyrifos was measured in a liquid scintillation counter (Beckham LS 600 series, USA) after mixing with 4 mL of Insta-Gel Plus. The mineralization rate was expressed as accumulated $^{14}CO_2$ as a percentage of the initial radioactivity and divided by the incubation period in days.

In the respiration tests, the CO_2 captured in the NaOH solution was determined by titrating the remaining alkali with 0.1 M HCl after precipitation of the carbonate with 0.1 M $BaCl_2$. TIM 850 Titration Manager equipment (Radiometer Analytical, Copenhagen, Denmark)

Table 1. Composition and Properties of the Materials Used in This Study

material	symbol	composition (% v/v)	C (%)	N (%)	C/N	pH	soluble carbon % dm	lignin % dm
urban compost	U	100	30.2	2.40	13	7.97	0.7	21.8
garden compost	G	100	32.8	2.34	14	8.18	0.3	33.4
citrus peel	C						4.0	1.4
straw	S						2.2	30.4
urban compost + citrus peel	U+C	87.5:12.5	32.4	2.60	12	6.73		
garden compost + citrus peel	G+C	87.5:12.5	31.8	2.26	14	6.41		
urban compost + straw	U+S	50:50	39.4	1.96	20	7.44		
garden compost + straw	G+S	50:50	32.3	1.43	23	6.87		
biomix straw + soil + peat	B1	50:25:25	18.0	0.35	51	5.66		
biomix straw + soil + peat	B2	25:50:25	7.3	0.23	32	5.94		
biomix straw + soil + peat	B3	12.5:62.5:25	3.8	0.18	21	5.99		
soil		100	1.0	0.11	9	6.82		

was used for the titrations. The respiration rate was expressed as accumulated mg CO_2 g $soil^{-1}$ and divided by the incubation period in days.

The incubation regime was 70 days at 20 °C. At the end of this period, all of the samples were analyzed to determine the final chlorpyrifos and TCP concentrations. The TCP accumulation rate was expressed as the percentage of the moles of TCP formed by moles of the initial extracted chlorpyrifos divided by the incubation period in days.

Effect of the Levels of Straw on the Dissipation of Chlorpyrifos in a Typical Biobed Mixture. As described for the experiment above, the dissipation of chlorpyrifos was monitored by measuring the mineralization of ^{14}C-chlorpyrifos over time and chlorpyrifos and TCP concentrations at the beginning and the end of the incubation period. The microbial activity was measured as respiration rate.

The biobed mixture consisted of topsoil, peat, and straw. Mixtures B1, B2, and B3 (**Table 1**) consisted of three levels of straw: 50, 25, and 12.5% v/v, respectively. The soil content was 25, 50, and 62.5% v/v, respectively, while the peat content in the three mixtures was constant (25% v/v). Soil alone was run as a control (**Table 1**). Triplicates of each mixture were used.

All of the other conditions were as described before with the exception that the incubation period was 62 days.

Analytical Methods. Chlorpyrifos and TCP were determined by extracting the samples vigorously overnight with 6 mL of acidified acetone per gram of substrate (*21*) (acetone + water + concentrated phosphoric acid, 98 + 1 + 1 by volume) on a shaking table. Samples were centrifuged at 358*g*, and the solution was collected. The extraction was repeated two more times for 30 min periods.

One milliliter of the pooled extract was centrifuged at 5411*g* for 15 min, and 0.5 mL of the supernatant was collected, diluted 1:1 with water, and analyzed by high performance liquid chromatography (HPLC). The chlorpyrifos recovery was >50% in the biomixes containing U-compost and >80% in those containing G-compost. The TCP recovery was >70% in all of the treatments.

The HPLC analysis was performed using an Agilent 1100 series chromatograph, equipped with a variable wavelength UV detector (wavelength 300 nm), and a C18 column, Zorbax SB-C18, 5 μm 4.6 × 150 mm, using the mobile phase 50% CH_3CN and 50% water/acetic acid (95.3/4.7; v/v) with a flow rate of 1.5 mL min^{-1}.

Degradation of Chlorpyrifos and TCP by Manganese Peroxidase (MnP). The degradation of chlorpyrifos and TCP was studied in separate trials using pure MnP. All reagents were sterilized by filtration (0.45 μM pore size) before use. The tests were conducted using loosely capped sterilized 8 mL glass vials, which were rotary shaken (150 rpm) in a water bath at 37 °C. Chlorpyrifos and TCP were added to the reaction mixture from a sterilized stock solution (400 μM) to give a concentration of 10 μg mL^{-1}. Samples were immediately analyzed by HPLC according to the method described.

The reaction mixtures (2 mL) were prepared in 20 mM Na tartrate buffer pH 4.5 and contained 0.4 mM $MnSO_4$, 0.1 mM H_2O_2, 1 mg MnP, 1% Tween 80, and chlorpyrifos or TCP. The trials were performed on seven replicates. The reaction was started by addition of the MnP, and an additional 0.25 mg of MnP was added after each sampling. At each sampling occasion, the chlorpyrifos and TCP concentrations were determined. The total reaction time was 9 days. Controls without H_2O_2, $MnSO_4$, and Tween 80 were also run, and the chlorpyrifos and TCP concentrations were measured at the beginning and at the end of the incubation period.

Chlorpyrifos and TCP concentrations were directly determined by HPLC according to the method described.

RESULTS

Chlorpyrifos Dissipation in Urban and Garden Composts Alone or Mixed with Citrus Peel or Straw. The U-compost had a 2.2-fold higher respiration rate than the G-compost, and the respiration in both composts increased with the addition of citrus peel or straw (**Table 2**). The mixtures with citrus peel were characterized by a 1.2-fold higher respiration rate than the mixtures containing straw. No significant differences were obtained in the dissipation rate of chlorpyrifos, which ranged between 0.96% and 1.34% per day (**Table 2**).

Table 2. Respiration Rate and Chlorpyrifos Dissipation Rate in the Different Materials

material	respiration rate[a] mg CO_2 g^{-1} d^{-1}	chlorpyrifos dissipation rate[b] % d^{-1}
U	3.184 ± 0.053	1.083 ± 0.175
G	1.450 ± 0.036	1.268 ± 0.044
U+C	5.184 ± 0.220	1.128 ± 0.013
G+C	4.170 ± 0.193	1.141 ± 0.043
U+S	4.324 ± 0.100	0.956 ± 0.092
G+S	3.488 ± 0.080	1.337 ± 0.158

[a] Respiration rate is expressed as the total CO_2 produced per gram sample divided by the incubation period in days. [b] Chlorpyrifos degradation rate is expressed as the total percentage of dissipation divided by the incubation period in days.

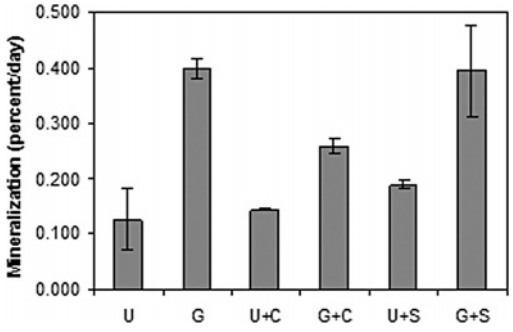

Figure 2. Mineralization rate of ^{14}C-chlorpyrifos in urban (U) and garden (G) composts alone or mixed with citrus peel (C) or straw (S). (Bars represent standard deviation of three measurements.)

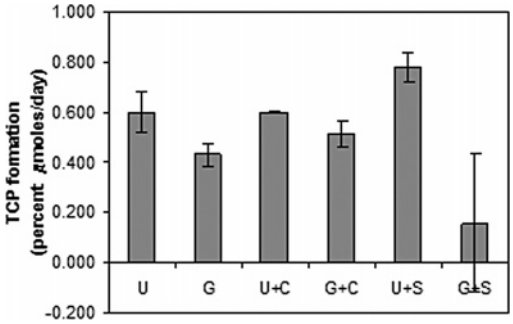

Figure 3. TCP accumulation rate in urban (U) and garden (G) composts alone or mixed with citrus peel (C) or straw (S). (Bars represent standard deviation of three measurements.)

However, some differences were observed in the mineralization rate of ^{14}C-chlorpyrifos (**Figure 2**). Despite the high standard deviation, it was possible to discern a tendency for higher mineralization rate in G-compost mixtures as compared to U-compost mixtures.

The highest mineralization rates were found in the G-compost and in the G+S mix (~0.40% per day), while the lowest value was found in the U-compost and in the U+C mix (~0.14% per day). Moreover, it was observed that the addition of citrus peel significantly decreased the mineralization rate in the G-compost as compared to G-compost alone.

For TCP, no significant differences were observed among the treatments, but a tendency for higher TCP accumulation rates was observed in the U-compost biomixes as compared to the G-compost biomixes (**Figure 3**).

Effect of the Levels of Straw on the Dissipation of Chlorpyrifos in a Typical Biobed Mixture. To assess the effect

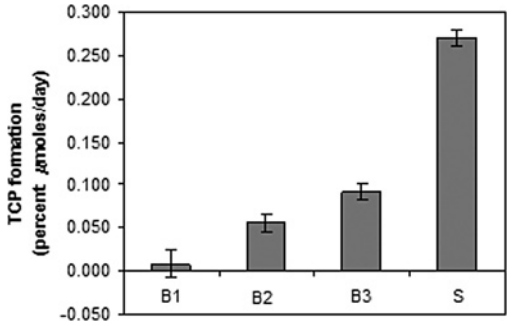

Figure 4. TCP accumulation rate in biobed mixtures with different levels of straw (S) and in the soil used in the mixtures. (Bars represent standard deviation of three measurements.)

Table 3. Respiration Rate and Chlorpyrifos Dissipation and Mineralization Rates in the Biobed Mixtures and the Soil

treatment	respiration rate mg CO_2 g^{-1} d^{-1}	chlorpyrifos dissipation rate % d^{-1}	chlorpyrifos mineralization rate % $^{14}CO_2$ d^{-1}
B1	1.899 ± 0.170	0.937 ± 0.320	0.224 ± 0.016
B2	0.557 ± 0.030	1.121 ± 0.024	0.218 ± 0.010
B3	0.432 ± 0.025	0.982 ± 0.226	0.198 ± 0.009
soil	0.028 ± 0.001	1.013 ± 0.064	0.245 ± 0.009

of straw on chlorpyrifos dissipation and TCP accumulation, we monitored changes in their concentration in a typical Swedish biobed mixture (straw−soil−peat) with increasing levels of straw (**Table 3**).

The respiration rate (1.899 mg CO_2 $g^{-1}d^{-1}$) was significantly higher in the biobed mixtures containing the highest amount of straw (B1, 50% v/v) as compared to the medium and the lower straw levels. The soil alone gave the lowest respiration rate (0.028 mg CO_2 $g^{-1}d^{-1}$).

No significant differences among the treatments were observed in the dissipation and mineralization rates of chlorpyrifos (**Table 3**).

However, a clear correlation ($r = 0.988$) was observed between the straw levels and the TCP concentration (**Figure 4**), with a lower TCP accumulation rate being observed with increasing levels of straw in the mixture. Indeed, the lowest TCP level (0.01% per day) was found in the B1 mixture containing 50% straw as compared to the B2 mixture with 25% straw (0.06% per day) and the B3 mixture with 12.5% straw (0.91% per day). The control with soil alone showed the highest TCP accumulation (0.25% per day).

Degradation of Chlorpyrifos and TCP by Manganese Peroxidase (MnP). Chlorpyrifos was degraded in both the presence and the absence of pure MnP (**Figure 5a**). However, TCP was only degraded in the presence of the enzyme, at a rate of 8.4% per day (**Figure 5b**).

DISCUSSION

Biobeds have to be adapted to Italian operating conditions, so there is a need to identify organic materials that can act in the same effective way as the original Swedish composition. Urban and garden composts alone or mixed with citrus peel or straw were used in the preliminary trials of an Italian version of the biobed, the biomassbed. Including a good selection of organic materials is important, because they determine the occurrence of microorganisms with specific catabolic activities involved in the degradation of pesticides and their metabolites.

The trials with the urban and garden composts alone were characterized by a high respiration activity. However, higher

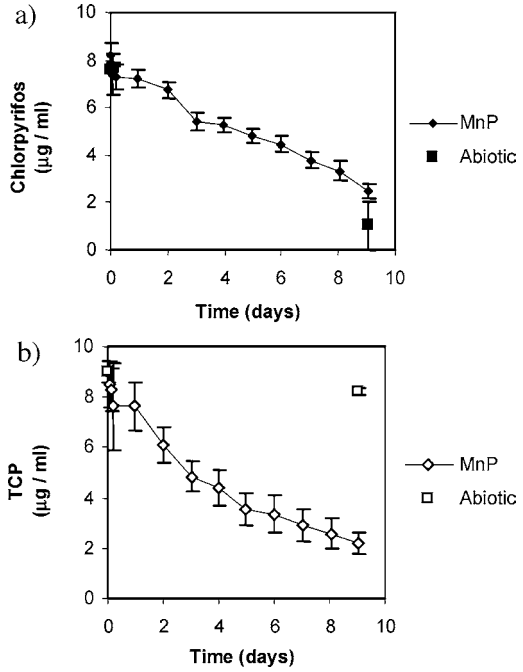

Figure 5. Degradation of (**a**) chlorpyrifos and (**b**) TCP by pure MnP in the presence of Tween 80. (Bars represent standard deviation of three measurements.)

respiration rates were observed in U-compost than in G-compost. We attributed this difference to a higher content of soluble carbon in the U-compost (0.7% dm) as compared to the G-compost (0.3% dm). When these two composts were amended with citrus peel or straw, the respiration rate increased significantly, with higher respiration observed in the presence of the citrus peel than with the straw. Again, the presence of higher soluble carbon in the citrus peel (4.0% dm) as compared to the straw (2.2% dm) probably explains this difference.

Despite the differences in respiration rate, no significant differences were observed in chlorpyrifos dissipation rate between the treatments (**Table 2**). Hence, the different composition of the substrates had no influence in the dissipation of the parent compound.

However, a tendency for different chlorpyrifos mineralization rates between treatments was observed (**Figure 2**). Chlorpyrifos mineralization was significantly higher ($p < 0.01$) in G-compost as compared to that in U-compost. In general, the treatments with G-compost showed a tendency for higher mineralization rates than the treatments with U-compost, despite the fact that the U-composts had higher respiration rates. The presence of citrus peel contributed to much higher respiration rates but did not increase mineralization of chlorpyrifos. On the contrary, the presence of the citrus peel decreased the mineralization of chlorpyrifos, as observed in the G+C treatment as compared to the G and G+S treatments (**Figure 2**). Furthermore, no correlation was found between respiration of the different treatments and chlorpyrifos mineralization rate.

In addition to their higher chlorpyrifos mineralization rate, all of the G-composts also showed a tendency for lower levels of the metabolite TCP as compared to the U-compost treatments (**Figure 3**). Overall, the G+S treatment showed the lowest levels of TCP, although values obtained were not significant due to the high SD.

A basic difference between the G- and U-composts is that the former compost is richer in lignocellulosic materials and hence in lignin (33.4% dm as compared to 21.8% dm for

U-compost). The addition of straw (30.4% lignin dm) to the composts further increased the lignin content.

According to these results, the higher chlorpyrifos mineralization rates and lower levels of TCP were correlated with the higher levels of lignin.

The major pathway of chlorpyrifos transformation involves cleavage of the phosphate ester bond, release of diethylthiophosphoric acid chain, and formation of TCP (*28*). In our study, we measured mineralization as the evolution of $^{14}CO_2$, which was assumed to have originated from the further degradation of the diethylthiophosphoric acid (**Figure 1**). All of the compost mixtures were able to dissipate chlorpyrifos, probably by releasing diethylthiophosphoric acid. However, the mixtures richer in lignocellulosic materials were more efficient at further degrading the side chain and producing $^{14}CO_2$. Moreover, these mixtures gave the smallest accumulation of TCP (**Figure 3**). It is known that TCP has antimicrobial activity (*20*, *24*) and the lower chlorpyrifos mineralization observed in the U-compost may have been due to the inhibition of diethylthiophosphoric acid degraders by TCP.

The antimicrobial properties of TCP are due to the presence of the chlorine atoms on the pyridinol ring. To break this structure, chlorine atoms have to be removed, and free chlorine is toxic to microflora (*29*). Indeed, several studies report an inhibition of chlorpyrifos-degrading microflora in the presence of its principal metabolite, TCP (*24*). As a chlorophenol, TCP degradation can be carried out by several bacteria and fungi, and it can occur aerobically (*30*) or via reductive dehalogenation (*31*), even if the latter mechanism does not always lead to a complete mineralization of the compound. Several studies report the efficacy of the lignocellulosic material in promoting chlorophenol degradation (*32*). Particularly effective chlorophenol degraders include ligninolytic microorganisms such as the white-rot fungi (*33*), which degrade lignin and produce manganese peroxidases. In the present study, the higher levels of lignin in the G-compost and the G+S mixtures may have favored the presence of lignin-degrading fungi, formation of peroxidases, and higher degradation of TCP. This in turn may have provided a less toxic environment for the growth of the diethylthiophosphoric acid degraders and their formation of $^{14}CO_2$.

However, it seems that a well-established and active lignin-degrading microflora is necessary to produce this effect, because the presence of straw in the U+S biomix was not sufficient to give lower TCP levels. The U-compost was richer in soluble carbon, and in the presence of straw the microorganisms may still prefer the more readily available carbon source.

In the experiment investigating a typical Swedish biobed mixture with different levels of straw, respiration rate was lower in the biobed mixtures as compared to the biomassbed compost mixtures due to the presence of the more complex carbon sources in the straw. However, the more striking result was that the TCP content in the mixtures was inversely correlated to the straw level ($r = 0.988$); that is, higher levels of straw gave lower amounts of TCP. The control with soil alone gave the highest amounts of TCP. Unlike the results with the compost mixes, we found no correlation between higher TCP levels and lower mineralization. However, it is important to point out that the levels of TCP in the typical biobed mixtures were much lower than the levels in the compost mixes, probably low enough to not affect mineralization.

The lower levels of TCP in the biobed mixtures may not be only a contribution from the activity of straw degraders but also from the peat, which can contribute with microorganisms able to degrade phenolic structures, or even from the soil, which even alone accumulated low levels of TCP. This particular soil has been periodically amended with straw, and this may explain its high activity toward TCP.

This second experiment clearly supported the importance of straw in getting low levels of TCP. However, to establish whether low levels of TCP were correlated with lignin degradation, we ran the trials with the lignin-degrading enzyme manganese peroxidase (MnP). In some earlier work, we had shown that lignin peroxidase and manganese peroxidase have the ability to degrade the herbicides isoproturon and bentazon (*6*, *8*, *9*) and isoproturon degradation has also been observed in straw cultures of *Phanerochaete chrysosporium* when the MnP activity is high (*6*).

Chlorpyrifos was rapidly degraded both in the presence and in the absence of MnP (60% in 9 days). Several studies have shown abiotic degradation of chlorpyrifos by chemical hydrolysis or photolysis (*19*, *21*).

On the other hand, TCP degradation only occurred in the presence of MnP, while no degradation of this metabolite occurred abiotically. The presence of Tween 80 in the reaction mixture suggests that a lipid peroxidation process may have been responsible for the degradation of TCP, as found for other pesticides (*6*, *8*, *9*).

In conclusion, chlorpyrifos is degraded efficiently without accumulation of the metabolite TCP in the presence of straw and a well-established lignin-degrading microflora. The lignin degradation system and its enzymes can degrade both chlorpyrifos and TCP. However, we cannot exclude the possibility that chlorpyrifos may also have been degraded by abiotic processes. Lignocellulosic materials such as straw and garden compost should be present in biomixes to be used in Italian conditions. Further studies should be done to adapt these results to field-scale systems.

ABBREVIATIONS USED

HPLC, high-pressure liquid chromatography; MnP, manganese peroxidase; U, urban compost; G, garden compost; C, citrus peel; S, straw; TCP, 3,5,6-trichloro-2- pyridinol; dm, dry matter.

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Supporting Information Available: Chlorpyrifos dissipation pathway (source: Racke (*16*)). This material is available free of charge via the Internet at http://pubs.acs.org.

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