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Effect of Biobed Composition, Moisture, and Temperature on the Degradation of Pesticides

MARIA DEL PILAR CASTILLO* AND LENNART TORSTENSSON

Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, SE75007 Uppsala, Sweden

Biobeds retain and degrade pesticides through the presence of a biobed mixture consisting of straw, peat, and soil. The effects of biobed composition, moisture content, and temperature on pesticide degradation were investigated in laboratory studies. Straw produced the main microbial activity in the biobed mixtures as strong positive correlations were observed between straw, respiration, and phenoloxidase content. Most pesticides investigated were dissipated by cometabolic processes, and their dissipation was correlated with respiration and/ or phenoloxidase content. More pesticides were more dissipated at biobed moisture levels of 60% water holding capacity (WHC) than at 30% and 90% WHC, while 20 °C gave higher dissipation rates than 2 and 10 °C. A straw:peat:soil ratio of 50:25:25% v/v is recommended in field biobeds since this produces high microbial activity and low pH, favorable for lignin-degrading fungi and phenoloxidase activity.

KEYWORDS: Biobeds; pesticides; degradation; white-rot fungi; phenoloxidases; manganese peroxidase; *Phanerochaete chrysosporium*

INTRODUCTION

Inadequate management of pesticides can lead to contamination of surface and groundwater. Danish (*1*−*3*), German (*4*−*7*), and Swedish (*8*) experiences have shown that point sources of pesticides often are most important for such pollution.

A major point source of contamination is spills during filling and cleaning of spraying equipment. These activities often are performed at particular on-farm sites due to the convenience of a water supply, and high concentrations of pesticide residues have been found at such sites (*1*). If spillages take place in a farmyard where the topsoil layer has been replaced by a layer of gravel and sand, there is an obvious risk of groundwater contamination from leaching.

The biobed, a simple and cheap on-farm construction intended to collect and degrade spills of pesticides (*9*, *10*), can be used to minimize the risk of pollution when handling such pesticides. In its original design, the biobed consists of a 60 cm deep pit in the ground with an impermeable clay layer (10 cm) at the base. The remaining volume is filled with a mixture of straw, peat, and soil, intended to give both sorption capacity for immobilization of pesticide spills and microbial activity able to degrade the chemicals. Finally, a grass layer covering the biobed regulates the moisture, probably by evapotranspiration, and can be used as an indicator of herbicide spills.

The composition and type of organic material present in the biobed are crucial for retention of chemicals as well as for the amount, activity, and genotypic and phenotypic versatility of microorganisms responsible for degradation of pesticides and their metabolites. A broad range of microbial activity is necessary to achieve degradation of pesticide mixtures.

Straw, topsoil, and peat are materials easily available to Swedish farmers, and a mixture of these is recommended for use in the biobed. Topsoil is rich in microorganisms and provides sorption capacity. Peat provides sorption capacity, and its water retention ability contributes to regulating the moisture in the biobed. Straw with its high lignin content stimulates growth of lignin-degrading fungi (such as white rot fungi) and formation of phenoloxidases. The lignin-degrading system of many of these microorganisms is activated by nutrient limitation, such as nitrogen or carbon deficiency. The phenoloxidases include peroxidases (e.g., manganese and lignin peroxidases) and polyphenoloxidases (e.g., laccases), which have broad substrate specificity and are able to transform a wide range of toxic compounds, including pesticides. The degradation of single pesticides by white rot fungi/peroxidases has been demonstrated in several studies (*11*−*15*). However, biobeds on farms are subjected to mixtures of pesticides.

In order to determine the optimal conditions for pesticide degradation in the biobed it is important to establish the proportions of materials in the biobed mixture giving the highest degradation efficiency. Moreover, the climate in Sweden can produce wide variation in temperatures and precipitation, and therefore, it is important to study the effect of temperature and moisture on biobed efficiency.

The present work investigated the effects of biobed composition, moisture, and temperature on the degradation of a mixture of pesticides in the laboratory. The effect of composition was studied by preparing biobed mixtures with different straw−

* To whom correspondence should be addressed. Phone: +46 18 67 32 93. Fax: + 46 18 67 33 92. E-mail: maria.castillo@mikrob.slu.se.

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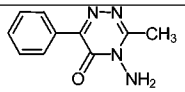
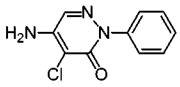
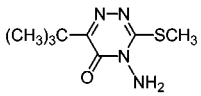
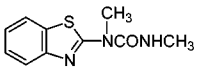
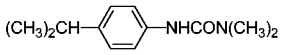
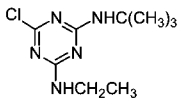
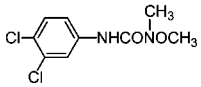
Table 1. Pesticides Used in the Study[a]

Active substance	Chemical name	Chemical formula	Water Solubility mg l^{-1}	Log Kow	Koc ml g^{-1}	Vapor pressure mPa (20°C)	Commer. name
Metamitron	4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one		1700	1.6	100	0.00086	Goltix WG
Chloridazon	5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone		340	1.19	120	<0.01	Pyramin DF
Metribuzin	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one		1050	1.6	60	0.058	Sencor
Methabenzthiazuron	*N*-2-benzothiazolyl-*N,N'*-dimethylurea		59	2.64	1100	0.0059	Tribunil
Isoproturon	*N,N*-dimethyl-*N'*-[4-(1-methylethyl)phenyl]urea		65	2.5	120	0.00315	Tolkan
Terbuthylazine	6-chloro-*N*-(1,1-dimethylethyl)-*N'*-ethyl-1,3,5-triazine-2,4-diamine		8.5	3.21	220	0.15 (25°C)	Folar 460 SC
Linuron	*N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea		63.8	3.0	400	0.051	Afalon SC

[a] Data obtained from *The Pesticide Manual*, 13th ed.; BCPC.

peat−soil proportions and spiking them with a mixture of pesticides. The degradation efficiency was assessed by monitoring the remaining pesticide concentrations and also relating them to the microbial activity as respiration and the phenoloxidase content in the biobed. Three levels of moisture (30%, 60%, and 90% of water holding capacity, WHC) and temperature (2, 10, and 20 °C) were evaluated in some of the treatments. Formulated pesticides with the active ingredients metamitron, chloridazon, metribuzin, methabenzthiazuron, isoproturon, terbuthylazine, and linuron were used as model substances. Degradation trials on single pesticides and mixtures of these were also run in pure sterile straw cultures of the white rot fungus *Phanerochaete chrysosporium*.

MATERIALS AND METHODS

Chemicals. Goltix WG, Sencor, and Tribunil were purchased from Bayer-Gullviks AB, Sweden. Folar 460 SC was delivered by Ciba-Geigy AB (Basel). Pyramin DF and Afalon SC were purchased from BASF, Germany. Tolkan was supplied by Rhône-Pulenc (Lyon).

Isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea), linuron (3-(3,4-dichloro-phenyl)-1-methoxy-1-methylurea), metamitron (4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one), methabenzthiazuron (1-benzothiazol-2-yl-1,3-dimethylurea), metribuzin (4-amino-6-*tert*-butyl-3-methyl-thio-1,2,4-triazin-5(4*H*)-one), chloridazon (5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone), and terbuthylazine (2-*tert*-butylamino-4-chloro-6-ethylamino-1,3,5-triazine) were supplied by Dr. Ehrenstorfer GmbH, Germany.

The active substances in each formulation and some of their physical and chemical properties are shown in **Table 1**.

MBTH (3-methyl-2-benzothiazolinone), DMAB (3-(dimethylamino) benzoic acid), and veratryl alcohol (3,4-dimethoxybenzyl alcohol) were supplied by Aldrich Chemical Co., Germany. All other chemicals were supplied by VWR International (former KEBO AB, Sweden).

Degradation of Pesticides in Pure Straw Cultures of *P. chrysosporium.* The white-rot fungus *P. chrysosporium* (BKM−F-1767) was used in the experiments. It was kept on malt agar slants at room temperature. A spore solution was made by adding a few milliliters of a 0.9% NaCl solution to the slants, shaking, and filtering twice through glass wool to separate mycelial residues. Malt extract (2%) was mixed with the spore solution to a concentration of 5×10^5 spores mL^{-1}. A 10 mL amount was cultured in 100 mL Erlenmeyer flasks at 37 °C for 8 days, and the mycelia were used to inoculate the straw cultures.

Wheat straw was used as a substrate. The straw was chopped and sieved (the fraction between 2 and 4 mm was used in the experiments). A 2 g amount of dry mass was weighed into 100 mL E-flasks, moistened with 3 mL of distilled water, sealed with cotton plugs, and autoclaved for 20 min at 120 °C. A nutrient solution (ammonium tartrate, manganese sulfate, and veratryl alcohol) was prepared and sterile-filtered through 0.2 μm filters, and 7 mL of this was added to the straw cultures to give a C/N ratio of 51 and a Mn and veratryl alcohol concentration of 50 and 5 $\mu g\ g^{-1}$ straw, respectively. After inoculation with the mycelia, the straw cultures were incubated at 20 °C for 8 days. On day 8, the flask cultures were divided into two sets. One set was spiked with single pesticides and the second with a mixture of pesticides.

In the first set metamitron, chloridazon, metribuzin, methabenzthiazuron, isoproturon, terbuthylazine, and linuron were added separately to the straw cultures to give a final concentration of 100 $\mu g\ g^{-1}$ straw dry weight (dw). These cultures were incubated at 20 °C for 28 days from the time of addition of the pesticides. Concentrations of the test chemicals were determined at the time of addition and the end of the incubation period.

In the second set metribuzin, methabenzthiazuron, and isoproturon were added to give 100 $\mu g\ g^{-1}$ straw dw each. The cultures were

Table 2. Some Characteristics of the Biobed Materials Used in the Experiments

material	pH	dry matter (%)	density (g L^{-1})	Org C (%)	N (%)	C/N ratio	CEC (meq 100 g^{-1})	clay (%)
topsoil	6.6	90.6	1121	1.6	0.14	12	16.2	17
straw		93.9	24.8	41.6	0.47	89		
peat	4.4	89.8	66.2	45.1	0.88	51		

Table 3. Mixture Composition (% v/v), C/N Ratio, and pH of the Different Biobed Mixtures

biobed mixture	straw (%)	peat (%)	soil (%)	C/N ratio	pH
1		10	90	14	6.5
2	60	10	30	26	6.5
3	60	30	10	43	5.5
4		90	10	42	4.7
5	60	20	20	33	5.9
6	30	60	10	43	5.0
7	30	35	35	26	5.9
8	10	70	20	35	4.9
9	50	25	25	30	5.9
10	15	30	55	20	6.0
11	25	50	25	32	5.3
12		100		51	4.4
13			100	12	6.9

incubated for 23 days from the time of addition of the pesticides, harvested on different occasions, and frozen (−20 °C) until analysis. Manganese peroxidase activity and pesticide concentrations were determined on these samples.

Biobed Materials. The materials used for the biobed mixtures (biomixtures) were soil, straw, and peat. The soil was collected from the 0−20 cm layer of an arable field in Uppsala (Ulleråker), sieved (<4 mm), and thoroughly mixed before use. Wheat straw (1−5 cm long) from a neighboring field was used in the experiments. Commercial garden peat (*Sphagnum*) was obtained from Hasselfors, Sweden. Some characteristics of these materials are shown in **Table 2**.

Biobed Mixtures and Incubation Conditions. The biobed mixtures were placed in 25 L plastic trays measuring 40 × 60 × 12 cm^3. Eleven different biobed mixtures and two controls (treatments 12 and 13 with 100% peat and 100% soil, respectively) were tested (**Table 3**). The proportion by volume of each component in the mixtures ranged from 0 to 100% except for the straw, for which the maximum proportion used in the mixtures was 60%. The final C/N ratio and pH of each mixture are also shown in **Table 3**.

With the help of the density values, the proportion (% v/v) of each component was measured. The biobed mixture was then thoroughly mixed in a cement mixer and the content placed in the 25 L plastic trays.

All test chemicals (**Table 1**) were mixed with the amount of water needed to attain the respective water content in the biobed. The concentration of each pesticide was 100 μg of active substance per gram of biobed mixture dw, so each biobed was loaded with a total of 700 μg of active substance per gram of biobed mixture dw.

Samples were taken at regular intervals (days 0, 14, 28, 42, 56, 70, 86, and 107) and analyzed for concentration of pesticides, basal respiration, and phenoloxidase content. The sampling was done by inserting a sharp hollow steel corer (2.5 cm diameter) into the biobed mixture. At least five cores were taken to collect a total of 250 g of sample for all analyses. The incubation period was 107 days.

All treatments were incubated at 20 °C and 60% WHC, except when the effects of moisture and temperature were being studied.

To study the effect of moisture on biomixture activity, three levels of moisture were tested (30%, 60%, and 90% WHC) in biobed mixtures 9 and 11 (**Table 3**). Each treatment was run in duplicate. In this study, the pesticide concentration, basal respiration, and phenoloxidase content were determined at the intervals explained above.

To study the effect of temperature on the biomixture activity, three replicates with biomixture 3 were prepared and incubated at 2, 10, and 20 °C. Pesticide concentration and phenoloxidase content were determined at the intervals explained above. Respiration was measured at 20 °C. No respiration measurements were made at 2 and 10 °C.

Basal Respiration. Biobed respiration was measured in a respirometer Respicond III (*16*) as CO_2 produced and absorbed in a 0.2 M NaOH solution. The subsequent decrease in conductivity of the hydroxide solution was used to calculate the rate of respiration. Respiration rate was then plotted against time, and the integrated area below the curve was taken to represent the total respiration during the incubation time. The size of the sample was 30 g, and the incubation time was 14 days.

Phenoloxidase Content. An extract from the biomixture samples was used for measuring phenoloxidase content. The sample (20 g) was weighed into an Erlenmeyer flask, and 40 mL of a 0.1 M succinate−lactate buffer (pH 4.5) was added. The flask was shaken at 100 rpm for 2 h, and 10 mL of the supernatant was centrifuged at 4000 rpm for 20 min. The supernatant was filtered twice, first through Whatman filter paper no. 3 and then through a 0.45 μm Schleicher & Scuell filter unit. The maximum potential activity, which is proportional to the phenoloxidase content, was measured using the MBTH/DMAB assay (*17*). This assay is normally used for measurement of Mn peroxidase (MnP) activity, but high lignin peroxidase (LiP) and laccase activity can interfere with the measurements. Because no correction was made for the possible presence of LiP and laccase activity, this measurement may represent the sum of MnP, LiP, and laccase activities and is expressed as phenoloxidase activity. The accumulated activity during the incubation period was taken as the total activity per biomixture and can be used as a measure of the phenoloxidase content in the biobeds since the assay quantifies the maximum activity under optimal conditions.

In the pure straw cultures of *P. chrysosporium* extraction was made with 40 mL of distilled water. Manganese peroxidase (MnP) activity was determined by the MBTH/DMAB assay (*17*) and lignin peroxidase by the veratryl alcohol assay (*18*). However, no lignin peroxidase activity was detected under the conditions tested and because *P. chrysosporium* does not produce laccases at the conditions tested (*19*), the activity measured in the MBTH/DMAB assay is therefore exclusively from MnP.

Analytical Procedures. The water holding capacity was measured by pouring samples of the respective biobed mixture into plastic tubes (3 cm diameter, 6 cm height) to about one-half of the height. The bottom of the tube was covered with nylon net. The tubes were left overnight in a water bath, after which they were allowed to drain freely for 5 h. The water content at 100% WHC was calculated after drying the samples at 105 °C overnight. The pH was determined in water (biomixture:water ratio of 1:2).

The concentration of the pesticides in the biobed material was measured by HPLC by a multianalysis method. A biomixture sample (10 g) was air dried at room temperature for 2 days. Methanol (25 mL) was added, and the sample was shaken for 30 min at 250 rpm. After standing overnight the sample was shaken again under the same conditions and centrifuged for 10 min (4000 rpm). The supernatant was filtered through an OOH Munktell filter paper. An aliquot (30 μL) of the filtrate was injected into the HPLC (model Waters 600 E multisolvent delivery system). The eluent was water:acetonitrile 70:30 with 2 mM sodium acetate. The flow rate was 1.8 mL min^{-1}, and the UV detector was set at a wavelength of 250 nm. A C-18 reversed phase column (Waters Rad-Pak/Nova-Pak C18-column, 100 × 8 mm) was used. The recovery efficiency of the chemicals was between 80% and 110%. The retention times for the respective pesticides were as follows: metamitron, 3.5; chloridazon, 3.80; metribuzin, 10.17; methabenzthiazuron, 13.59; isoproturon, 18.69; terbuthylazine, 39.82; linuron, 51.72.

The concentration of the pesticides in the pure straw cultures of *P. chrysosporium* was also measured by HPLC. The straw cultures were freeze dried for 48 h and extracted with 50 mL of HPLC-grade methanol, left for 15 min in an ultrasonic bath, and then gently shaken for 30 min. The samples were left overnight and shaken once again. The extracts were filtered through OOH Munktell filter paper, and 4 mL of the extracts was evaporated and dissolved in 4 mL of water and two drops of 6 M HCl. The clear phases were cleaned on a C18 column and used for HPLC analysis. The UV detector was set at 280 nm, the

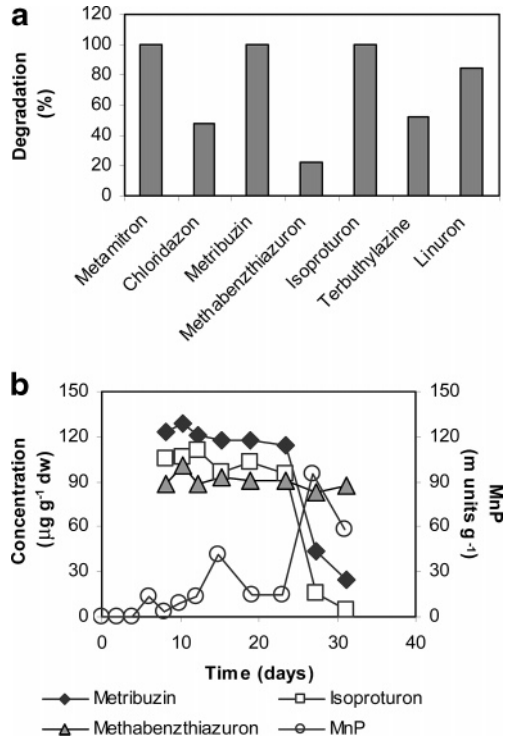


Figure 1. Degradation of pesticides in pure straw cultures of *P. chrysosporium*: (**a**) degradation of single pesticides (incubation time 28 days) and (**b**) degradation of a mixture of three pesticides (incubation time 23 days).

injection volume was 30 μL, and the flow rate was 1.8 mL min^{-1}. The column was a Waters Rad-Pak/Nova-Pak C18-column, 100 × 8 mm. The mobile phase differed among the pesticides; 2 mM sodium acetate plus different amounts (%) of acetonitrile was used for metamitron (30%), methabenzthiazuron (30%), metribuzin (30%), isoproturon (30%), chloridazon (25%), linuron (40%), and terbuthylazine (40%).

Data Analyses. The specific dissipation rate constants (SDRC) were calculated assuming that the degradation followed first-order kinetics and derived by linear regression analysis of the natural logarithm of the residual concentration against time of incubation. Significant differences from zero values were identified using the Student's *t* test ($P < 0.05$).

RESULTS

Degradation of Pesticides in Pure Straw Cultures of *P. chrysosporium*. No metamitron, metribuzin, or isoproturon residues were found in the pure straw cultures of *P. chrysosporium* after 28 days of incubation when added alone. Linuron was 84%, terbuthylazine was 52%, chloridazon was 48%, and methabenzthiazuron was 22% degraded (**Figure 1a**).

In the cultures with a mixture of three pesticides the degradation of isoproturon was not affected by the presence of metribuzin and methabenzthiazuron and no isoproturon was detected at the end of the incubation period (23 days). Metribuzin was not degraded completely as in a single culture, and 20% of it still remained in the mixed cultures. Methabenzthiazuron showed no degradation when added in a mixture compared with 22% degradation in a single culture. Moreover, a sharp decrease in the concentration of isoproturon and metribuzin coincided with the highest MnP amount in the cultures, suggesting involvement of this lignin-degrading enzyme in the degradation of the pesticides.

Effect of Biobed Composition on C/N Ratio, pH, Basal Respiration, and Phenoloxidase Content. The different biobed mixtures tested covered a broad range of C/N ratios and pH

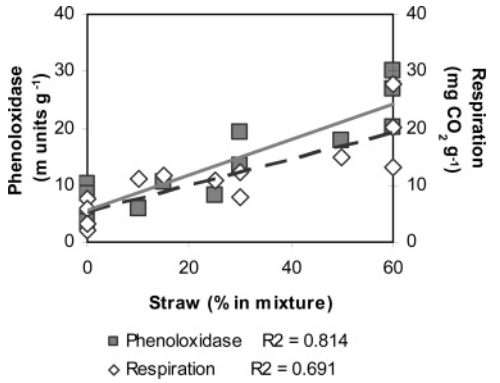


Figure 2. Significant correlations ($P < 0.05$) between basal respiration and phenoloxidase content with percentage of straw in the biobed mixture.

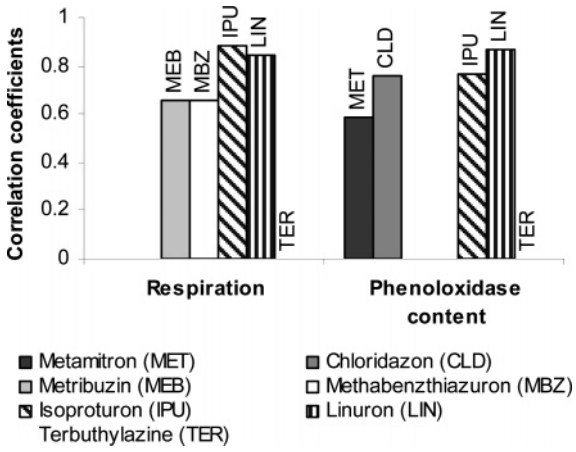


Figure 3. Correlation coefficients between specific dissipation rate constants and respiration and phenoloxidase content (significant correlation at $P \leq 0.05$).

values (**Table 3**). The highest C/N ratio (*51*) and lowest pH (4.4) were observed in the treatment with peat alone (biobed 12). Moreover, a negative correlation was found between the pH of the biomixture and the peat content ($r = -0.96$), while the C/N ratio was positively correlated with the peat content ($r = 0.80$).

No significant correlation was found between the straw content and the C/N ratio or the pH. However, basal respiration and enzyme content were positively correlated with the amounts of straw in the mixture (**Figure 2**).

Dissipation of the Pesticides in the Different Biomixtures. The dissipation rate (expressed as SDRC) was correlated with the microbial activity linked to the straw for most of the pesticides. With the exception of terbuthylazine, the dissipation of which was correlated with the peat levels ($r = 0.826$), the dissipation of all the other pesticides was correlated with either the respiration or the phenoloxidase content (**Figure 3**). Metribuzin ($r = 0.660$) and methabenzthiazuron ($r = 0.658$) SDRC were positively correlated with the respiration levels, while metamitron ($r = 0.585$) and chloridazon ($r = 0.761$) SDRC were positively correlated with the phenoloxidase content. Isoproturon and linuron SDRC were positively correlated with both respiration and phenoloxidase and showed correlation coefficients between 0.762 and 0.883 (**Figure 3**).

Effect of Moisture on Pesticide Dissipation, Respiration, and Phenoloxidase Content. The effect of moisture on the dissipation of several pesticides as well as on the basal respiration and phenoloxidase content was tested in biobed mixtures 9 and 11. Moisture gave higher dissipation rates in

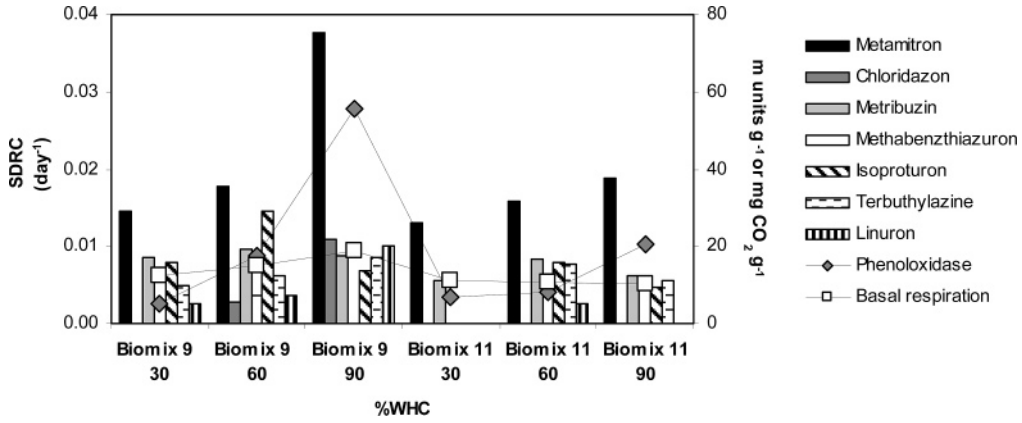


Figure 4. Effect of moisture on the specific dissipation rate constant (SDRC, days^{-1}) of each pesticide and on respiration (mg CO_2 g^{-1}) and phenoloxidase content (m units g^{-1}) in two biobed mixtures. Straw:peat:soil ratio (% v/v) 50:25:25 (biomixture 9) and 25:50:25 (biomixture 11). Incubation time 107 days at 20 °C.

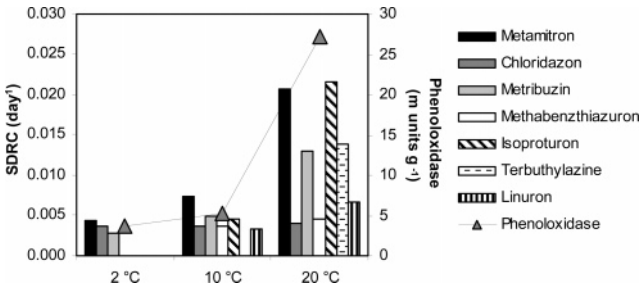


Figure 5. Specific dissipation rate constant (SDRC, days^{-1}) of the pesticides studied and phenoloxidase content at 2, 10, and 20 °C in biobed mixture 3 (straw:peat:soil ratio 60:30:10% v/v). Incubation time 107 days at 60% WHC.

biomixture 9 (straw:peat:soil ratio 50:25:25% v/v) than in biomixture11 (ratio 25:50:25% v/v). In addition, more pesticides were dissipated in biomixture 9 compared with biomixture 11, especially at the lowest WHC level of 30% (**Figure 4**). At this low level only metamitron and metribuzin were dissipated in biomixture 11. All pesticides were dissipated at 60% WHC in biomixture 9, while chloridazon and methabenzthiazuron were not dissipated at the same moisture content in biomixture 11. At 90% WHC in biomixture 9 the dissipation rate (as SDRC) of some of the pesticides increased significantly compared with that at 60% WHC, while that of methabenzthiazuron and isoproturon decreased. In biomixture 11 at 90% WHC the SDRC decreased for some of the pesticides (metribuzin, isoproturon, and terbuthylazine), while others were not dissipated at all (chloridazon, methabenzthiazuron, and linuron). Chloridazon and methabenzthiazuron were not dissipated at any WHC level in biomixture11.

Phenoloxidase content increased with increasing moisture content in both of the biobed mixtures. However, higher amounts were observed in biomixture 9 compared with biomixture 11 (**Figure 4**).

Effect of Temperature on Pesticide Dissipation, Respiration, and Phenoloxidase Content. Higher temperatures gave higher dissipation rates for most of the pesticides in biobed mixture 3 (straw:peat:soil ratio 60:30:10% v/v). At 2 °C only metamitron, chloridazon, and metribuzin were dissipated, while at 10 °C all pesticides except terbuthylazine were dissipated. The highest dissipation rates were observed at 20 °C, where all pesticides were dissipated (**Figure 5**).

Temperature had a significant effect on the phenoloxidase content. It increased 1.4-fold when the temperature increased from 2 to 10 °C and 5.2-fold when the temperature increased from 10 to 20 °C (**Figure 5**).

DISCUSSION

Basal Respiration and Phenoloxidase Content Correlated with Straw Levels. The straw produced the main activity for the dissipation of pesticides in the biobed mixture. The microbial activity measured as basal respiration and phenoloxidase content was positively correlated with the levels of straw in the biobed mixture, and the dissipation of six of the seven pesticides studied was significantly correlated with either basal respiration or phenoloxidase content.

Respiration and phenoloxidase content were positively and linearly related to each other ($R^2 = 0.488$), showing that not all respiration processes are correlated with the phenoloxidase content. For example, readily available carbon sources can contribute to respiration but do not necessarily depend on phenoloxidase content. However, cellulose and hemicellulose released after the degradation of lignin are carbon sources that contribute to respiration processes and lignin degradation is linked to the phenoloxidases. Straw contains both readily available carbon sources and those that are linked to lignin degradation, which probably explains why straw content is correlated with both respiration and phenoloxidase content.

Correlation between Pesticide Dissipation and Phenoloxidase Content and Respiration. Degradation of metamitron, chloridazon, isoproturon, and linuron was correlated with phenoloxidase content. The fact that metamitron was effectively degraded (100%) in the pure straw cultures of *P. chrysosporium*, where a significant production of MnP was observed, suggests that these enzymes may be at least partly responsible for the dissipation of metamitron in the biomixtures. This correlation with phenoloxidase content may explain the results obtained by Allen and Walker (*20*), who found that soil microbial respiration was not significantly correlated with metamitron degradation, although it is known that microorganisms are involved in the degradation of this herbicide. They concluded that the estimate of microbial activity used in their study might not have given a true indication of the activity of the microorganisms that degrade metamitron.

Degradation in soils of phenylurea pesticides such as isoproturon and linuron is considered to be cometabolic (*21*−*23*). This was confirmed by our results, where both isoproturon and linuron degradation were correlated with cometabolic processes such as those expressed in basal respiration and phenoloxidase content. Phenoloxidase activity is an expression of the cometa-

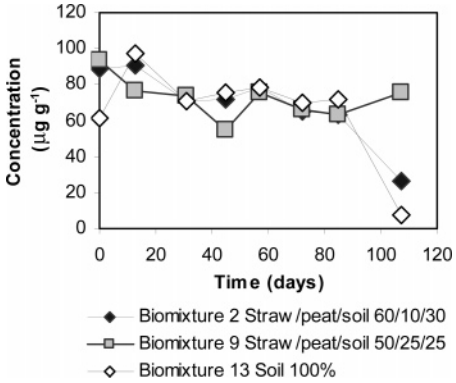


Figure 6. Chloridazon degradation in biomixtures 2, 9, and 13 (straw:peat:soil ratio 60:10:30; 50:25:25, and 0:0:100% v/v, respectively).

bolic degradation of lignin, while respiration has been used to predict cometabolic decomposition of pesticides (*24*). The pure straw cultures of *P. chrysosporium* in the present study also confirmed the effective degradation of isoproturon by fungal peroxidases such as MnP and showed that the presence of other pesticides does not interfere with its degradation (**Figure 1**). This result agrees with our earlier studies, where isoproturon concentration and MnP and lignin peroxidase (LiP) activities were monitored in sterile straw cultures of *P. chrysosporium* (*14*). Isoproturon was degraded efficiently, and both MnP and LiP activities were detected in the cultures. Further in vitro tests with both pure LiP and MnP showed that both enzymes were able to degrade isoproturon by completely different mechanisms and routes (*14*). In the present study linuron was also effectively degraded (84%) in the straw cultures of *P. chrysosporium* when added alone. However, when added together with other pesticides in the biobed mixtures, dissipation was slow. Moreover, the SDRC of linuron in the biomixtures was lower than that of the other pesticides (data not shown). Low pH values can give low linuron degradation rates, as shown by Heinonen−Tanski (*23*), who reported that in slightly acidic Finnish soils increasing the soil pH to 6.7 by liming stimulated linuron degradation rate by increasing microbial activity. Furthermore, the degradation of linuron is dependent on the herbicide concentration (*25*), and lower initial concentrations degrade more rapidly than high initial values. A combined effect of low pH, high initial concentration, and presence of other pesticides may have slowed down linuron dissipation in the biobed materials tested here.

Chloridazon dissipation was also correlated with phenoloxidase content, although low dissipation rates were observed in most of the biomixtures tested (data not shown). The pure straw cultures of *P. chrysosporium* showed that chloridazon was also degraded in these ligninolytic cultures, although at a lower rate than the other chemicals, as only 48% of chloridazon was degraded compared with total degradation of pesticides as metamitron, metribuzin, and isoproturon (**Figure 1a**). In soils, the degradation of chloridazon often occurs metabolically (*26*, *27*) as also indicated from the degradation kinetics, with increasing degradation rates in treatment 13 with only soil (**Figure 6**). However, in biomixtures containing straw/peat/soil it seems that dissipation occurs in two ways: either (a) by a slow cometabolic process correlated with straw and phenoloxidase content (biomixture 9, **Figure 6**) or (b) by a sequential cometabolic/metabolic process in which the cometabolic process (of approximately 80 days) precedes the metabolic process (biomixture 2, **Figure 6**). Lag phases of 8−10 days were observed in studies by Fan et al. (*26*), who tested the effects of the presence of other pesticides on the degradation of chloridazon in soil. Capri et al. (*27*) also showed that the lag phase occurring before the metabolic degradation of chloridazon in silty clay loam soil varied from 10 to 30 days, depending on the temperature and moisture content of the soil. Longer incubation times may be necessary to observe a metabolic degradation of chloridazon in most of the biobed mixtures in the present study. The length of the lag phase is probably negatively correlated with the initial amount of chloridazon-degrading microorganisms, the amount of which must reach a certain threshold before degradation is discernible. In addition, the low nitrogen levels in all biobed mixtures could have limited the growth of the chloridazon degraders and hence increased the lag phase.

Metribuzin dissipation was exclusively correlated with respiration activity in the biomixtures. This agrees with results of Allen and Walker (*20*), who showed that soil respiration as well as metribuzin availability in solution were important for its degradation. However, we expected its dissipation to be correlated with phenoloxidase content, as observed when it was added both alone and in a mixture in the pure straw cultures of *P. chrysosporium*, although the degradation rate was slower when it was in the mixture. From these results it seems that the presence of other pesticides may have diminished the extent of metribuzin degradation, probably by competition for the ligninolytic enzymes from the other chemicals. Similar results have been shown in other studies. For example, lower degradation rates of some pesticides in biobeds when added in mixtures compared with individually have been reported by Fogg et al. (*28*). Furthermore, when studying the effect of co-applied herbicides on the degradation rate of phenmediphan, chloridazon, and metamitron, Vischetti et al. (*29*) observed that the degradation rate of each herbicide was negatively influenced by the presence of other pesticides with similar functional groups.

Methabenzthiazuron was the least dissipated in the biobed mixtures tested (data not shown). Azam et al. (*30*) also observed that this chemical was fairly resistant to microbial transformation and that any degradation occurring was by cometabolic processes. Regardless of the reason for this slow dissipation, it could be a matter of concern if it leads to long persistence in the biobed system. The slow dissipation could be due to the high initial concentration used in this study (100 μg g^{-1}). Studies in field biobeds have shown that methabenzthiazuron concentration decreased to levels under the limit of detection in a period of 1 year. The initial concentration of methabenzthiazuron in these farm biobeds fluctuated between 0.10 and 0.22 μg g^{-1} (*9*). Regardless of this possible concentration effect, the low water solubility and high partition coefficient of methabenzthiazuron probably favor binding to the organic matter present in the biobed and thereby reduce its bioavailability but also its mobility and thereby its risk for contamination. However, further studies are required in order to optimize methabenzthiazuron degradation in biobeds in the presence of other pesticides.

The kinetics of terbuthylazine dissipation was typical first order, and the highest dissipation was observed in biomixture 12 (peat alone) (**Table 3**). The SDRC was positively correlated with the peat level ($r = 0.826$) and the C/N ratio ($r = 0.913$) but negatively correlated with topsoil amount ($r = -0.772$) and pH ($r = -0.864$). Thus, high amounts of peat decrease the pH of the biobed mixture, which favors the dissipation of terbuthylazine. However, no correlations were found with straw, respiration, or phenoloxidase content despite the fact that pure straw cultures of *P. chrysosporium* gave a 52% degradation of terbuthylazine (**Figure 1a**).

The dissipation of terbuthylazine in biobed mixtures may be explained by several processes, e.g., adsorption, chemical hydrolysis, and biological degradation. Terbuthylazine belongs to the group of triazine herbicides, which are weak bases with low water solubility and low pK_a. At low pH triazine herbicides are positively charged, giving high adsorption to soils with high proportions of organic matter (*31*). The high level of organic matter and the low pH in the biomixtures tested may contribute to the sorption of triazine pesticides; however, this may not be relevant for terbuthylazine because of its low pK_a. Chemical hydrolysis of triazine herbicides with the consequent formation of hydroxylated metabolites is also favored at low pH. For example, it is widely accepted that the atrazine dechlorination reaction in soil is a soil-catalyzed chemical process, while *N*-dealkylation reactions are biologically mediated (*32*). These chemical transformations are strongly pH dependent with both acid and alkaline conditions promoting hydrolysis of atrazine. However, it seems that biologically mediated hydrolysis can be widespread and significant in groundwater and soil (*32*). It was not possible to determine which type of process was responsible for the dissipation of terbuthylazine in the biomixtures tested on the basis of the current data, but since no correlation was observed with the phenoloxidase content or respiration, it can be speculated that other processes might have been more important.

Effect of Moisture and Temperature on Pesticide Dissipation, Phenoloxidase Content, and Respiration in Biobed Mixtures. Higher phenoloxidase contents were observed in biomixture 9, which had double the straw content of biomixture 11. However, the activity in biomixture 11 was not always one-half that in biomixture 9, as could be expected. The presence of higher levels of peat in biomixture 11 could have inhibited the phenoloxidases by several mechanisms.

Phenoloxidases can occur in free extracellular form in soils and be easily extracted by different ways, but they can also be bound and partially protected in carriers such as clay minerals, organomineral complexes, and humic acids (*33*). However, the degradation of peat produces phenols, which can act as phenoloxidase inhibitors (*11*). It has been reported that tannins and polyuronic acids of *Sphagnum* peat act as enzyme inhibitors (*34*). The presence of peat may also cause complexation of phenoloxidases with humic acids and preserve them in the soil solution, albeit with reduced activity due to the presence of tannins, polyuronic acids, and phenols (*35*). However, it has also been reported that certain humic acid fractions can inhibit phenoloxidase activity (*36*).

Peat is an important part of the biobed mixture because it can control the moisture of the system. However, it is important to distinguish between water retention and water availability. For example, peat has high water retention (high water holding capacity), but a proportion of this water may be held so tightly that it is not available for microorganisms and the degradation processes (*37*). Therefore, high amounts of peat are not recommended in biobed mixtures because of its negative effect on pesticide dissipation and phenoloxidase and respiration activities.

Different microbial environments can arise at the three moisture levels tested in this study. Low levels of moisture, such as 30% WHC, may limit the microbial activity and the amounts of pesticides in solution. Moisture levels of 60% WHC may give enough water for microbial processes, solubilization of pesticides, and pore space for oxygen to support aerobic processes. At 90% WHC, conditions near water saturation are expected with associated oxygen deficiency in the system. Under these conditions aerobic microbial processes will be limited and anaerobic processes may prevail (*38*). However, higher solubilization of pesticides can be expected.

The dissipation of pesticides with high water solubility and anaerobic degradation processes therefore probably will be favored at high WHC. This is the case for metamitron (*39*) and linuron (*23*, *40*). In contrast, metribuzin degradation appears to be slower in anaerobic conditions compared with aerobic or semiaerobic field conditions (*41*).

Aerobic environments may be more important for pesticides such as methabenzthiazuron and isoproturon, which dissipated better at 60% WHC. Degradation of isoproturon mainly occurs in aerobic environments. Little is known about the potential biodegradation of phenylurea herbicides under anoxic conditions (*42*).

Enhanced water solubilization may be more important for chloridazon and terbuthylazine, which showed higher SDRC with increasing moisture. This agrees with the results of Capri et al. (*27*) and Sahid et al. (*43*), who also, respectively, observed that higher soil moisture levels gave higher chloridazon and terbuthylazine dissipation rates.

In general, at moisture levels of 60% WHC more pesticides were degraded when the peat levels were not too high to immobilize water. Aerobic processes may be also favored at this moisture level.

Higher temperatures gave higher dissipation rates for most of the pesticides in biobed mixture 3 (straw:peat:soil ratio 60:30:10% v/v). Increasing temperatures may have enhanced the solubility of the pesticides but also microbial activity, as observed for phenoloxidase content. No effect of temperature was seen on the dissipation rate of chloridazon. Other factors may be more important for chloridazon dissipation, for example, development of chloridazon degraders, which may not be favored at the low nitrogen contents in biobed mixtures.

Composition and Conditions for Optimal Degradation of Pesticide Mixtures. The three components of the biobed mixture have an important role on the dissipation of pesticides. The straw is the main substrate for pesticide degradation and microbial activity, especially from lignin-degrading fungi, producers of phenoloxidases. The broad specificity of this system makes it suitable for the degradation of mixtures of pesticides, as shown by our results where the dissipation of most of the pesticides was correlated with phenoloxidase content and/or basal respiration. In addition, both activities increased with higher levels of straw in the biomixture. Therefore, the biobed mixture should contain high levels of straw. However, in practice it is not possible to have more than 50% straw by volume in field biobeds due to difficulties in achieving a homogeneous mixture.

The presence of peat contributes to sorption capacity, moisture control, and also abiotic degradation of pesticides, as observed for terbuthylazine. However, peat levels of 50% or more decrease microbial activity, perhaps by immobilizing water needed for microbial activity. On the other hand, too low levels of peat allow the pH of the biobed mixture to increase. Peat levels of 25% by volume give a final pH of about 5.9, suitable for lignin-degrading fungi.

The final 25% by volume in the biobed mixture is made up of topsoil, which contributes with both sorption capacity and pesticide degrading microorganisms, especially those with the ability to metabolically degrade such chemicals. However, due to the high C/N ratio in the biobed mixture (to favor lignin-degrading fungi), these processes may be restricted.

Moisture levels of 60% WHC have been shown to be optimum for the dissipation of most pesticides. Lower levels

will restrict microbial activity and solubilization of pesticides. Higher levels, near saturation conditions, may increase water solubility and bioavailability of pesticides and enzymes but may limit oxygen to support aerobic degradation. Moreover, it may create a risk for transport of chemicals outside the biobed. However, keeping the moisture at 60% WHC in farm biobeds may be a difficult task, and simple methods of controlling moisture are needed.

From the results it can be inferred that high pesticide dissipation is expected to occur during the spring and summer seasons in Sweden. Dissipation rates will decrease in the autumn, but they will be still important. During winter the dissipation will probably be limited to the more soluble pesticides.

In summary, a straw:peat:soil ratio of 50:25:25% v/v is recommended for the biomixture composition of field biobeds. Such a biobed mixture has a low pH, favorable for lignin-degrading fungi and phenoloxidase production and activity.

ABBREVIATIONS USED

SDRC, specific dissipation rate constant; MnP, manganese peroxidase; LiP, lignin peroxidase; WHC, water holding capacity; MBTH, 3-methyl-2-benzothiazolinone; DMAB, 3-(dimethylamino) benzoic acid; HPLC, high-performance liquid chromatography.

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