

Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalothrin under tropical climate conditions

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Abstract

BACKGROUND: After the rinsing of spray equipment, the rinsing water contains polluting products. One way to avoid pollution is to bring the rinsing water over a purification system, a biological bed. The system consists of an impermeable tub filled with a biomix substrate that facilitates biodegradation of pesticides. Usually, straw is one component of the biomix. The objective of this study was to assess the efficiency of an unusual substrate, bagasse, a residue of sugar cane, for the degradation of three pesticides, glyphosate, malathion and lambda-cyhalothrin.

RESULTS: Results showed that more than 99% of malathion and glyphosate were degraded in 6 months. In the biological bed, the DT₅₀ value for malathion was 17 days, for glyphosate 33 days and for lambda-cyhalothrin 43 days. The degradation rate of aminomethylphosphonic acid (AMPA) residues from the degradation of glyphosate was slower than that of the other pesticides (DT₅₀ 69 days). Finally, the innocuousness of the biomix after 6 months of degradation was confirmed by biological tests.

CONCLUSIONS: Although the degradation rates of the three pesticides in the present bagasse-based system were similar to those under temperate conditions, the degradation conditions were improved by comparison with those in soil under the given tropical conditions. Further benefits of this system are pesticide confinement, to avoid their dispersion in the environment by liquids or solids, and a lower overall cost. Finally, possibilities for optimising the bagasse-based system (e.g. management of the water content and nature of the biomix) are discussed.

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Keywords: biological bed; pesticide; biodegradation; bagasse; tropical climate

1 INTRODUCTION

Water contaminated by pesticides after the cleaning and rinsing of spray equipment is usually disposed of at one spot on the farm. This polluted water can leach out into ditches and rivers and into the water table by streaming and/or infiltration.^{1–3} There is still a risk of ground and surface water pollution, even if all precautions are taken when the tank is filled and washed.⁴ Biological systems have been developed to degrade pesticides and thus reduce the contamination of ground and surface waters. Biofilters, Phytobacs[®], biological reactors and biobeds are efficient techniques to reduce contamination generated when spray equipment is rinsed.^{1,3,5,6} For instance, in 55 days it is possible to reduce the pesticide concentration from an initial 100 000 µg L⁻¹ to 0.5 or even 0.1 µg L⁻¹.⁷ Moreover, compared with existing

industrial processes, the biological systems appear to be cheap and efficient systems suited to agricultural environmental issues.^{3,4}

The main factor determining the efficiency of biological systems is the type of substrate (biomix). A mix composed of straw, peat or compost and soil in proportions of 2:1:1 is currently used in this kind of purification system.^{4,7–13} The biomix retains pesticides, makes them available for biodegradation and creates optimal conditions for degradation by microorganisms.¹⁴ The straw serves as a substrate for lignin-degrading microorganisms. Peat or compost increases the pesticide retention capacity of the biomix, enhances microbial activity and increases water-binding capacity.^{3,4,10,15} The C/N ratio is also important for microbial activity. The optimal ratio is between 25 and 35.¹⁶ At a ratio higher than 40,

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the development of microorganisms is limited and degradation is slower.¹⁶ The type of soil texture used in the biomix appears to be of less importance.¹³ The use of local soils is thus possible and even recommended, as the soil provides an inoculum of microbiological populations suitable for degrading the pesticides normally used on the farm.³ Finally, the biomix enables the molecules to be retained and degraded more rapidly than in soil alone.^{10,12,13}

The second factor affecting the efficiency of biological systems is water availability, which is a limiting factor for pesticide biodegradation.^{8,14} Monitoring the moisture content of the biomix ensures adequate humidity.

Other factors such as pH and temperature are also important for biological activity. The optimal pH for bacterial development is between 7 and 8.5. The optimal temperature is between 20 and 30 °C.¹⁵

Biological systems have mainly been studied under temperate climates. The most common pesticides cited in the literature are isoproturon, chlorpyrifos, chlorothalonil and mecoprop.^{6–10,13,17,18} However, pollution also occurs under tropical conditions, e.g. by glyphosate or its metabolite aminomethylphosphonic acid (AMPA) and malathion,¹⁹ but no solutions, notably based on biological systems, have been tested under these tropical conditions where climate, pesticides and biomix components differ. In practice, the choice of the components of the biomix depends on locally available materials, soils and compost, which are different from those in temperate climates. Under tropical conditions, bagasse, the fibrous portion of sugar cane that remains after the juice has been extracted, is a widely available organic source that has high lignin content and could replace straw. However, the efficiency of a biomix with bagasse instead of straw has not previously been studied.

The aim of this study was to assess the efficiency of an unusual substrate, a biomix made with bagasse, with a low C/N ratio, in a biological system for the degradation of pesticides under real farm conditions. The Phytobac[®] system³ was chosen as a model because of its capacity to confine pesticides (owing to its impermeability), efficiency, ease of use and low cost. The Phytobac[®] system consists of an impermeable hole in the ground filled with a substrate composed of soil (70%) and straw (30%). In the present system, referred to as a biological bed, straw is replaced with bagasse. The authors chose to study tropical arboriculture using the pesticides glyphosate, lambda-cyhalothrin and malathion, as they have rarely or never been studied in biological systems. However, the results of the study could have an impact beyond orchards, as these pesticides are used in other types of agriculture, market gardening for example. In the present study, waste treatment corresponded to real residual quantities accumulated after 6 months of treatment on an orchard of 1 ha. The biological bed was covered and made impermeable to avoid infiltration of the pesticides into the soil and to allow

the water supply to be controlled.¹⁰ Pesticides were added only once. Degradation was monitored through monthly analyses. To check the innocuousness of the biomix with respect to current norms, and to enable subsequent application on the fields, ecotoxicological tests were performed on the biomix after 6 months of degradation.

2 MATERIALS AND METHODS

The experiment took place at the CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) research station at Vieux-Habitants, 97 119 Guadeloupe, France (longitude 61° 45' 37" W, latitude 16° 3' 58" N).

2.1 Dimensions of the biological bed

The biological bed was a hole dug in the ground with concrete walls coated with impermeable paint and topped with a metal roof. The internal dimensions were 2 m long, 1 m wide and 0.80 m deep. The biological bed was filled with biomix to a height of 65 cm, which is equivalent to a volume of 1300 L.

2.2 Biomix

The biomix used was a mixture of soil and bagasse in a proportion of 1 volume of soil to 3 volumes of bagasse. The soil was alluvial and came from a citrus orchard located at the research station. The biomix was composted for 2 months before it was added to the biological bed. The biomix had a density of 0.5 g L⁻¹; 650 kg of biomix was added to the biological bed for a volume of 1.3 m³. Before the addition of pesticides, the soil, the bagasse and the composted biomix were analysed (Table 1). Granulometry was determined according to AFNOR NF X31-107 norms and expressed as a percentage of mineral matter. Water-holding capacity (WHC) was determined according to AFNOR NF U44-170 norms (March 1981). It was expressed as a volumetric water content, by multiplying the ratio of the mass of water to the mass of dry material (soil + bagasse) by the bulk density of the latter. Organic carbon was measured by dry combustion on a Thermo-Electron CN 2100 according to NF ISO 10 694 (1995 F) norms. Total nitrogen was measured by dry combustion on a Thermo-Electron CN 2100 according to NF ISO 13 878 (1995 F) norms. The C/N ratio was then obtained by calculation. The cationic exchange capacity (CEC) was obtained according to NF X 31–130 norms (December 1999).

The theoretical values for the biomix were estimated from the data for the soil and the bagasse (Table 1). For granulometry, the estimated values for the biomix are the same as those for the soil, as the percentage was expressed as a percentage of mineral matter and there was no mineral matter in bagasse. For organic carbon and total nitrogen, which were needed for calculation of organic matter and of the C/N ratio, the values for

the biomix were estimated by means of Eqns (1) to (3):

$$X_{\text{bio}} = (X_{\text{soil}} \times d_{\text{soil}} \times V_{\text{soil}} + X_{\text{bag}} \times d_{\text{bag}} \times V_{\text{bag}}) / (d_{\text{soil}} \times V_{\text{soil}} + d_{\text{bag}} \times V_{\text{bag}}) \quad (1)$$

$$V_{\text{bag}} = 3 \times V_{\text{soil}} \quad (2)$$

Combining Eqns (1) and (2) gives

$$X_{\text{bio}} = (X_{\text{soil}} \times d_{\text{soil}} + X_{\text{bag}} \times d_{\text{bag}} \times 3) / (d_{\text{soil}} + d_{\text{bag}} \times 3) \quad (3)$$

where X_{bio} , X_{soil} and X_{bag} are the percentages of C or N in the biomix, the soil and the bagasse respectively, and d_{soil} , V_{soil} and d_{bag} , V_{bag} are the densities and volumes of the soil and the bagasse respectively. Firstly, for granulometry, it was observed that the estimated values for sand were larger than the measured values, and that the estimated values for clay and silt were consequently lower. This was probably due to the sedimentation of sand in the sample, leading to a decrease in the percentage of sand and hence to an increase in the percentage of silt and clay. Secondly, it was noted that the estimated value for organic matter was of the same order as the measured value. Both of these values were far lower than the value for bagasse. This was due to the fact that the weight ratio of bagasse to biomix was only 12.5% owing to the very low density

of bagasse (Table 1), and in spite of the fact that the volume of bagasse was 3 times higher than the volume of soil when the biomix was first made. Thirdly, a gap between the estimated and measured values of N and hence the C/N ratio was noted. The most obvious explanation was the quantity of N brought by the pesticides, but calculation showed that this quantity was too small (lower than 0.01%) to explain the gap. No other explanation can be suggested.

2.3 Pesticides

The following pesticides were chosen because they are commonly used: glyphosate (herbicide; GlyphosTM 360 g L⁻¹ SL); lambda-cyhalothrin (insecticide; KarateTM 100 g L⁻¹ EC); malathion (insecticide; JoseolTM 100 g L⁻¹ EC).

The pesticides were sprayed in the biological bed in the following amounts (C_0 , $\mu\text{g AI kg}^{-1}$ dry biomix): glyphosate $295 \times 10^3 \mu\text{g kg}^{-1}$, malathion $136 \times 10^3 \mu\text{g kg}^{-1}$ and lambda-cyhalothrin $370 \mu\text{g kg}^{-1}$. The physicochemical properties of the pesticides are listed in Table 2.

When a biological bed is used on a farm, good farming practice supposes a preliminary rinse of spray equipment in the field corresponding to a dilution of 1:5. The water resulting from the second rinse of the spray equipment should be poured into the biological bed, leading to a final dilution of 1:25.²⁰ However, in order to keep a safety margin, it was

Table 1. Composition of soil, bagasse and initial biomix

	Soil	Bagasse	Initial Biomix	
			Estimated ^a	measured
Clay %	16.9	–	16.9	25.5
Silt %	31.6	–	31.6	37.8
Sand %	51.5	–	51.5	36.8
pH	6.8	–		7.7
Maximum Water Holding Capacity (WHC) (m m ⁻¹)	0.62	0.34		0.53
Organic matter %	1.98	74.2	10.0	14.4
C %	1.15	43	5.8	8.4
N %	0.10	0.50	0.15	0.70
C/N	11.3	85.8	39.6	12.0
Cationic Exchange Capacity (CEC) me.100g ⁻¹	18.7	–		30.8
Density	1.2	0.05		0.5

^a the estimated values for the biomix were calculated from the data for the soil and the bagasse.

Table 2. Physicochemical characteristics of pesticides used^a

Pesticide	Water solubility (mg L ⁻¹)	K_{oc} (L kg ⁻¹)	DT ₅₀ (soil) (d)	Henry's constant (Pa m ³ mole ⁻¹)
Glyphosate	10 500 (pH 2)	3598	1–130	2.1×10^{-7}
AMPA	–	3640	76–240	–
Malathion	145	93–1800	1–25	2.8×10^{-3}
Lambda-cyhalothrin	5×10^{-3} (pH 6.5)	157 000	2–40	0.02

^a Source of data: **for glyphosate, AMPA and malathion:** database Agritox of AFSSA (Agence Française de Sécurité Sanitaire des Aliments) – Direction du végétal et de l'environnement, 2005 (<http://www.dive.afssa.fr/agritox/index.php>); **for lambda-cyhalothrin:** European Commission report, Directorate E – Public, animal and plant health, Unit E1 – Legislation relative to crop products and animal nutrition. Lambda-cyhalothrin 7572/VI/97-Final-25 January 2001 (http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1-24_en.pdf).

decided to maximise the pesticide concentrations. Hence, the concentrations of the pesticides that were added to the biological bed corresponded to a single rinse of the spray equipment, i.e. a dilution of 1:5. The final quantities added to the biological bed were calculated as follows: firstly, the amount of each individual pesticide remaining in the spray equipment after one treatment was determined from the concentration of each pesticide recommended by the supplier and the volume remaining in the spray equipment after treatment; secondly, this value was multiplied by the number of treatments applied on a citrus farm over a 6 month period for each pesticide, i.e. three treatments for glyphosate, one treatment for malathion and one treatment for lambda-cyhalothrin. Finally, the three pesticides were simultaneously and homogeneously sprayed onto the surface of the biological bed using a watering can. The total volume was 110 L, corresponding to a single rinse of a tank. Given the maximum water-holding capacity (WHC) of the biomix ($0.53 \text{ m}^3 \text{ m}^{-3}$), this quantity ensured aerobic conditions in the biological bed.

2.4 Measurement and calculation

2.4.1 Monitoring of pesticides

After the original application of pesticides, the concentrations of the three pesticides were measured every month up to 6 months, on 24 February, 24 March, 3 May, 26 May, 30 June and 24 July 2006. At 3 and 6 months, samples were removed from three different depths: 0–15 cm, 15–30 cm and 30 cm to the bottom. Samples were immediately frozen and sent to France for analysis. Glyphosate was extracted by water (10 g of biomix in 100 mL of water), and a 3 mL sample was taken, derivatised with 9-fluorylmethyl chloroformate (FmocCl) and then analysed by fluorescent high-performance liquid chromatography (NH_2 column $250 \times 4.6 \text{ mm}$, flow 1 mL min^{-1} , isocratic conditions: 23% v/v acetonitrile + 77% v/v water containing 0.2% H_3PO_4 , fluorimeter setting: Ex: 260 nm, Em: 310 nm). Malathion and lambda-cyhalothrin were extracted by accelerated solvent extraction (ASE 200) in warm conditions and under pressure: dichloromethane + acetone at 100°C and 120 bar. Malathion and lambda-cyhalothrin were analysed by mass spectroscopy. Extraction levels were 67% for lambda-cyhalothrin, 81% for malathion, 24% for glyphosate and 29% for AMPA. The extraction levels for glyphosate and AMPA were quite low because the extraction process from such a fibrous mixture is not as efficient for these molecules as for the other two.²¹ The laboratory in charge of analysis obtained a coefficient of variation of 16.3% for the extraction rates of glyphosate (concentrations used 10 and $20 \mu\text{g kg}^{-1}$; three replicates). Because the absolute values of the concentrations obtained for glyphosate and AMPA were not of high accuracy, the concentrations were mainly considered in relative terms, using variables based on the ratios of absolute

values (degradation rates; relative amount in each layer).

Degradation rates (K) were calculated for each pesticide in the biological bed according to the following equation:

$$\text{Ln}(C) - \text{Ln}(C_0) = -Kt \quad (4)$$

where C_0 is the initial concentration ($\mu\text{g kg}^{-1}$ dry biomix), C is the concentration at time t ($\mu\text{g kg}^{-1}$ dry biomix), K is the rate constant (day^{-1}) and t is the time (days). For each pesticide, K is the slope of the linear regression of $\text{Ln}(C)$ against t , calculated for the six sampling dates. The confidence interval for each K came from the regression analysis.

On the same basis, an apparent K (K_{app}) was estimated for each layer, using data from samples taken at 3 and 6 months, according to the following equation:

$$\text{Ln}(C_{6\text{m}}) - \text{Ln}(C_{3\text{m}}) = -K_{\text{app}}(t_{6\text{m}} - t_{3\text{m}}) \quad (5)$$

where $C_{6\text{m}}$ is the concentration at 6 months and $C_{3\text{m}}$ is the concentration at 3 months. Because only two points were available, it was not possible to calculate a confidence interval. In addition, it was difficult to compare the K_{app} for each layer with the K value for the entire biomix owing to possible pesticide transfers between layers over the course of the experiment. Hence, it is speculated that the degradation rate was probably overestimated in the surface layer (degradation plus pesticide loss by leaching) and underestimated in the bottom layer (degradation partly offset by the supply of pesticide from leaching).

Finally, at 3 and 6 months, the relative amount of each pesticide in each layer of the biomix (0–15 cm, 15–30 cm and 30 cm to the bottom) was calculated according to Eqns (6) and (7):

$$\begin{aligned} WP_i / (WP_1 + WP_2 + WP_3) = CC_i \times WB_i / (CC_1 \\ \times WB_1 + CC_2 \times WB_2 + CC_3 \times WB_3) \end{aligned} \quad (6)$$

As the heights of each layer were about the same, it was assumed that $WB_1 = WB_2 = WB_3 = WB$, giving

$$\begin{aligned} WP_i / (WP_1 + WP_2 + WP_3) = CC_i / (CC_1 \\ + CC_2 + CC_3) \end{aligned} \quad (7)$$

where WP_i is the mass of a given pesticide in layer i , CC_i is the concentration of a given pesticide in layer i and WB_i is the mass of biomix in layer i .

2.4.2 Chemical and biological characteristics of soil, bagasse and biomix

Before the pesticides were added, the carbon and nitrogen contents of the organic matter after combustion were analysed in the soil, bagasse and biomix. At 3 and 6 months after the supply of pesticides, the biomix was analysed at three different depths: from 0 to 15 cm, from 15 to 30 cm and from

30 cm to the bottom. Each sampling was repeated twice.

Before the pesticides were added, the microbial biomass in the soil and in the biomix was determined by fumigation with chloroform and extraction with potassium chloride.²² At 3 and 6 months after the addition of pesticides, the biomix was analysed at three different depths: 0 to 15 cm, 15 to 30 cm and 30 cm to the bottom. Each sampling was repeated twice.

2.4.3 Soil tension and humidity

Tensiometers were positioned in the middle of the biomix at depths of 15 and 50 cm below the surface. Pressure captors (Captors SDEC SKT850T; SDEC France, Reignac sur Indre, France) connected to a CR10X data acquisition system (Campbell Scientific, Shepshed, Leicestershire, UK) were used for automatic data acquisition. Captors were calibrated in the biomix using manual measurements of water height. Data were collected every minute and averaged over 1 h.

A relation between tensiometric values and soil humidity [Eqn (8)] was established by calculating the water volume based on five samples:

$$\text{VWC} = 1.8 \times 10^{-3} S + 0.20 \quad (8)$$

where VWC is the volumetric water content (%) and S is the tensiometer measurement (cm water). The biomix water content was estimated using this relationship.

Water was supplied regularly with a watering can to maintain humidity at about 20% of the biomix volume, corresponding to 40% of the maximum WHC (Fig. 1). A piezometer was placed in the system to check for the formation of a water layer deep in the biological bed.

2.4.4 Temperature

Temperature probes (108-005; Campbell Scientific, Shepshed, Leicestershire, UK) were positioned in the middle of the biological bed at depths of 15 and 50 cm below the surface. These probes were connected to a data acquisition system (CR10X; Campbell Scientific). Data were collected every minute, and mean temperature was calculated over 1 h.

2.4.5 Potential evapotranspiration (PET)

PET values in mm day^{-1} came from the data of the meteorological station located at the CIRAD research station at Vieux-Habitants and were calculated using the Penman–Monteith²³ formula according to the meteorological survey in Guadeloupe (CIRAD, <http://rainette.cirad.fr>)

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (9)$$

where ET_0 is the reference evapotranspiration (mm day^{-1}), R_n is the net radiation at the crop surface ($\text{MJ m}^{-2} \text{day}^{-1}$), G is the soil heat flux density ($\text{MJ m}^{-2} \text{day}^{-1}$), T is the mean daily air temperature at 2 m height ($^{\circ}\text{C}$), u_2 is the wind speed at 2 m height (m s^{-1}), e_s is the saturation vapour pressure (kPa), e_a is the actual vapour pressure (kPa), $e_s - e_a$ is the saturation vapour pressure deficit (kPa), Δ is the slope of the vapour pressure curve ($\text{kPa}^{\circ}\text{C}^{-1}$) and γ is the psychrometric constant ($\text{kPa}^{\circ}\text{C}^{-1}$).

2.5 Final innocuousness of the biomix

To check the innocuousness of the biomix after pesticide degradation, two ecotoxicological tests were carried out according to the ISO-11268-1 and ISO 11269-2 norms 6 months after the pesticides had been added.^{24,25} The first norm was an acute toxicity test on

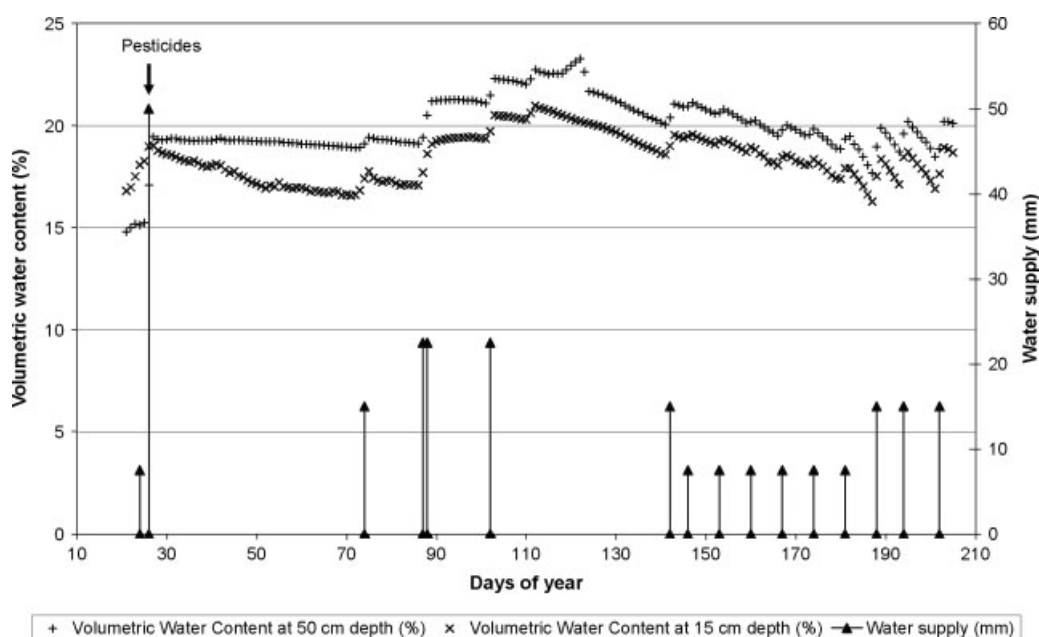


Figure 1. Volumetric water content and water supply in the biological bed during the experiment.

earthworms *Eisenia fetida* (Savigny), and the second a phytotoxicity test on *Sorghum bicolor* (L.) and *Brassica napus* (L.). The control was the biomix without pesticides. Unpaired Student tests were performed at a level of 5% to compare the biomix after 6 months of degradation, with one control in each case.

3 RESULTS

3.1 Changes in environmental conditions

3.1.1 Microbial biomass

Between the time the biomix was placed in the biological bed and 3 months after the pesticides were added, the volume of microbial biomass increased from 177 to 303 mg C kg⁻¹. No significant reduction in microbial biomass was observed 6 months after pesticides had been added, and it remained at 263 mg C kg⁻¹. This value was higher than the initial value in the biomix (177 mg C kg⁻¹), and was much higher than the microbial biomass present in the soil (51.6 mg C kg⁻¹).

3.1.2 Soil

The organic carbon content decreased regularly during the course of the experiment (Fig. 2), corresponding

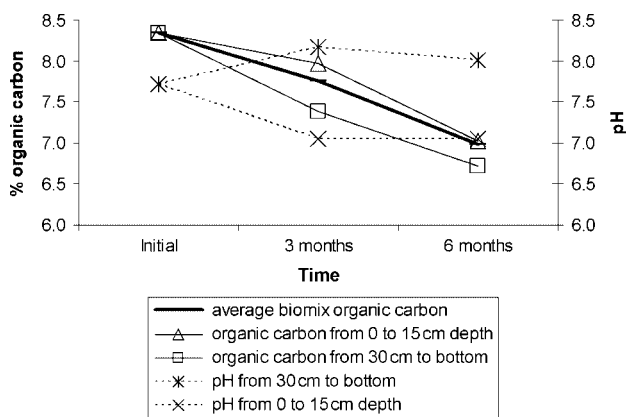


Figure 2. Changes in organic carbon and pH during the experiment.

to a loss of 0.22% per month and reflecting a continuous process of mineralisation. The C/N ratio (total carbon/total nitrogen) of the organic matter in the initial biomix was 12.0; this ratio decreased and finally stabilised after 3 months at about 10. This value is typical for such a fibrous mixture, but is lower than the optimal value for compost, which is usually between 25 and 35, as mentioned by Godden.¹⁶ The cationic exchange capacity (CEC) at 3 and 6 months remained stable throughout the experiment, at about 35 meq 100 g⁻¹.

The pH varied with depth during the course of the experiment, being lower at a depth of 15 cm than at 50 cm; the mean pH at 3 and 6 months was 7.15 (SD = 0.08) at a depth of 15 cm and 8.09 (SD = 0.08) at a depth of 50 cm (Fig. 2). These high pH values may have favoured the development of bacteria instead of fungi,¹⁴ and were probably due to the absence of peat. The consequence for pesticide degradation was difficult to assess: on the one hand, it may have favoured pesticide degradation, as bacteria are mainly involved in this phenomenon;¹⁵ conversely, it may have decreased pesticide degradation by fungi. No significant difference in soil organic carbon content with depth was observed: mean C at 3 and 6 months was 7.53% (SD = 0.13) at a depth of 15 cm, and 7.05% (SD = 0.33) at 50 cm.

3.1.3 Temperature

External mean daily temperature varied between 25 and 28 °C (Fig. 3). These temperatures were similar to the experimental incubation conditions used for the study of pesticide degradation¹⁴ and thus appeared to be favourable for the correct functioning of the biological bed. Consequently, in contrast to temperate regions, external temperature was not a limiting factor in the present study.

There was a marked increase in the temperature of the biomix, which reached 43 and 38 °C at

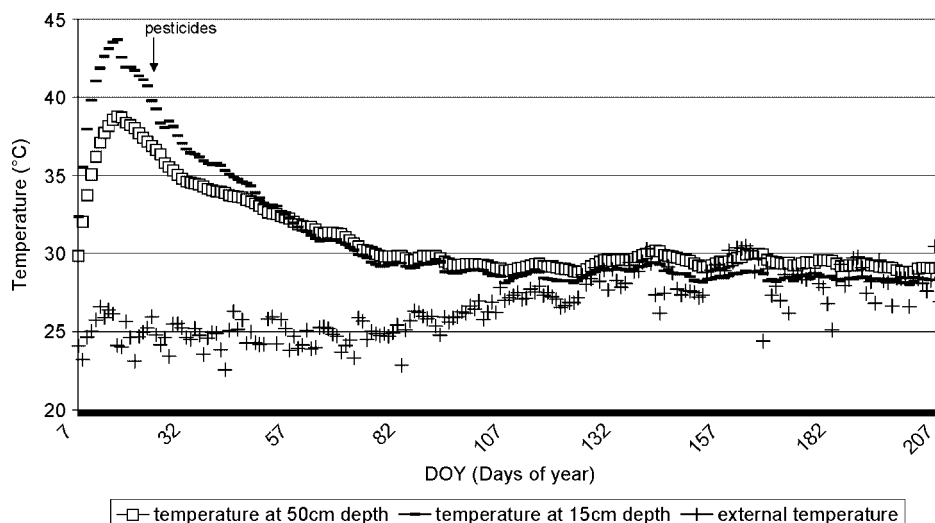


Figure 3. Changes in external temperature and in biological bed temperatures at 15 and 50 cm depth.

depths of 15 and 50 cm, respectively, 9 days after the biomix had been placed in the biological bed (Fig. 3). This rise in temperature, which corresponded to the first supply of water, was undoubtedly due to the increase in biological activity in the biomix¹⁴ and revealed that composting was still in progress. At such temperatures, enzymatic activity can be reduced because of enzyme denaturation.¹⁴ However, no analysis was performed during the temperature peak, and thus no relation could be established with a parallel peak in microbial biomass. Subsequently, 4 months after the biomix had been placed in the biological bed, the temperature of the biomix decreased and approached external temperatures. Pesticides were poured into the biomix 10 days after the temperature peak.

3.1.4 Potential evapotranspiration (PET)

Figure 4 shows that evaporation from the biological bed was lower than PET. This phenomenon can be explained on the one hand by the formation of an isolating layer, like mulch, on the surface of the biomix, which limited evaporation. On the other hand, covering the biological bed with a metal sheet protected the biomix from direct solar radiation and certainly limited evaporation. Finally, the volumetric water content fraction of the biological bed was stable throughout the experiment (on average, 20% ± 4%) (see Fig. 1).

3.2 Pesticides

3.2.1 Degradation

After 6 months of processing, the biological bed had degraded 99% of malathion and glyphosate and 90% of lambda-cyhalothrin. AMPA was the most abundant molecule after 6 months (1149 µg kg⁻¹) (Fig. 5).

Malathion was degraded the fastest. Its degradation rate (K), $40.4 \times 10^{-3} \text{ day}^{-1}$, was twice that of the other molecules (21.2×10^{-3} , 15.8×10^{-3} and $10.0 \times 10^{-3} \text{ day}^{-1}$ for glyphosate, lambda-cyhalothrin

and AMPA respectively). Given their confidence interval (Fig. 5, Table 3), there was no significant difference in the degradation speeds of glyphosate and lambda-cyhalothrin. For AMPA, the degradation rate was due to both formation and degradation processes. The degradation rate of AMPA was lower than that of the other molecules. The characteristics of the linear regression used to calculate the degradation rates were as follows: for malathion, multiple $R^2 = 0.99$, for glyphosate $R^2 = 0.89$, for lambda-cyhalothrin $R^2 = 0.84$ and for AMPA $R^2 = 0.98$.

Finally, by comparing Tables 2 and 3, an overlap was noted between on the one hand the confidence intervals of DT₅₀ values (degradation time for 50% of the initial quantity) of glyphosate, malathion, lambda-cyhalothrin and AMPA as calculated from the present results (Table 3) and on the other hand the intervals of DT₅₀ values in soil that are cited in the literature (Table 2).

3.2.2 Pesticide distribution in the biomix

At least 77% of the pesticides were concentrated in the top 30 cm of the biomix throughout the experimental period (Table 4). The main reason was that all pesticides have a high K_{oc} (the distribution coefficient of a pesticide between soil organic matter and the aqueous phase) (see Table 2). Pesticides were retained in the surface layer, and little was leached by water supplies.

Degradation was faster in the middle of the biomix than in the surface layer for all molecules. Indeed, the mean proportion of the four molecules in the middle layer of the biomix was 39.7% (SD = 5.2%) after 3 months of degradation (Table 4). In the same layer, the mean proportion of the four molecules was only 14.9% (SD = 5.0%) after 6 months of degradation. In comparison, the mean proportion of the four molecules in the surface layer was 53.6% (SD = 6.9%) after 3 months and 71.9% (SD = 9.7%) after 6 months of degradation. According to the calculation of the apparent degradation rate (K_{app} , Section 2.4.1) for each layer (Table 5), K_{app} was about twice as high in the middle layer than in the surface layer. This can be explained by the fact that the surface layer, which is like a mulch in a dry environment, was unable to ensure favourable conditions for biological activity and consequently for degradation. Finally, it is not possible to interpret the low K_{app} values in the bottom layer, as

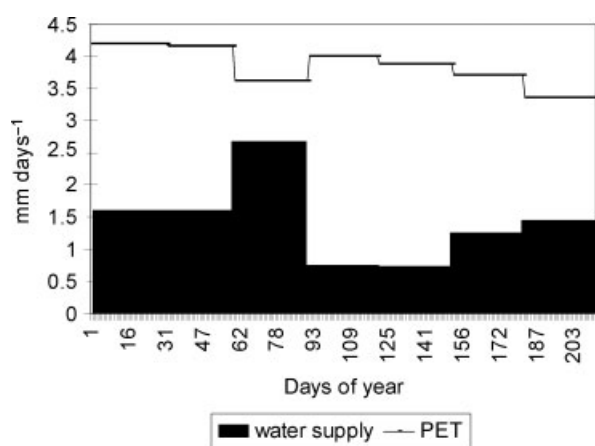


Figure 4. Comparison between PET (mm day⁻¹) and water supply (mm day⁻¹) during the experiment. A unit system in mm day⁻¹ was chosen to express and compare the two variables; for this reason, the volume of water supplied was divided by the number of days between two supplies.

Table 3. DT₅₀ and degradation rates in the biological bed^a

Pesticide	DT ₅₀ in biological bed (days)	K (day ⁻¹)
Glyphosate	33 [22; 62]	$21.2 \pm 10.2 \times 10^{-3}$
AMPA	69 [57; 88]	$10.0 \pm 2.1 \times 10^{-3}$
Malathion	17 [15; 20]	$40.4 \pm 5.6 \times 10^{-3}$
Lambda-cyhalothrin	43 [27; 110]	$15.8 \pm 9.5 \times 10^{-3}$

^a Maximum and minimum values of DT₅₀ were calculated using the confidence interval of degradation rates, K .

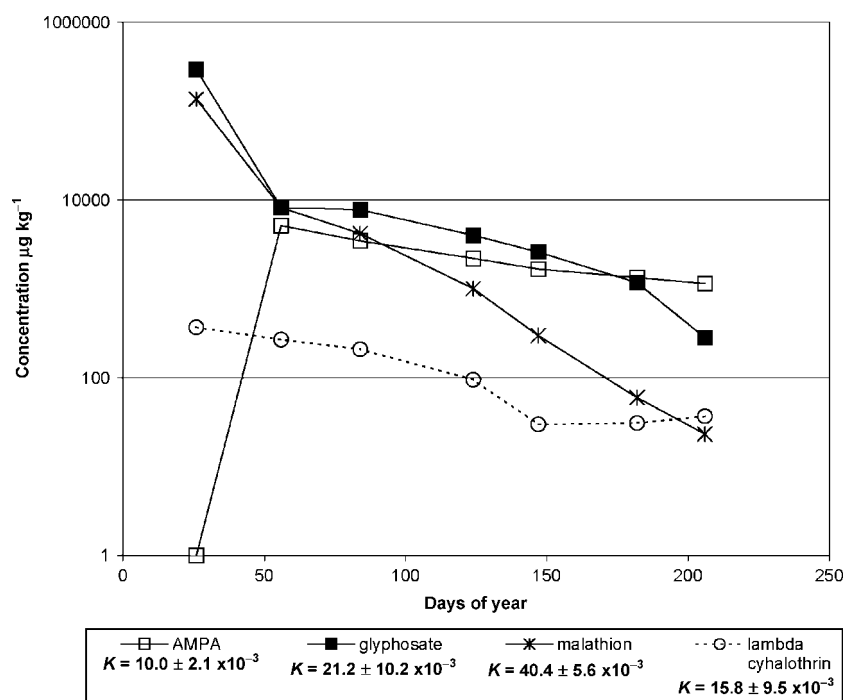


Figure 5. Pesticide degradation in the bagasse–soil biomix. For AMPA, the degradation speed resulted both from formation due to degradation of glyphosate and from real degradation of AMPA; K is the degradation rate (day^{-1}); the number after ‘ \pm ’ gives the confidence limits at a level of 95%.

Table 4. Pesticide distribution in the biological bed (amount of each pesticide in each layer as a percentage of the total of that pesticide) after 3 and 6 months of degradation

Time after pesticide addition	Depth of layer	AMPA	Glyphosate	Malathion	Lambda-cyhalothrin
3 months	Surface (0–15 cm)	42.5	44.7	72.6	54.5
	Middle (15–30 cm)	43.6	45.7	24.1	45.5
	Deep (30 cm to bottom)	13.9	9.6	3.3	0
6 months	Surface (0–15 cm)	56.3	68.1	100	63.1
	Middle (15–30 cm)	20.8	20.7	0	18.0
	Deep (30 cm to bottom)	22.9	11.2	0	18.9

Table 5. Amount of pesticide to kg of dry biomix ($\mu\text{g kg}^{-1}$) after 3 and 6 months of degradation, and apparent degradation rate ($K_{\text{app}} \text{d}^{-1}$) in each layer of the biological bed

	Depth of layer	AMPA	Glyphosate	Malathion	Lambda Cyhalothrin
Amount at 3 months ($\mu\text{g kg}^{-1}$)	Surface (0 to 15 cm)	2817	5389	2200	156
	Middle (15 to 30 cm)	2885	5508	730	130
	Deep (30 to bottom)	918	1154	100	0
Amount at 6 months ($\mu\text{g kg}^{-1}$)	Surface (0 to 15 cm)	1941	579	70	70
	Middle (15 to 30 cm)	716	176	0	20
	Deep (30 to bottom)	790	95	0	21
$K_{\text{app}} \times 10^{-3} (\text{d}^{-1})$	Surface (0 to 15 cm)	4.5	27.2	42.0	9.8
	Middle (15 to 30 cm)	17.0	42.0	80.4	22.8
	Deep (30 to bottom)	1.8	30.5	56.2	–

they were probably underestimated owing to the fact that pesticide leaching from upper layers partly offset degradation (see Section 2.4.1)

3.3 Toxicity tests

Table 6 shows that no significant differences were observed between the control biomix (without pesticides) and the biomix after 6 months of pesticide degradation, in the total mass of 14-day-old *S. bicolor*

seedlings and in the number of living earthworms *E. fetida* after 14 days in the biomix. In contrast, the total mass of 14-day-old *B. napus* seedlings was significantly higher in the biomix than in the control biomix. This was probably due to a more fibrous texture in the control biomix than in the biomix at 6 months. In conclusion, after 6 months of degradation, the biomix was not toxic to either earthworm, *S. bicolor* or *B. napus*.

Table 6. Results of toxicity tests on earthworms and seedlings. Comparison between the biomix without pesticides (control) and the biomix with pesticides after 6 months of degradation

	Control biomix (without pesticides)	Biomix after 6 months of pesticide degradation	Probability P^a
Mass of 14-day-old <i>Brassica napus</i> seedlings (g)	0.50	0.73	0.04 ^b
Mass of 14-day-old <i>Sorghum bicolor</i> seedlings (g)	0.65	0.98	0.17 ^c
Mean of live earthworms after 14 days	9.50	9.50	1 ^c

^a Unpaired Student tests were performed at a level of 5%.

^b Significant difference.

^c No significant difference.

4 DISCUSSION

In this study it has been shown that the use of bagasse compost in a biological bed system is efficient in degrading glyphosate, malathion and lambda-cyhalothrin. Notably, the biological bed degraded more than 99% of glyphosate and malathion after 6 months. Lambda-cyhalothrin had the slowest degradation rate. This can be explained by the molecular structure of lambda-cyhalothrin, which contains two aromatic cycles and many tertiary carbons. These factors do not facilitate degradation.¹⁴ Thus, lambda-cyhalothrin persisted in the environment longer than glyphosate and malathion. Its high K_{oc} strengthened this feature.

The method used to calculate the degradation rates included volatilisation processes, which may be high for malathion and lambda-cyhalothrin, as they are very volatile molecules²⁶ relative to their Henry constants (Table 2) which are higher than 2.65×10^{-5} . Conversely, according to the same criteria, glyphosate is a non-volatile molecule. Bedos *et al.*²⁷ reported that volatilisation can be limited by the availability of the molecule, which depends on its fixation on the substrate. Fixation in the biomix is facilitated by its high organic matter content. Thus, volatilisation should be limited in the case of lambda-cyhalothrin because its K_{oc} is the highest ($157\,000 \text{ L kg}^{-1}$) (Table 2). However, for malathion, which has the lowest K_{oc} value, the volatilisation process should artificially increase the calculated degradation rate. Anyway, the pesticide degradation rate in the biological bed was similar to data on degradation in soil that are cited in the literature. Thus, in comparison with average soil conditions, the biological bed did not accelerate pesticide degradation.

The maximum total biomass carbon observed in the biological bed was 300 mg C kg^{-1} , which corresponds to data observed in poor sandy soils (200 mg C kg^{-1}), as reported by Chaussod *et al.* in Calvet *et al.*,¹⁴ whereas values in soils considered to be rich in organic matter are about 900 mg C kg^{-1} . However, 300 mg C kg^{-1} is higher than the value in the soil used for the biomix, which was 50 mg C kg^{-1} . This suggests an improvement in degradation conditions in comparison with direct supplies in the alluvial soil. This content is also comparable with that in cultivated andosols rich in organic matter (carbon content in andosols

3–10%; total biomass carbon content between 100 and 300 mg kg^{-1}).²⁸

There are several possible explanations for the limited efficiency of the biological bed. The first explanation is related to the biomix used. The use of local material resulted in a biomix with a C/N ratio of 12, i.e. lower than the optimal values (25–35) for a compost cited by Godden.¹⁶ Additionally, notwithstanding a favourable effect on bacterial development, the high pH of the biomix was detrimental to fungal activity, and probably resulted in an overall restricted pesticide degradation by microorganisms, in spite of the use of lignin-rich bagasse substrate which should have favoured the development of fungi. The second possible explanation is that temperature can influence the degradation rate of each molecule. However, given the temperatures recorded in the present study, conditions appeared to be favourable for correct functioning of the biological bed, as the pesticides were added after the temperature peak that occurred after the biomix had been placed in the biological bed. The third possible explanation is the difficulty in maintaining suitable humidity in the biological bed. Firstly, humidity was probably not sufficient: 40% of WHC in the present experiment, which is lower than the 50% of WHC considered to be optimal by Henriksen *et al.*¹⁰ Secondly, humidity was not homogeneous: simultaneous drying out of the surface layer and accumulation of surplus water in the deep layer were observed. This phenomenon can be explained by the hydrodynamic characteristics of the biomix, which is a highly porous medium with a high drainage capacity but no capillary rise capacity. As a consequence, because pesticides were preferentially fixed in the surface layer, the least favourable degradation conditions occurred in the layer in which the pesticides were preferentially fixed.

It may be possible to improve the system. Firstly, the biomix can certainly be improved, as values of about $600 \text{ mg biomass C kg}^{-1}$ have been observed in soils in Guadeloupe.²⁸ One option consists in increasing the C content in the organic matter, which would improve biological activity.³ Adding bovine, caprine or poultry manure or even vegetable waste compost to the soil + bagasse mixture in a proportion of 1:1:2 (manure or compost:soil:bagasse) could increase the organic matter carbon content, which would favour

microbial activity while maintaining a neutral to slightly basic pH, which is optimal for bacterial development.^{15,29} De Vleeschouwer *et al.*³⁰ showed that the organic component of the manure mix was more efficient than vegetable waste compost in terms of pesticide retention and degradation.

Secondly, management of the water content of the biomix can also be improved. One solution may be regularly to mix the biomix to obtain a homogeneous water content in the biological bed and good aeration, which could improve microbial activity. Another solution would be to compact the biomix to reduce macroporosity and facilitate capillary rise. A third solution would be to grow grass on the surface, which would mobilise the water at the bottom of the biological bed. This would be particularly important if a bigger waste treatment capacity were necessary.

5 CONCLUSIONS

This study has demonstrated that the use of a bagasse-based biomix in a biological bed system can degrade glyphosate, malathion and lambda-cyhalothrin, which are widely used pesticides even under tropical conditions. The results showed that more than 99% of malathion and glyphosate were degraded after 6 months. The degradation rate of these two molecules was higher than that of lambda-cyhalothrin, with a mean DT₅₀ of 17 days in the biomix for malathion, 33 days for glyphosate and 43 days for lambda-cyhalothrin. Finally, the degradation rate of AMPA was the slowest of all the pesticides tested (69 days). After 6 months, AMPA predominated in the biomix at a concentration of 1149 µg kg⁻¹, as opposed to 283 µg kg⁻¹ for glyphosate and 30 µg kg⁻¹ for the two others. As under tropical climates the outside temperature rarely drops below 15 °C, it was hypothesised that temperature was not a limiting factor for pesticide degradation. In spite of these favourable conditions, pesticide degradation in a biological bed did not appear to be higher than European references. The reasons are probably related to (i) the nature of the biomix, (ii) the insufficient water content of the biomix, (iii) the disparity in degradation conditions and pesticide distribution with depth (the degradation rate was higher in the middle layer than in the surface layer where pesticides were preferentially fixed and where the environment was drier) and (iv) possibly excessively high temperatures that were destructive for enzymes. Mixing the biomix might improve this system, as might adding bovine, caprine or poultry manure or vegetable waste compost. Other advantages of this system are its capacity to confine pesticides, and thus avoid their dispersion in the environment by liquids or solids, and its low cost. Finally, the innocuousness of the biomix after 6 months of degradation was confirmed by biological tests. Therefore, the use of bagasse in a biological bed system in tropical areas appears to be an efficient, practical and ecological way to reduce farm pollution.

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