Research Article

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# Degradation of isoproturon and bentazone in peat- and compost-based biomixtures

**Laura Coppola,[a*] Maria del Pilar Castillo[b] and Costantino Vischetti[a]**

## Abstract

**BACKGROUND: The composition and properties of a biomixture used in a biobed are decisive for pesticide sorption and degradation. This study was performed to investigate the capability of compost-based substrates in mixtures with citrus peel and vine branch straw and peat-based substrates in mixtures with soil and vine branch straw at different levels in order to degrade isoproturon and bentazone.**

**RESULTS: Dissipation and mineralisation rates of both pesticides were determined, and metabolic activity was followed as respiration. Compost-based substrates showed faster pesticide dissipation in the presence of lignocellulosic materials, as in garden compost and vine branch straw. The increasing content of vine branch straw in peat-based substrates does not seem to affect dissipation of the parent compounds. Low mineralisation rate was observed in all treatments.**

**CONCLUSION: Higher pesticide degradation was observed in the lignocellulosic substrates, probably because of the development of lignin-degrading microorganisms which have shown to be robust and are able to degrade recalcitrant pesticides.**


**Keywords:** biodegradation; mineralisation; compost; biobed; isoproturon; bentazone

## 1 INTRODUCTION

Unsatisfactory management of pesticides and other chemicals can give rise to residues in surface water and groundwater and in large volumes of soil. One of the main sources of contamination is represented by the filling and cleaning of agricultural spray equipment and accidental spillages at farm level.[1,2] To prevent pollution from this kind of contamination, Torstensson and Castillo[2] proposed biobed systems that aimed at retaining and degrading pesticides. A biobed should have three important characteristics: high sorption capacity; microbial pools to degrade pesticides; organic carbon sources to support microbial activity. Such systems, simple in design, employ inexpensive and easily available organic biomixtures. The desired outcome of biobed utilisation is the sorption and complete mineralisation of the parent compound. However, incomplete mineralisation can also happen, leading to metabolites that may be more persistent or even more toxic than the original compound.[3]

Different organic biomixtures can be introduced in a biobed. In its original design, the biobed mixture, used in several northern European countries, consisted of straw (wheat or barley), peat and soil. Straw is the main substrate for pesticide degradation and microbial activity, especially of lignin-degrading fungi (e.g. white rot fungi) producing phenoloxidases (i.e. peroxidases and laccases). Peat contributes to sorption capacity, moisture control and also abiotic degradation of pesticides. Finally, soil provides sorption capacity as well as microorganisms other than ligninolytic fungi that are capable of degrading pesticides.[4,5]

In southern European countries, peat is not easily available, and research has been carried out in order to demonstrate that urban and garden compost could be an effective substitute.[6,7] Compost-based substrates are known to host several microorganisms with different pesticide-degrading activities, and they have also been demonstrated to have a good sorption ability for a wide variety of pesticides, with a retention capacity of over 90%.[8]

The herbicides isoproturon, a phenyl urea, and bentazone, a diazinone, are widely used in agriculture to kill a large spectrum of weeds, and are also often detected in surface water and groundwater at concentrations higher than the EU limit for drinking water of 0.1 $\mu g\ L^{-1}$.[9–11]

Isoproturon is a hydrophobic pesticide that is slightly soluble in water (70 $mg\ L^{-1}$ at 25 °C) and has a polar reactive group. Isoproturon half-life in soil was estimated to be 12 days in the laboratory at 20 °C. It is a rather mobile pesticide with a $K_{oc}$ of 122 $L\ kg^{-1}$.[12] Reuter and Scheunert[13] have reported that isoproturon forms a considerable amount of unextractable residues in soil, the levels of which vary depending on the humification of organic matter.

Bentazone is an acidic herbicide that is ionised in aqueous solution to form anionic species. The FOOTPRINT Pesticide Properties Database[12] reported bentazone's half-life in soil as

* Correspondence to: Laura Coppola, Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy. E-mail: l.coppola@univpm.it

a Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Ancona, Italy

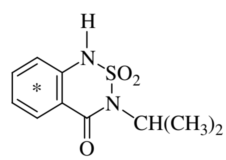
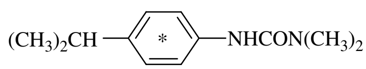
b JTI – Swedish Institute of Agricultural and Environmental Engineering, Uppsala, Sweden

**Table 1.** Composition and properties of the materials used in the study

| Material | Symbol | Composition (% v/v) | C (%) | N (%) | C/N | pH | Soluble carbon (% dm) | Lignin (% dm) |
|---|---|---|---|---|---|---|---|---|
| Urban compost | A1 | 100 | 30.2 | 2.40 | 13 | 7.97 | 0.7 | 21.8 |
| Garden compost | A2 | 100 | 32.8 | 2.34 | 14 | 8.18 | 0.3 | 33.4 |
| Citrus peel | C | | | | | | 4.0 | 1.4 |
| Straw (vine branch) | S | | | | | | 2.2 | 30.4 |
| Urban compost + citrus peel | A3 | 87.5 : 12.5 | 32.4 | 2.60 | 12 | 6.73 | | |
| Garden compost + citrus peel | A4 | 87.5 : 12.5 | 31.8 | 2.26 | 14 | 6.41 | | |
| Urban compost + straw | A5 | 50 : 50 | 39.4 | 1.96 | 20 | 7.44 | | |
| Garden compost + straw | A6 | 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 | | |

**Table 2.** Pesticides used in the study

| Active substance | Chemical name | Chemical formula[a] | Water solubility[b] (mg $L^{-1}$) | $K_{oc}$[b] (L $kg^{-1}$) | $DT_{50}$[bc] (days) |
|---|---|---|---|---|---|
| Bentazone | 3-Isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide |  | 570 | 51 | 45 |
| Isoproturon | *N*,*N*-Dimethyl-*N*′-[4-(1-methylethyl)phenyl]urea |  | 70 | 122 | 12 |

[a] * = $^{14}C$ labelled.
[b] Data from Footprint.[12]
[c] Time for 50% decomposition in soil in the laboratory at 20 °C.

45 days in the laboratory at 20 °C. Bentazone is considered to be a mobile pesticide with a $K_{oc}$ of 51 L $kg^{-1}$.

Several studies have reported the ability of biobed mixtures to degrade isoproturon and bentazone. In particular, it has been observed that the degradation of isoproturon and bentazone is related to fungal-lignin degrading activity,[14,15] which increases as a result of the presence of straw.[4] On the other hand, Fogg *et al.*[16] have reported the effect of initial concentration on pesticide degradation rate in biobeds composed of topsoil, compost and wheat straw. They found that isoproturon degradation was quicker in topsoil alone than in biomixtures, probably because previous treatments of isoproturon applied to this soil, as part of normal agricultural practices, have resulted in the proliferation of microbial communities specially adapted to use isoproturon as an energy source.

The present work was carried out to assess the degradation of isoproturon and bentazone in peat- and compost-based biomixtures, and to assess the effect of adding different carbon sources on the degradation of the two pesticides. In particular, peat-based biomixtures, typical of northern Europe, were studied by varying the relative content of straw and soil. On the other hand, urban and garden compost were used to create biomixtures that are typical of southern Europe. Two different carbon sources, i.e. citrus peel and straw, for the activation of compost biomass were compared. In order to assess the amount of pesticide degradation, mineralisation was measured as $^{14}CO_2$ evolved in the different substrates over time. In addition, basal respiration in biomixtures was followed during the experiment to check the behaviour of the microbial biomass activity.

## 2 MATERIALS AND METHODS

### 2.1 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. Chopped 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 1.6% organic carbon, pH 6.6, collected in Uppsala, Sweden. The peat mould (*Sphagnum*) came from Econova Garden AB, Sweden. All 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 and soluble carbon and lignin content of each material and their mixtures are presented in Table 1.

### 2.2 Chemicals

The pesticides used in this study and some of their properties are shown in Table 2. The unlabelled isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] and bentazone [3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide] were supplied by

Sigma Aldrich, Riedel-de-Haëh, Germany. Both chemicals had >99% purity. The $^{14}C$-isoproturon and $^{14}C$-bentazone with a purity of >97% were supplied by the Institute of Isotopes Co., Budapest. Insta-Gel Plus was provided by Perkin Elmer, USA. All the other chemicals used in this work were supplied by VWR International AB.

### 2.3 Degradation of isoproturon and bentazone in compost-based and peat-based biomixtures

The dissipation of isoproturon and bentazone was monitored by measuring the mineralisation of $^{14}C$-isoproturon and $^{14}C$-bentazone over time and the respective pesticide concentration at the beginning and at the end of the incubation period. The microbial activity was measured as $CO_2$ production.

The compost-based biomixtures tested in this trial consisted of urban or garden compost alone or were mixed with citrus peel or straw as in the following treatments: (a) urban compost (A1); (b) garden compost (A2); (c) urban compost plus citrus peel (A3); (d) garden compost plus citrus peel (A4); (e) urban compost plus straw (A5); (f) garden compost plus straw (A6). The citrus peel was added in a proportion of 12.5% v/v and the straw at 50% v/v (Table 1).

The peat-based biomixtures 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 also run as control (Table 1).

Triplicates of each mixture were used. Controls consisting of the respective biomixtures without addition of pesticides were run.

All the materials were passed through a 2 mm sieve and thoroughly mixed. Triplicates of each biomixture sample (2 g) were weighed into 100 mL plastic jars. The samples were spiked with isoproturon (cold, 100 μg g$^{-1}$ and $^{14}C$-labelled, 126 000 dpm) and bentazone (cold, 100 μg g$^{-1}$ and $^{14}C$-labelled, 44 000 dpm) in separate trials. The pesticides were added as methanol solution, as suggested by Brinch *et al.*,[17] by treating a subsample (25%) with each herbicide, mixing thoroughly, allowing the solvent to evaporate and finally mixing again with the remainder of the sample. Controls consisting of the respective biomixtures without any pesticides were also run. The water content was kept at 60% of the water-holding capacity.

The plastic jars were fitted 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 mineralisation and respiration were measured.

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

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) was used for the titrations. The respiration rate was expressed as accumulated mg $CO_2$ g$^{-1}$ biomixture.

For each pesticide in each biomixture studied, the incubation period was interrupted when no significant metabolic activity was revealed in the substrates. For this reason, the incubation period was 35 and 28 days for the isoproturon and bentazone trials respectively in the compost-based biomixtures, and 36 days for both isoproturon and bentazone trials in the peat-based biomixtures.

At the end of the trials, all samples were analysed to determine the final isoproturon and bentazone concentrations. Data were expressed as percentage of the pesticide degraded at the end of the experiments.

### 2.4 Analytical methods

Isoproturon and bentazone were determined by extracting the samples overnight with 6 mL methanol g$^{-1}$ substrate on a shaking table. Samples were centrifuged at 358 × *g*, and the solution was collected. The extraction was repeated for two more 30 min periods.

A quantity of 1 mL of the pooled extract was centrifuged at 5411 × *g* for 15 min. The clear supernatant was analysed by high-performance liquid chromatography (HPLC) using the Agilent 1100 series equipped with a variable-wavelength UV detector, and performed with C18 columns, Zorbax SB-C18 5 μm, 46 × 150 mm.

Isoproturon was detected using a mobile phase of 50% $CH_3CN$ and 50% ultrapure water with a flow of 1.2 mL min$^{-1}$ for 20 min and a detection wavelength of 240 nm.

The mobile phase for bentazone was 90% $KH_2PO_4$ and 10% $CH_3CN$ with a flow of 1.0 mL min$^{-1}$ for 20 min and a detection wavelength of 230 nm. The isoproturon and bentazone recoveries were >93%.

### 2.5 Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) using SAS (v.8.2), and least significant differences (LSDs) were derived at $P = 0.05$.

## 3 RESULTS AND DISCUSSION

Degradation and mineralisation rates of isoproturon and bentazone were investigated in two biomixture groups. Also, to assess the impact of the pesticides on microbial activity, basal respiration with and without application of pesticides was followed. The compost-based biomixtures (A-biomixtures) consisted of urban and garden compost alone or were mixed with citrus peel or vine branch straw. However, the peat-based biomixtures (B-biomixtures) consisted of peat mixed with different levels of vine branch straw and soil.

Isoproturon and bentazone degradation in the A- and B-biomixtures are shown in Fig. 1. Results refer to the percentage of pesticide disappearance after 35 days for isoproturon and after 28 days for bentazone in compost-based biomixtures, and after 36 days for both pesticides in peat-based biomixtures. Generally, higher specific degradation was observed in the compost-based biomixtures compared with the peat-based biomixtures, even if in the bentazone treatments the experimental time differed between compost- and peat-based substrates by 8 days. The specific degradation percentage of isoproturon in the compost-based biomixtures ranged between 27 and 85%, compared with a 27–33% range in the peat-based biomixtures (20% soil alone). Meanwhile, the specific degradation rate of bentazone ranged between 6 and 85% in the compost-based biomixtures and between 36 and 38% in the peat-based biomixtures (19% soil alone).

Within the compost-based biomixtures, a tendency for higher degradation of isoproturon in garden compost mixtures compared

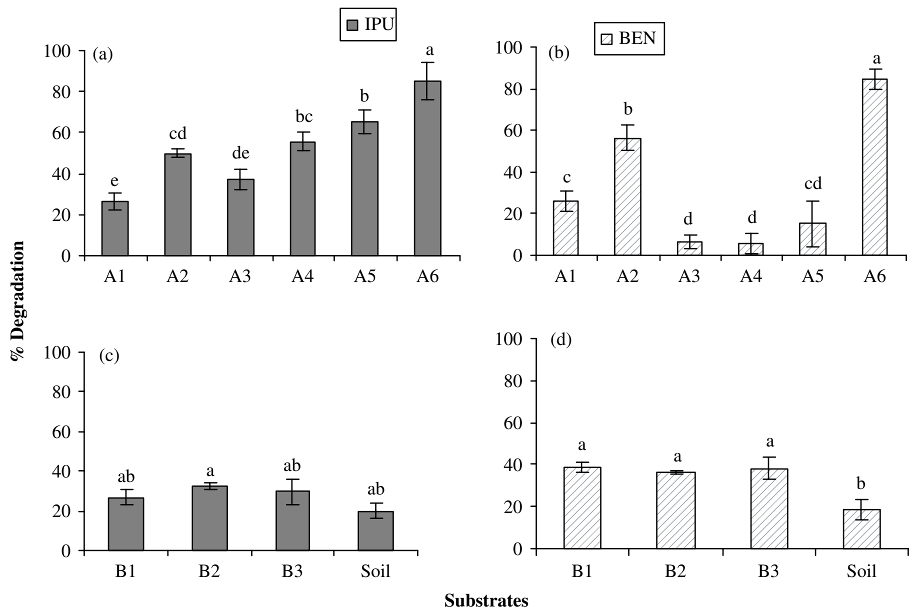



**Figure 1.** Percentage degradation after an incubation period of 35 days for IPU (a) and 28 days for BEN (b) in compost-based substrates, and 36 days for both IPU (c) and BEN (d) in peat-based substrates. Urban compost alone (A1) and mixed with citrus peel (A3) or vine branch straw (A5); garden compost alone (A2) and mixed with citrus peel (A4) or vine branch straw (A6); biobed mixture at different levels of straw: 50% (B1), 25% (B2), 12.5% (B3). Lower-case letters represent LSD at $P < 0.05$. IPU = isoproturon; BEN = bentazone.

with the respective urban compost mixtures was observed (Fig. 1a). Indeed, LSD analysis showed significant ($P = 0.05$) differences between A1–A2, A3–A4 and A5–A6 substrates. The addition of straw and citrus peel increased the degradation in both compost mixtures, showing a higher effect of straw than of citrus peel (A3–A5 and A4–A6 substrates).

A broad microbial pool was able to degrade isoproturon, as all A-composts showed degradation ability. However, the presence of lignocellulosic materials, as in the garden compost and in straw, may provide specific microflora and enzymatic activity that enhance isoproturon degradation. Indeed, these results are in accordance with several works[6,15,18] where the importance of the presence of lignocellulosic materials in biomixtures to degrade this pesticide has been demonstrated.

A different trend was observed for bentazone, where not all biomixtures showed their ability to degrade it (Fig. 1b). The highest degradation was observed in garden compost alone (A2, 56%) and in the garden compost amended with straw (A6, 85%), while the lowest degradation was observed in biomixtures containing citrus peel (A3, 6%; A4, 6%). Again, the presence of lignocellulosic materials seems to play an important role in the degradation of bentazone, which may be due to the presence of fungal lignin-degrading enzymes, as shown before by Castillo *et al*.[19,20]

Within the peat-based biomixtures, no significant differences were observed between treatments in the degradation of the two herbicides. The increasing content of vine branch straw in the peat-based substrates does not seem to affect degradation, as no significant differences between B1, B2 and B3 were observed for either pesticide, which could indicate that, in the present work, 12.5% of vine branch straw is sufficient to degrade isoproturon and bentazone. These results do not agree with previous work by Castillo and Torstensson,[4] which showed that increasing levels of straw correlated positively with isoproturon degradation. However, the incubation time (107 days) in that work was longer than the incubation time in this study. Moreover, the biomixture contained wheat straw. This suggests structural differences between the vine branch and wheat straw, as observed for other herbaceous crops.[21] Lignin structure, cellulose and hemicelluloses levels, monomeric content and composition are some of the factors that can have an effect on the ability of the biomixture to degrade pesticides because they affect the level of lignin-degrading enzymes. It is thus probable that the metabolic activity of microorganisms in vine branch biomixtures is lower than that in wheat straw biomixtures.

For a better understanding of the disappearance mechanisms of isoproturon and bentazone from the studied substrates, mineralisation expressed as $^{14}CO_2$ that evolved during incubation is reported in Fig. 2. Mineralisation of bentazone and isoproturon in this study was always low. This may be because mineralisation might have been impeded by a cometabolic step, with the formation of $^{14}$C-metabolites, which could be more easly bound to organic matter and be less available for degraders.[22–25]

In spite of low mineralisation, significant differences were found for isoproturon within compost-based substrates. Isoproturon was mineralised faster in the lignocellulosic substrates as those containing garden compost (∼5%) and compared with the ones containing urban compost (∼3%). This may be due to microbial production of some lignin-degrading enzymes that are involved in mineralisation and are able to degrade the aromatic ring, as reported by other authors.[15]

In the peat-based biomixture, isoproturon mineralisation was mainly determined by the activity in the soil, as the control with soil alone gave a high value of 8%, and B1, which contained a lower amount of soil (12.5%), gave a lower value of 5%. This might be explained by the fact that the soil used in this study had a remarkable microbial activity that was able to degrade even high-molecular-weight polyaromatic hydrocarbons,[26] and that may have developed after a long-term treatment with straw.

No significant bentazone mineralisation was observed in the compost-based biomixtures, even though high degradation was

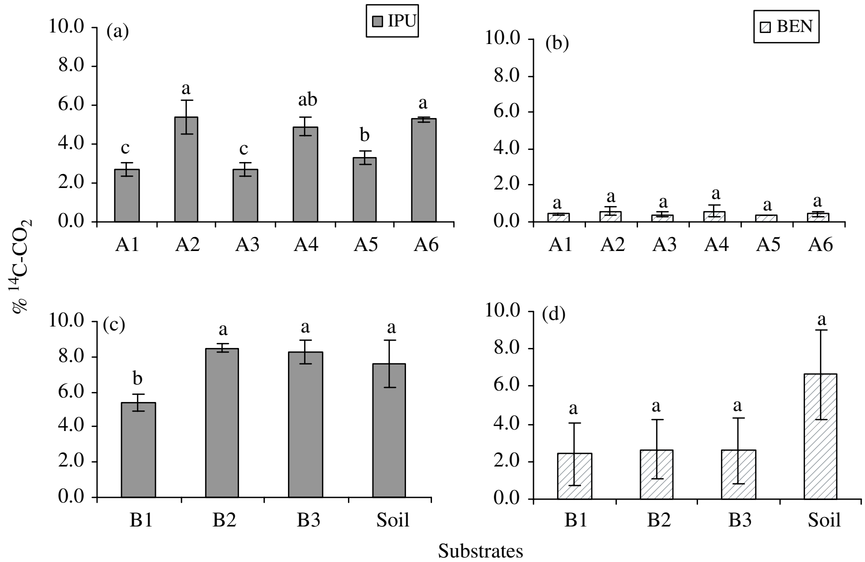



**Figure 2.** Percentage cumulative mineralisation evolved during an experimental period of 35 days for $^{14}$C-IPU (a) and 28 days for $^{14}$C-BEN (b) in compost-based substrates, and 36 days for both $^{14}$C-IPU (c) and $^{14}$C-BEN (d) in peat-based substrates. Urban compost alone (A1) and mixed with citrus peel (A3) or vine branch straw (A5); garden compost alone (A2) and mixed with citrus peel (A4) or vine branch straw (A6); biobed mixture at different levels of straw: 50% (B1), 25% (B2), 12.5% (B3). Lower-case letters represent LSD at $P < 0.05$. IPU = isoproturon; BEN = bentazone.

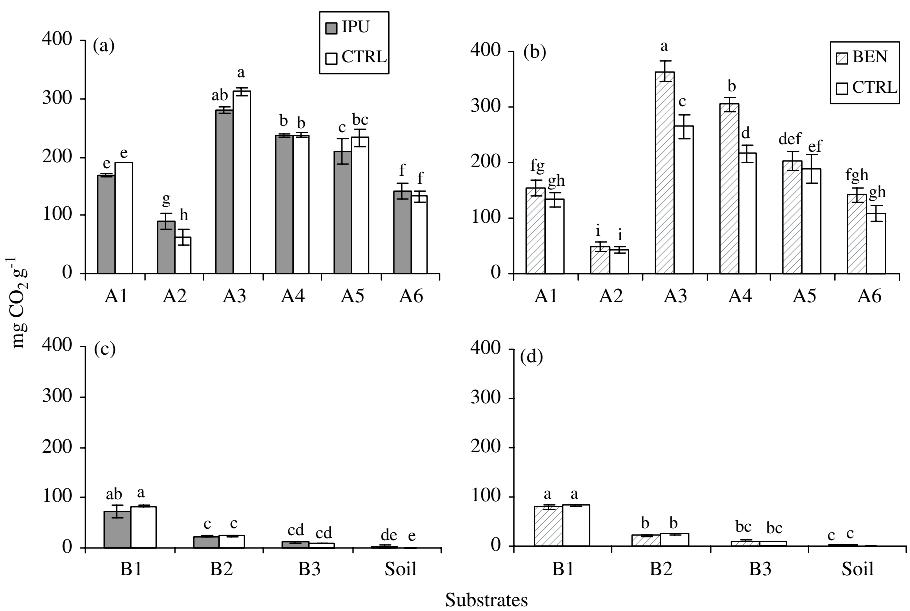



**Figure 3.** Cumulative respiration (mg $CO_2$ g$^{-1}$) evolved during an experimental period of 35 days for IPU (a) and 28 days for BEN (b) in compost-based substrates, and 36 days for both IPU (c) and BEN (d) in peat-based substrates, with respect to the control. Urban compost alone (A1) and mixed with citrus peel (A3) or vine branch straw (A5); in garden compost alone (A2) and mixed with citrus peel (A4) or vine branch straw (A6); in biobed mixture at different levels of straw: 50% (B1), 25% (B2), 12.5% (B3). Lower-case letters represent LSD at $P < 0.05$. IPU = isoproturon; BEN = bentazone; CTRL = control.

observed in garden compost alone (A2) and amended with straw (A6) (Fig. 1b). Knauber *et al.*[27] found a decrease in the total amount of bentazone mineralisation with an increase in total carbon content, in spite of an increase in the total number of culturable microorganisms. They explain this as a consequence of reduced bioavailability due to the binding of bentazone metabolites to organic matter. Accordingly, in the present study, slow mineralisation was found in the compost-based substrates, which are characterised by a high carbon content.

No significant differences in the mineralisation of bentazone were found in the peat-based substrate, regardless of the percentage of organic carbon added as straw biomixture. Again, in this case, this may be due to a reduction in bioavailability of metabolites caused by the organic matter.

The metabolic activity of the substrates of each biomixture was followed as basal respiration (Fig. 3). Higher respiration was observed in the compost-based substrates (A-biomixtures) than with the peat-based substrates (B-biomixtures), probably owing to the presence of more easily degradable carbon sources in the A-biomixtures. Also, the compost biomixtures may have had an already developed and active microbial population, as they were approximately 4–5 months old at the start of the trials. The

B-biomixtures were freshly prepared at the beginning of this study, and the microbial activity was expected to be low because it has been observed that young biomixtures have low sorption capacity and microbial activity in field biobeds.[4]

Within the A-biomixture group, higher respiration was observed in the urban compost (A1, A3, A5) than in the garden compost biomixtures (A2, A4, A6). Also, the addition of citrus peel or straw significantly increased the respiration rate. The higher content of soluble carbon in the urban compost (0.7% dm) compared with garden compost (0.3% dm) and in citrus peel (4.0% dm) compared with straw (2.2% dm) may explain the differences.

Within the B-group, the respiration rate was positively correlated with the straw levels in the biomixtures, and the control with soil alone gave the lowest respiration, confirming that soil amendment with a carbon source enhances metabolic activity. Moreover, respiration activity in isoproturon and bentazone treatments showed no significant differences compared with the untreated samples in both A- and B-biomixtures, suggesting that the microbial activity was not affected by the addition of the pesticides. However, an exception was observed with the addition of bentazone in A3-biomixtures (urban compost + citrus peel) and A4-biomixtures (garden compost + citrus peel). In these two biomixtures, respiration was significantly higher in the presence of bentazone. As reported by Anderson and Domsh,[28] an increase in respiration could also be related to a toxic effect caused by the pesticides which provide microbial stress. This evidence, taken together with the low degradation of bentazone in these substrates, suggests a toxic effect of the pesticide on the microbial population.

## 4 CONCLUSIONS

In conclusion, both biomixture groups tested showed a good degradation capability against the two pesticides, and they are recommended for biomixture compositions in the biobed field.

The biomixtures in the A-group showed different degradation capability, indicating that the best composition seems to be represented by the garden compost amended with lignocellulosic residues.

The B-group substrates showed similar degradation capability, highlighting that a further addition of vine branch straw is not useful in the degradation of the two parent compounds. Moreover, even though 12.5% of the straw seemed to be enough for the degradation of isoproturon and bentazone, no conclusion can be drawn concerning the effective degradation of the pesticides, as no metabolite accumulation was measured. Further studies are needed to evaluate the effect of the lignocellulosic residues on metabolite degradation.

The presence of lignocellulosic materials enhances the degradation capacity of the biomixtures, probably because of the development of lignin-degrading microorganisms which have shown to be more robust than the microbial population supported in citrus peels which is more sensitive to the toxic effects of highly soluble pesticides.

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