

Vermiremediation of Biomixtures from Biobed Systems Contaminated with Pesticides

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Received: 30 March 2020; Accepted: 30 April 2020; Published: 2 May 2020

Abstract: Biobeds bioremediation systems are effectively used for minimizing pesticide point-source contamination. For keeping the biobed effectiveness, its biomixture needs to be replaced every so often. The exhausted biomixtures can contain pesticide residues and so they require a special treatment before being discharged into the environment. In this study, we explore the potential of vermioremediation for cleaning up biobed biomixtures contaminated with pesticides. Two biomixtures composed of soil:peat:straw (P) and soil:vermicompost of wet olive cake: olive tree pruning (O), contaminated with high loads of four pesticides, were used. Vermicomposting was carried out by *Eisenia fetida* earthworms for 12 weeks. Results showed that 50% and 70% of the earthworms colonized the contaminated P and O biomixtures, respectively, but the number of alive earthworms decreased with time just as their weight. The colonization of biomixtures did not significantly affect the dissipation of imidacloprid and tebuconazole, but increased 1.4 fold the dissipation of oxyfluorfen in both biomixtures and that of diuron in biomixture P. Although the presence of high loads of pesticides and the composition of the biomixtures limited the vermioremediation, satisfactory results were obtained for diuron and oxyfluorfen. Complementing vermioremediation with other remediation practices could improve the efficiency of this technology.

Keywords: *Eisenia fetida*; contaminated biomixtures; dehydrogenase activity; pesticide residues; dissipation

1. Introduction

Biobeds are biopurification systems used to minimize pesticide point-source contamination. Currently, these systems are widely expanded worldwide, accounting for more than 10,000 units, especially in European and South American countries. These systems have an active biomixture that adsorbs pesticides in organic matter and enhances their microbial degradation. The most commonly used biomixture includes soil, peat and straw (25:25:50 vol %)—original Swedish biobed [1]. However, in recent years, peat and straw are being efficiently replaced by local organic wastes from agricultural, agro-industrial and energy production sectors in order to reduce costs and favor the recycling of local wastes [2–4]. Biomixtures used in the biobeds should be replaced every five to eight years (periodically) in order to maintain the overall efficiency of this biopurification system. After usage, the exhausted biomixtures can contain residues of pesticides. For that reason, they should be considered as hazardous wastes and thus, need special treatments. Among others, dispersion, landfill disposal and incineration are techniques applicable to the exhausted biomixtures but, in general, they are expensive and transfer pesticide residues to other compartments. Therefore, decontamination of exhausted biomixtures is essential prior to their final disposal or prior to their possible reuse to enhance biodegradation in new biobeds [5]. Previous studies revealed that, after 76 days of composting and maturation, 70% of bentazon, 93% of linuron, 90% of metalaxyl and 20% of bifenthrin

were dissipated from a contaminated biomixture used in a biopurification system [6]. Karas et al. [7] showed that bioaugmentation with pesticides degrading bacteria removed 83% and 97% of thiabendazole and imazalil, respectively, from residual biomixtures. Nevertheless, when the suitable microbial inocula for the degradation of pesticide residues are not available, composting can also be a valuable alternative.

Vermicomposting, which involves the joint action of earthworms and microorganisms under aerobic and mesophilic conditions, is a useful biotechnological process for recycling, biodegradation, biostabilization and biomaturation of a great variety of organic wastes [8–10]. Among others, *Eisenia fetida* is the most common epigeic earthworm used in vermicomposting processes, characterized by its ubiquity, high reproduction rate, short life cycle, and high tolerance to changing conditions [11]. In addition, this earthworm is one of the more useful species for ecotoxicological testing, being a sensitive indicator of polluted substrates [12]. Although the main objective of that biotechnology is obtaining soil organic amendments—vermicomposts—for agricultural purposes [13,14], both the final product and the earthworms themselves have been used for bioremediation of soils and organic wastes contaminated with organic pollutants and heavy metals. These biological strategies are included in the term of vermiremediation, which is considered as a promising, low-cost, aesthetically pleasing, and eco-friendly technology [15].

Despite the limited information regarding vermiremediation, the vermicomposting has proven to be an effective process to remove organic pollutants (polycyclic aromatic hydrocarbons, pesticides), even in some complex substrates as sewage sludges [16,17] or heavy metals in coal fly ashes [18]. In addition, the use of vermicompost as soil amendment reduced the availability and mobility of potential toxic metals [19,20] and pesticides [21], which favors their elimination in soils [22,23]. Finally, different studies have reported that the inoculation of polluted soils with different earthworm species, including *Eisenia fetida*, directly or in combination with organic matter, enhanced the degradation and the removal of a broad range of environmental contaminants [12,15,24]. However, the vermiremediation has certain limitations, because high contaminant concentrations and residual fractions of very toxic compounds in soils and organic wastes may negatively affect the survival of the earthworms used [12].

The effectiveness of vermiremediation to eliminate environmental pollutants in different matrices is the scientific basis for its application in decontaminating exhausted biomixtures from biobed systems. In this context, the feasibility of using *E. fetida* into a vermicomposting process to remove pesticides in the residual contaminated biomixtures from two biobed systems developed at pilot scale was investigated. The potential of *E. fetida* for degrading pesticides was determined by comparison with the dissipation of pesticides in the same two biomixtures in the absence of earthworms. In addition, for the purpose of comparison with the vermicomposting process, the same two biomixtures, but non-contaminated, were also included in this study.

2. Material and Methods

2.1. Uncontaminated and Contaminated Biomixtures

Two non-contaminated (N) and contaminated (C) biomixtures were used. The first biomixture type was composed of soil:peat:straw (P) and the second consisted of soil: vermicompost from wet olive cake:olive tree pruning (O), at 25:25:50 v:v:v, each. The corresponding two contaminated biomixtures, CP and CO, came from a previous study that aimed to evaluate the efficiency of the biobed systems composed of P and O biomixtures in the removal of five pesticides applied at high doses (diuron, imidacloprid, tebuconazole, oxyfluorfen and dimethoate) [2]. The concentration of pesticides determined in these residual biomixtures is presented in Table 1.

Table 1. Concentration (mg kg^{−1}) of pesticide residues remaining in the two biomixtures. Values (dry mass) are means ± SD (n = 3).

	Diuron	Imidacloprid	Tebuconazol	Oxifluorfen	Dimethoate
CP	0.89 ± 0.14	23.2 ± 0.5	24.0 ± 1.6	42.9 ± 2.4	nd
CO	0.55 ± 0.02	28.2 ± 0.5	42.3 ± 3.0	66.8 ± 5.4	nd

CP: Contaminated biomixture of soil:peat:straw. CO: Contaminated biomixture of soil, vermicompost from wet olive cake:olive pruning. nd: not detected.

2.2. Experimental Layout

Fifty gram (dw) samples of each biomixture were placed, in triplicate, in 500 cm³ cylindrical containers. In each container, and separated with a mesh, 15 g of horse manure as an initial habitat for the earthworms was placed on top of each biomixture, and 23 non-clitellated earthworms of the species *Eisenia fetida*, weighed each between 208 and 222 mg (5 g of total biomass), were inoculated to this material (+E). In addition, the two contaminated biomixtures were assayed without inoculation of earthworms (−E). To prevent losses of pesticide residues from the biomixtures, plastic trays were placed under each container and leachates were returned into the respective container, although, no leachates were collected during the experiment. All eighteen containers were kept in darkness at 24 ± 1 °C for 12 weeks, maintaining the moisture conditions (75–80%) of the biomixtures by periodical watering.

During the vermicomposting period, the number and weight of non-clitellated and clitellated earthworms of each biomixture were measured biweekly as follows: after separation of the horse manure layer, the container was turned out and the earthworms from the biomixture were collected by hand-sorting, counted, weighed and examined for sexual conditions. A sample of the biomixture was taken and stored at −20 °C for dehydrogenase (Dhase) activity analysis. Then, all earthworms and biomixture were returned into the container. The same procedure was used, biweekly, to collect samples from the non-inoculated contaminated biomixtures for Dhase activity analysis. After 12 weeks, the earthworms were removed. Samples from both the initial and final biomixtures were removed and stored in plastic bags at −20 °C until analysis.

2.3. Pesticides Analysis in the Biomixtures

The extraction of the pesticide residues from the contaminated biomixtures, at the initial and final incubation time, was carried out with some variations, using the QuEChERS method according to Delgado-Moreno et al. [2] and Castillo-Diaz et al. [25]. Briefly, 3 g of each biomixture were weighed into a 50-mL centrifuge tube, and 5 mL of acetonitrile was added. The mixture was vortexed for 1 min, and then, added with 1 g of QuEChERS EN Puch (Agilent Technologies, Santa Clara, CA, USA). Then, the sample was centrifuged at 3000 rpm for 5 min. Interferences were removed from the sample by transferring 1 mL of the supernatant to a tube containing 1.5 g of amine dispersive SPE, Fruit, and Veg EN (Agilent Technologies, Santa Clara, CA, USA) and 3 mL of acetonitrile. After 1 min of stirring, it was centrifuged under the conditions described above, and 0.8 mL of the supernatant were removed and re-dissolved up to 2 mL with milliQ water. The sample was passed through 0.2 micron PTFE syringe filter (Thermo Fisher Scientific Inc. Waltham, MA, USA) prior to HPLC analysis. Recoveries of the extraction method ranged from 86% to 102%, depending on the pesticide and the biomixture, with relative standard deviation never exceeding 5%.

The pesticides were analyzed by HPLC-DAD (series 1100, Agilent Technologies, Santa Clara, CA) on a Zorbax RX-C8 column (5 mm, 2.1 × 150 mm) (Agilent Technologies, Santa Clara, CA, USA), connected to an Eclipse XDB-C8 (5 mm, 2.1 × 12.5 mm) precolumn (Agilent Technologies, Santa Clara, CA, USA). The mobile phase was acetonitrile and milliQ water at pH 3. A solvent gradient was used from 20% to 70% of acetonitrile. The mobile phase flow was 0.2 mL min^{−1}, the injection volume 10 µL and the oven temperature 40 °C. The wavelengths were 210 nm for metalaxyl, dimethoate, diuron, and oxyfluorfen, 215 nm for tebuconazole, and 270 nm for imidacloprid as described in Delgado-Moreno et al. [2]. The limit of quantification was 0.1 mg kg^{−1}.

2.4. Chemical and Dehydrogenase Activity in the Biomixtures

All the analyses were performed in triplicate according to validated methods as briefly described below. The pH and electrical conductivity (EC) were measured with a glass electrode using a 1:10 sample:water (dw:v) ratio. Total organic carbon (TOC) and total nitrogen (N) were determined, after acid digestion of samples, with a LECO TruSpec CN analyzer (LECO Corporation, St. Joseph, MO, USA). Water soluble carbon (WSC) was extracted by mechanical shaking at 60 °C for 1 h with distilled water (1:10 sample:water; dw:v), and then, determined with potassium dichromate and sulfuric acid at 160 °C for 30 min. Subsequently, the amount of Cr^{3+} produced by the reduction of Cr^{6+} was quantified spectrophotometrically at 590 nm [26]. Dehydrogenase activity was determined by quantifying idonitrotetrazolium formazan (INTF) produced after INT reduction from idonitrotetrazolium formazan (INTF), according to the method published by Garcia et al. [27].

2.5. Statistical Analysis

All statistical analyses were conducted at a confidence level > 95% ($p < 0.05$) using SPSS 21 statistical software (IBM Corp., Armonk, NY, USA). All results are the means of three replicates. Differences in earthworm colonization and biomass, and dehydrogenase activity between the different biomixtures in each vermiremediation time were statistically analysed using a one-way analysis of variance (ANOVA) and a Tukey post-hoc comparison test. Moreover, the homoscedasticity was checked to the one-way ANOVA analyses. Differences in physical-chemical properties (pH, WSC, EX, TOC and N) between the initial and final vermiremediation time for each biomixtures were tested with a paired-sample *t*-test.

3. Results and Discussion

3.1. Development of Earthworms in the Different Biomixtures

In the non-contaminated biomixtures (NP and NO) at week two, more than 50% of the non-clitellated earthworms, had migrated from the horse manure to the biomixture, reaching 100% at week six of vermicomposting (Figure 1). The mean of the individual weight of alive earthworms in non-contaminated biomixtures diminished slightly from 308 ± 33 mg earthworm⁻¹ in NP and 357 ± 10 mg earthworm⁻¹ in NO at the second week, to 251 ± 5 mg earthworm⁻¹ in NP and 298 ± 11 mg earthworm⁻¹ in NO at the sixth week (Figure 2). In the NP biomixture, all earthworms lacked a developed clitellum, whereas in NO biomixture, 15% reached sexual maturity at week four. As a consequence, three new hatched earthworms appeared at week 10 in the NO biomixture. At the end of the experimental period, the percentage of earthworms diminished slightly, due to the death of some earthworms inhabiting both biomixtures. The remaining earthworms showed lower weights (210 ± 40 mg earthworm⁻¹ in NP and 245 ± 22 earthworm⁻¹ in NO) and none of them had a clitellum. The loss of weight and the presence of a clitellum only in the 15% of the earthworms in the NO biomixture reveal the limited feasibility of the two non-contaminated biomixtures to be used in vermicomposting processes. This might be due to the fact that both mixtures are constituted by soil (25%), stable organic materials (peat or vermicompost, 25%) and lignocellulosic organic wastes (straw or pruning, 50%), which could not ensure adequate nutritional resources for the optimal growth and reproduction of the epigeic earthworm used in this experiment [28]. Therefore, the population dynamics observed in those biomixtures were different from that recorded in traditional vermicomposting processes using substrates composed only of raw organic wastes [8].

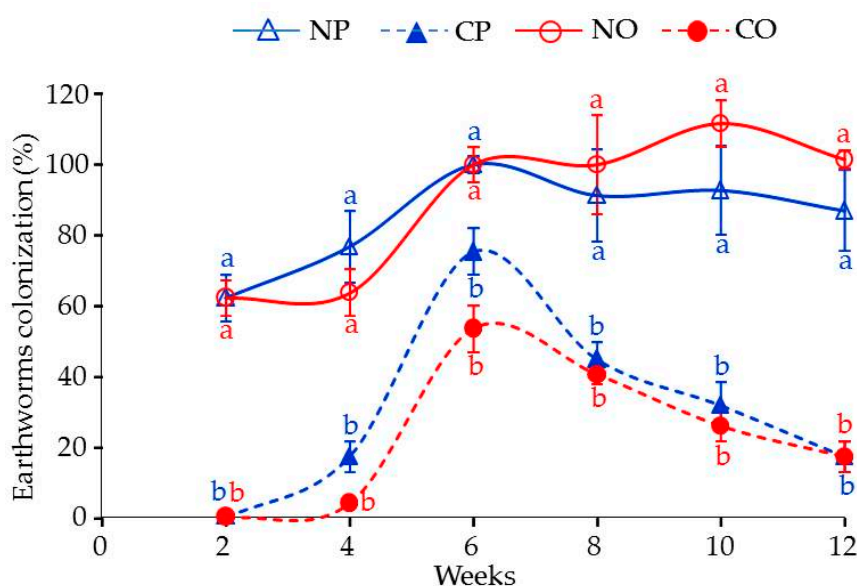


Figure 1. Percentages of earthworm colonization in the non-contaminated (NP and NO) and contaminated (CP and CO) biomixtures at each vermicomposting time. Error bars represent the standard deviation ($n = 3$). Different letters indicate significant differences between biomixtures ($p < 0.05$) at each vermicomposting time.

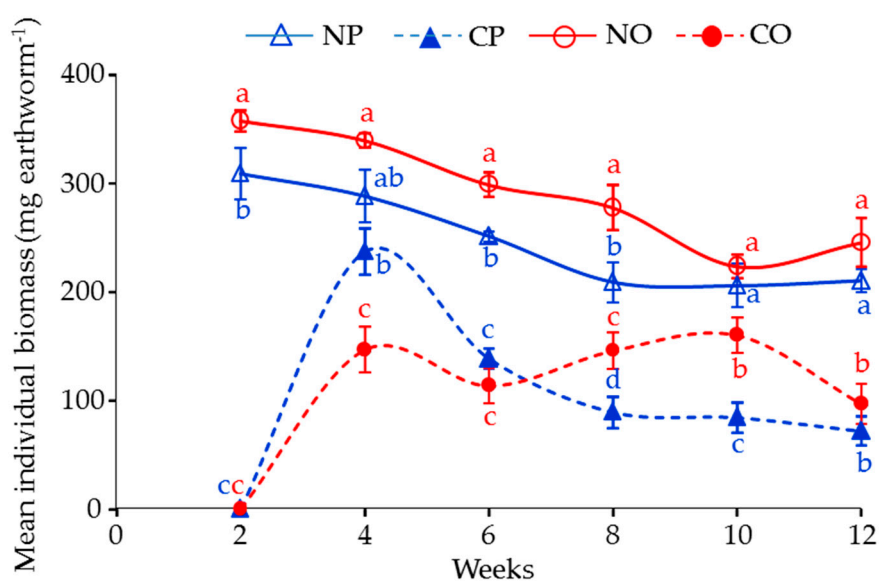


Figure 2. Evolution of the individual earthworm weight in the non-contaminated (NP and NO) and contaminated (CP and CO) biomixtures at each vermicomposting time. Error bars represent the standard deviation ($n = 3$). Different letters indicate significant differences between biomixtures ($p < 0.05$) at each vermicomposting time.

At week six, more than 70% and 50% of the non-clitellated earthworms, inoculated in the horse manure colonized the contaminated biomixtures CP and CO, respectively (Figure 1). Their individual weights were significantly lower (138 ± 10 mg earthworm⁻¹ in CP and 114 ± 17 mg earthworm⁻¹ in CO) than those recorded in the non-contaminated biomixtures, and, none of the earthworms had a clitellum (Figure 2). The number of earthworms diminished significantly after six weeks and finally most of them died remaining only a 17% with very low weight, 72 ± 13 mg earthworm⁻¹ in CP and 97 ± 13 mg earthworm⁻¹ in CO. The low weight and the death of most of the earthworms suggest that the residuals-contaminated biomixtures are not suitable habitats for the earthworm population due,

mainly, to the high concentration of pesticide residues remaining in the contaminated biomixtures (Table 1). Besides, imidacloprid is a toxic neonicotinoid insecticide for *E. fetida* [29], which can also be an important factor contributing to the negative effects observed on the earthworm population. Those concentrations exceeded largely the 14 day lethal concentration 50 (LC50) value established for *E. fetida* in an artificial soil test at 10.7 mg kg⁻¹ of imidacloprid [30]. Wang et al. [31] observed, after 14 days, non-survival *E. fetida* earthworms in an artificial soil exposed to 3.78 mg kg⁻¹ of imidacloprid. Fernández-Gómez et al. [32] indicated that imidacloprid concentrations >2 mg kg⁻¹ depressed *E. fetida* growth, whereas at concentration >10 mg kg⁻¹, the high mortality caused impeded the vermicomposting of cattle manure. Concentrations in an artificial soil above 0.5 mg kg⁻¹ imidacloprid produced also a significant induction of sperm deformity of *E. fetida* [33]. On the other hand, although concentrations of tebuconazole and oxyfluorfen residues were high in CP and CO biomixtures (Table 1), both pesticides have a low toxicity for *E. fetida*, with 14 days-LC50 values in artificial soils of 287 mg kg⁻¹ for tebuconazole and >500 mg kg⁻¹ for oxyfluorfen [34,35]. Finally, the concentration of diuron was more than 25 times lower, and, therefore, it might have less impact than tebuconazole and oxyfluorfen on the viability of colonizing earthworms in the contaminated biomixtures.

3.2. Evolution of Dehydrogenase Activity during the Vermiremediation Process

During the vermicoremediation process, the Dhase activity, which is an intracellular enzyme used as an indicator of the overall microbial activity in composting and vermicomposting processes [9,36], was significantly higher in biomixtures of vermicompost and olive pruning (O) (between 1.7 and 2.4 fold) than in those including peat and straw (P) (Figure 3). Vermicompost, unlike peat, hosts a wide diversity of microorganisms [37,38], which could explain the higher activity recorded in O biomixtures. On the other hand, the non-contaminated NP and NO biomixtures had higher Dhase activities (between 1.6 and 2 fold) than the contaminated ones. Comparatively, no significant differences of Dhase activities were observed in the contaminated biomixtures, in the presence (+E) or absence (−E) of earthworms. The lower Dhase activities are directly related to the concentrations of pesticides detected in the contaminated biomixtures, which may repress the enzyme activity, as has been previously described for soils [39,40].

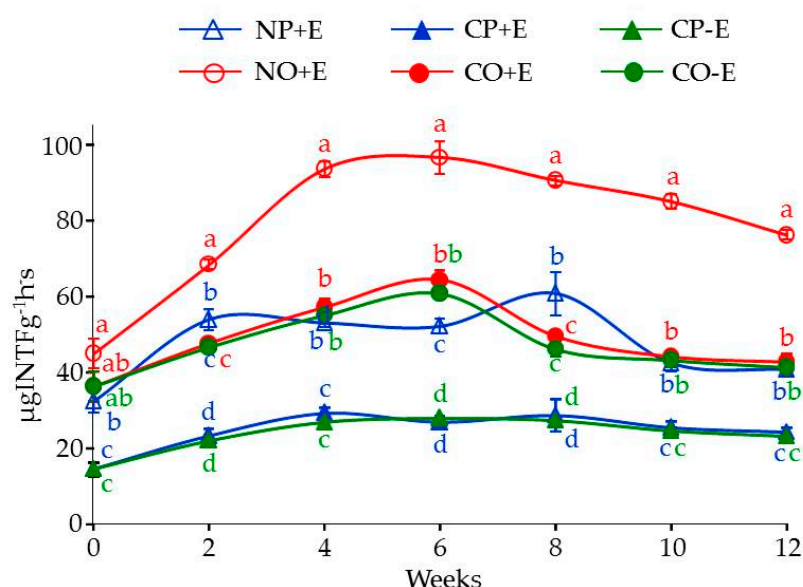


Figure 3. Dehydrogenase activity during the vermicoremediation process. Error bars represent the standard deviation (n = 3). Different letters indicate significant differences between biomixtures ($p < 0.05$) at each vermicoremediation time.

Regarding the evolution of this enzyme during the vermicoremediation period, it increased during the first weeks in all biomixtures composed of vermicompost and olive pruning (O) and reached the

maximum value at week 6, being higher in the non-contaminated NO biomixture (Figure 3). Thereafter, Dhase activity steadily declined until the end of the vermiremediation process. In these biomixtures, the Dhase activity pattern was similar to that observed in other vermicomposting processes [9,37]. In contrast, the fluctuations observed in Dhase activity (increases and decreases) throughout the vermiremediation period were less pronounced in biomixtures with peat and straw (NP+E, CP+E and CP-E), due, probably, to its lesser abundance of microorganisms.

In conclusion, these results reveal that the microbial activity during the vermiremediation process was more dependent on the composition and the presence of pesticide residues in the biomixtures than on the earthworm development in the biomixtures.

3.3. Chemical Changes in the Different Biomixtures

The chemical properties of the initial biomixtures were altered after the vermiremediation process (Table 2). The electrical conductivity values changed slightly in all assayed biomixtures. The TOC content significantly diminished in non-contaminated and contaminated biomixtures containing vermicompost and olive pruning (O), whereas non-significant reductions were observed in the biomixtures containing peat and straw (P) (Table 2). The same effects were observed for the WSC, which represents the most easily metabolizable organic matter [41]. Values of WSC were between 3 and 4 times higher in the O biomixtures than in the P mixtures. The most significant diminution of TOC and WSC content in the O biomixtures after the vermiremediation process must be attributed to their relatively higher Dhase activity or microbial activity, with regard to P biomixtures (Figure 3). As occurs in other biodegradation processes of organic wastes [9,42,43], the reduction in TOC and WSC may be, mainly, attributed to heterotrophic microorganisms present in the biomixtures, which mineralize and transform carbon compounds into simpler forms, and then into CO₂. In addition, the digestion and fragmentation of the organic matter of the biomixtures by earthworms might increase its surface area, which would favor its accessibility for microorganism and microbial enzymes [8], increasing the organic matter mineralization. This fact could explain the lower TOC and WSC contents recorded in the contaminated biomixtures and treated with earthworms (CP+E, CO+E) compared to those without earthworms (CP-E, CO-E). Nitrogen concentrations significantly increased in all biomixtures that had an earthworm population. In other vermicomposting processes, these increments have been attributed to the release of N by earthworms through mucus, enzymes or nitrogenous excretory substances [41,44].

Table 2. Chemical analysis of the biomixtures at initial (I) and final (F) times.

Biomixture ^a		pH			EC dS m ⁻¹			TOC g kg ⁻¹			WSC g kg ⁻¹			N g kg ⁻¹		
		I	F	t-test	I	F	t-test	I	F	t-test	I	F	t-test	I	F	t-test
NP	+E	7.0	7.1	0.42	3.0	2.7	0.07	139	131	0.15	2.2	2.0	0.15	7.1	8.9	0.01 *
	-E	7.1	7.2	0.43	3.5	3.1	0.02 *	114	108	0.39	1.3	1.1	0.10	6.4	7.3	0.04 *
CP	+E	7.1	7.2	0.42	3.5	3.5	0.99	114	112	0.72	1.3	1.2	0.18	6.4	6.2	0.44
	-E	7.1	7.2	0.42	3.5	3.5	0.99	114	112	0.72	1.3	1.2	0.18	6.4	6.2	0.44
NO	+E	8.1	8.0	0.18	1.5	1.3	0.13	151	104	0.00 *	9.6	7.2	0.01 *	8.4	13.3	0.00 *
	-E	8.1	8.0	0.18	1.5	1.3	0.13	151	104	0.00 *	9.6	7.2	0.01 *	8.4	13.3	0.00 *
CO	+E	8.1	7.8	0.09	2.3	1.6	0.01 *	143	125	0.01 *	6.5	4.1	0.00 *	12.5	13.6	0.07
	-E	8.1	7.9	0.04	2.3	2.3	0.99	143	138	0.18	6.5	5.8	0.01 *	12.5	12.9	0.61

EC: electrical conductivity; TOC: total organic carbon; WSC: water soluble carbon. Values (dry mass) are mean of three replicates. *t*-Test: P values of paired-sample t-test. * Significant differences between the initial and final time of the biomixtures. ^a Refer to text for explanation of biomixtures abbreviations.

3.4. Dissipation of Pesticide Residues in the Biomixtures

Regarding to the non-colonized biomixtures by *E. fetida* (−E), after 12 weeks of incubation, the amount of pesticide residues dissipated in the CO biomixture was between 1.7 and 3.9 fold higher than in CP, except for oxyfluorfen, which showed similar dissipation in both biomixtures (Figure 4). In the CO biomixture, the dissipation of imidacloprid, tebuconazole and oxyfluorfen residues was similar and ranged from 45.7% to 55.3%, while 100% of diuron was dissipated. By contrast, in CP, the percentage of dissipation ranged from 11.8% to 58.8% and followed the trend tebuconazole < imidacloprid < oxyfluorfen < diuron. As indicated above, the CO biomixture had Dhase activity levels higher than the CP biomixture (Figure 3) and, therefore, it is biologically more active, enhancing the dissipation of the pesticides. In addition, the CO biomixture has vermicompost, which contains a relative high abundance of microorganisms than the peat, but also, like compost, has higher richness of xenobiotic-degraders that can degrade these pesticides to less toxic compounds [25,45,46].

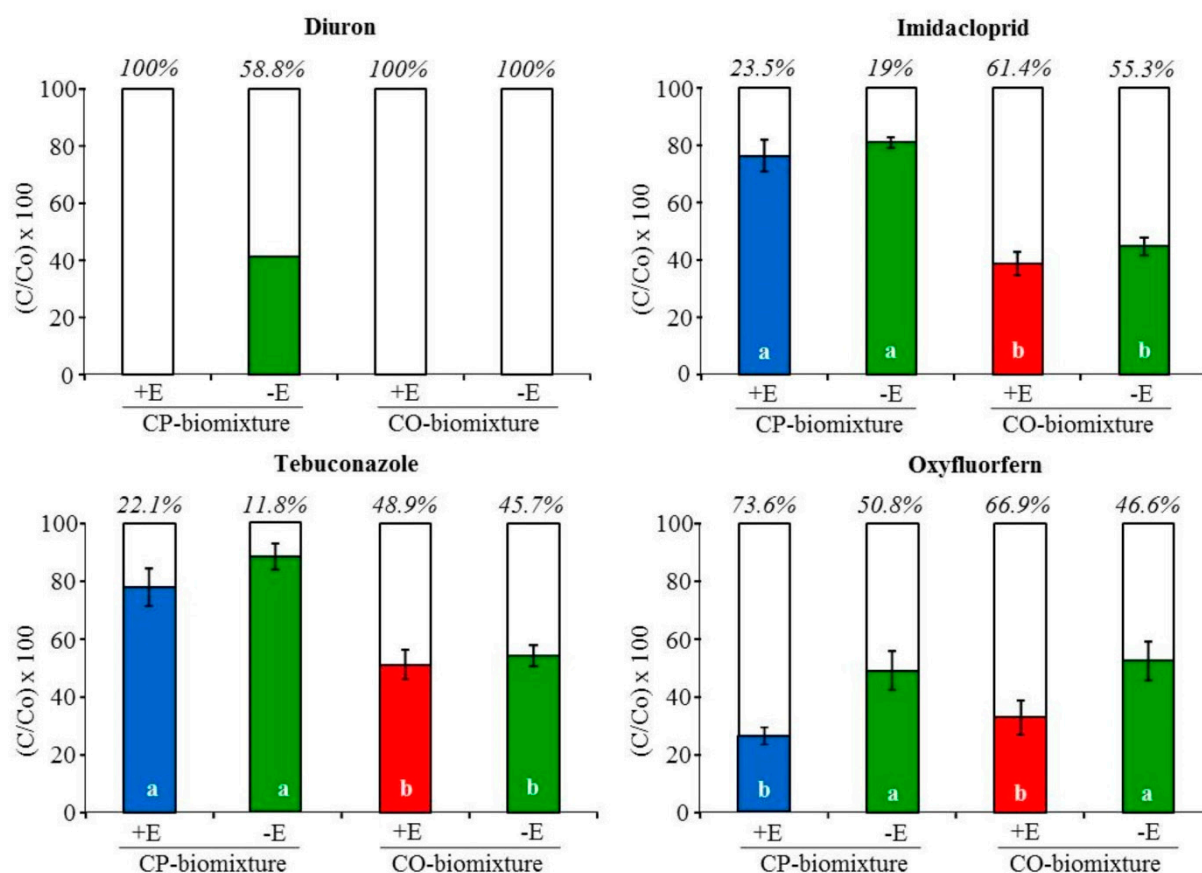


Figure 4. Relative concentration ($C/C_0 \times 100$) of residues (colored bars) and dissipated percentage (white bars) of diuron, imidacloprid, tebuconazole and oxyfluorfen in the CP and CO biomixtures, treated (+E) or not (−E) with *E. fetida* after 12 weeks of vermicomposting. Error bars represent the mean standard error. For each pesticide residue, means followed by the same letter do not significantly differ ($p < 0.05$).

The colonization of these biomixtures by *E. fetida* (+E) did not significantly ($p > 0.05$) affect the dissipation of imidacloprid and tebuconazole residues. Neither was the dissipation of diuron residues affected in the CO biomixture, but it increased in CP. By contrast, the presence of *E. fetida* (+E) enhanced the dissipation of oxyfluorfen residues 1.4 fold in both biomixtures (Figure 4).

It is well known that earthworms can stimulate the pesticide degradation in soils and organic wastes [15,47], through different interdependent mechanisms: (i) release of pesticide-detoxifying enzymes in their gastrointestinal tract [48], (ii) stimulating the growth and activity of microorganisms by increasing their contact with pesticide residues [12], and (iii) secretion of mucus, which would

improve the nitrogen concentration in the substratum, enhancing the growth of degrading microorganisms [16]. This last mechanism could explain the higher dissipation of diuron observed in CP+E biomixture regarding to CP-E (−E). As shown in Table 2, the presence of earthworms increased the concentration of N in both biomixtures, CP and CO. This increase may have favored the activity of diuron degrading microorganisms already in the biomixtures, more noticeable for CP biomixture with lower N content. In a previous study of our research group, it was demonstrated that diuron degradation was enhanced in biomixtures added with indigenous microorganisms previously exposed to diuron residues [5].

In the case of oxyfluorfen, it is a persistent and hydrophobic (Log Kow 4.7) pesticide that renders to be adsorbed in the organic matter of the biomixture. Since earthworms ingest these organic particles, it might explain the reduction of oxyfluorfen residues in the biomixtures with earthworms. As neither the extraction of pesticide residues in earthworms nor the detection of metabolites were carried out, we cannot elucidate if oxyfluorfen degradation occurred in the digestive tract of the earthworms.

Imidacloprid and tebuconazole dissipation was not enhanced in the presence of earthworms, which might attend to different reasons. As we mentioned previously, imidacloprid is highly toxic for *Eisenia fetida* [33], which prevents earthworms degrading this compound. Tebuconazole, for its part, is the most persistent of the studied pesticides and it may form bound residues with clay fraction of the soil [49] and organic matter that limited the degradation by *Eisenia fetida*.

In general, the dissipation of pesticide residues in our study was similar or slightly lower than that recorded in the composting of exhausted biomixtures, although in those studies, other types of pesticides were analyzed and the biomixtures contained other organic substrates [6,7], which might explain the differences observed.

4. Conclusions

The biotransformation of the biomixtures for 12 weeks, using or not the earthworms of the *E. fetida* species, was revealed as an emergent and effective strategy to dissipate the pesticide residues contained in the assayed exhausted biomixtures. In our study, the presence of *E. fetida* in the contaminated biomixtures had a limited effect on the dissipation of pesticide residues. This could be due to the inadequate development of the earthworm population surviving in the contaminated biomixtures as a consequence to the high concentration and toxicity of the pesticide residues, especially imidacloprid. Despite this, higher dissipation of oxyfluorfen was observed in the biomixtures colonized with earthworms than in the non-colonized biomixtures. Similarly, the presence of earthworms enhanced the dissipation of diuron in biomixture containing peat and straw. Thus, it might be expected that the vermiremediation will be useful when the exhausted biomixtures to be decontaminated do not contain pesticide residues at toxic levels for the earthworms. In addition, supplementing vermiremediation with other bioaugmentation techniques or with the addition of nutrients could improve the yield of the bioremediation of exhausted biomixtures containing high loads of pesticides with different properties. It must also take into account that the pesticide also contributes to modify the microbiological profile of the exhausted biomixtures, which depends not only on the biomixture's composition, but also on the residual concentration of the pesticides.

Author Contributions: Conceptualization, L.D.-M. and E.R.; Methodology, L.D.-M., R.N. and E.R.; Software, R.N.; Validation, L.D.-M., R.N. and E.R.; Formal Analysis, L.D.-M. and R.N.; Investigation, L.D.-M., R.N. and E.R.; Resources, L.D.-M. and R.N.; Data Curation, L.D.-M. and R.N.; Writing—Original Draft Preparation, R.N.; Writing—Review and Editing, L.D.-M. and E.R.; Visualization, L.D.-M. and E.R.; Supervision, L.D.-M. and E.R.; Funding Acquisition, E.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Agencia Estatal de Investigación (AEI) of the Spanish Ministry of Science and Innovation and co-funded by the European FEDER program (project CTM2017-86504-R).

Acknowledgments: The authors wish to thank Celia Cifuentes for her technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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