# *Are there any risks of the disposal of pesticide effluents in soils? Biobed system meets ecotoxicology ensuring safety to soil fauna*

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# Are there any risks of the disposal of pesticide effluents in soils? Biobed system meets ecotoxicology ensuring safety to soil fauna

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## Abstract

The biobed is a purification system, which reduces soil pollution for receiving pesticide residues from handling and washing machinery in agricultural areas. The aims of this study were (1) to assess ecotoxicity effects over time to soil fauna, posed by Lorsban® 480 BR (Chlorpyrifos) and Dithane® NT (Mancozeb) residues when disposed of in a biobed system compared with two subtropical soils, and (2) to assess ecotoxicity effects over time to soil fauna simulating an accidental spillage with Lorsban® 480 BR at the biobed. A semi-field experiment was conducted for 420 days in southern Brazil, testing continuous disposal of washing pulverization tanks in biobeds, Typic Haploperox or Typic Hapludults. In addition, different biobeds received a single dose (1 L) of Lorsban® 480 BR to simulate an accidental spillage. Chronic ecotoxicity tests were performed using *Folsomia candida*, *Eisenia andrei*, and *Enchytraeus crypticus* in different sampling times for both experiments. *F. candida* was the most sensitive species. The biobed system was able to eliminate effects from residues of both pesticides over time in all species, which did not happen in both natural soils. In accidental spillage simulation, even 420 days after contamination, *F. candida* did not show reproduction. The biobeds can be a feasible alternative for the disposal and treatment residues of pesticides, also for handling and washing pesticides activities. The system was efficient in promoting degradation and reducing ecotoxicity effects posed by Lorsban® 480 BR and Dithane® NT for soil fauna. It is a safe alternative to avoid soil contamination.

**Keywords** Biopurification system · Waste treatment · Pesticides · Ecotoxicity assessment · Soil invertebrates.

## Introduction

Pesticides have been used worldwide to eliminate pests and prevent diseases in crop areas. However, there are potential risks to terrestrial and aquatic ecosystems associated by their use—and not only related to field misapplication but also due to accidental spillage and washing equipments in inappropriate places (Reichenberger et al. 2007; Pérez-Parada et al. 2018; Zhang et al. 2018). In Latin America, residues from washing tanks are commonly disposed in soil, distant from aquatic ecosystems. However, this method may offer risks to the environment, possibly causing soil and water contamination, since normally, there is no clear indication of the distance to be taken from water sources, consequently causing a risk to environmental quality.

In order to achieve a successful effluent management, some processes have been developed to prevent and mitigate pesticide impacts (Monaci et al. 2009). Among the proposed strategies, the deposition of residues in biobeds is the most adopted in many countries (Monaci et al. 2009;

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Diez 2010; Karas et al. 2016). However, besides some biobeds experience in Chile (Urrutia et al. 2015), the information in tropical countries is scarce or under development (Dias et al. 2020). Brazilian legislation concerning the management of pesticide residues only embraces cleaning and returning the packages (Brasil 1989). Furthermore, it does not deal specifically with the effluents from the washing of spray tanks.

A biobed system has been developed as a pit in the soil, filled with biomixture—a mixture of straw, peat, and agricultural soil at the proportion of 2:1:1. On top of this, the system also receives vegetation cover on its surface. The preparation of pesticide dilutions and sprayer handling is performed on this system, which receives remaining spray solutions, accidental spillages, and residues from machinery washing (Fogg et al. 2003; Fait et al. 2007; Roffignac et al. 2008; Castillo et al. 2008).

After its useful lifetime, the substrate biomixture can be composted and then disposed of in a landfarming (Castillo et al. 2008). The guideline for biobeds in The United Kingdom (UK Government 2014), establishes that it is possible to receive up to 15.000 L of dilute residues of pesticides washings in 12 months, however, the standard does not provide information on the maximum concentration of pesticides. Since there are several entrances in the system and molecules degradation over time, it is not possible to know specifics concentrations in the residues from diluted and mixtures of pesticides.

Despite the biobed efficiency, pesticide degradation have been already reported, as well as changes in microbial community structure (Vischetti et al. 2008; Mukherjee et al. 2016; Castro-Gutiérrez et al. 2017). However, additional studies are necessary to determine the time needed until the biomixture could be disposed of in soil with no risk to soil fauna. This is important, mostly due to the potential risks posed by co-metabolites from the degradation process, which could be even more soluble and toxic than the original molecule, as in the case of Chlorpyrifos (Anwar et al. 2009; Fernández-Alberti et al. 2012).

Soil fauna is responsible for important roles on ecosystem services, such as nutrient cycling; decomposition and mineralization of organic matter; maintenance of the trophic chain; biological control, among others (Frouz et al. 2013; Marichal et al. 2017). Therefore, macro and mesofauna are important protection goals and has been used as ecotoxicological indicators in pesticide risk assessment (EFSA 2007). Pesticides can pose risks to survival and reproduction of soil fauna, as demonstrated by ecotoxicity tests (Natal-da-Luz et al. 2012; Leitão et al. 2014; Pelosi et al. 2014). It is recognized that pesticides and their metabolites could affect non-target organisms like earthworms, collembolans, spiders, and isopods (Fountain et al. 2007; Jordaan et al. 2012; Natal-da-Luz et al. 2012; Morgado et al. 2016; Zortéa et al. 2018). Although research has shown that the point contamination, derived from the recurrent handling of concentrated pesticides at the same place, may be as harmful, effects of the continuous disposal of these effluents on soil, or even in biobeds are uncertain, especially in subtropical conditions (Carter 2000; Mukherjee et al. 2016).

This study aimed to assess the risks over time of two pesticides (Dithane® NT—Mancozeb, and Lorsban® 480 BR—Chlorpyrifos) when discarded continuously on biobeds and two natural soils (Typic Haploperox and Typic Hapludults), using standardized ecotoxicity tests with soil fauna (*Eisenia andrei*, *Folsomia candida*, and *Enchytraeus crypticus*). We also assessed the time required to eliminate toxic effects of biomixture to soil organisms in a worst-case scenario of accidental spillage with Chlorpyrifos (Lorsban® 480 BR), using the same soil fauna organisms as bioindicators of ecotoxicity effects.

## Material and methods

The biobed system experiments were carried out at the Experimental Station of the Brazilian Agricultural Research Corporation (Embrapa) Grape & Wine of Vacaria, RS, Brazil (28°30′49″ S and 50°52′58″ W, 986 m). The average monthly temperature in December in the city of Vacaria (RS, Brazil) ranges from 11.4 to 20.6 °C, and it has a rainfall regime of 1789 mm (Cardoso et al. 2012), with mild summer and more rigorous winter.

### Soils

The soils used were a Typic Hapludults and a Typic Haploperox (Soil Survey Staff 2014). The first was sampled at Campo Belo do Sul, SC, (27°52′28″ S and 50°39′23″ W, altitude 947 m) in an area of native pasture, and the second was sampled at Embrapa Uva & Vinho Research Station, Vacaria, RS (28°30′49″ S and 50°52′58″ W, an altitude of 986 m), in an old apple orchard area without application of pesticides for 4 years. The collected soil samples were air dried, sieved (4-mm mesh) and then stored at the laboratory until the use. Soil chemical and physical properties are shown in Table 1. Organic matter was determined by wet oxidation with potassium dichromate and measured by titration; total organic carbon (TOC) was determined by dry combustion in a CHNS Vario EL Cube elemental analyzer; pH in water was determined in a soil solution ratio 1:1 wt: vol, with a glass electrode. Available Ca and Mg were extracted with KCl 1 Mol $L^{-1}$ and measured by atomic absorption spectrometry. Available P and K were extracted by Melich-1 and measured by colorimetric spectrophotometry (P) and flame spectrometry (K). Available Cu was determined with HCl 0.1 M extractor, Fe with

Ammonium Oxalate 0.2 M extractor (at pH 3.0). They were measured by atomic absorption spectrometry. The particle size distribution was determined with a Robinson pipette and with Calgon dispersant. All these methods were performed according to Tedesco et al. (1995). Water holding capacity (WHC) was determined according to ISO (2012a).

## Pesticides and experimental procedure

The commercial formulations of the products at wastewater were from Dow Agro (currently Corteva). In this study, we used the insecticide Lorsban® 480 BR (48% a.i. $L^{-1}$) with the organophosphate Chlorpyrifos as active ingredient (a.i.), and the fungicide Dithane® NT (80% a.i. $L^{-1}$) with dithiocarbamate Mancozeb as a.i. Physical and chemical characterization of these active substances is shown in Table 2. Products were applied in an apple orchard at EMBRAPA, according to agriculture recommendations (Table S1). The wastewater residues (containing Lorsban® 480 BR and/or Dithane® NT) from the pulverization tank (50 L) were used to contaminate the biobed and the soil systems.

To simulate the disposal of pesticide effluents over time and to compare the toxicity to soil fauna between a biobed system and soil disposal, we used 360-L water tanks. Three different systems were simulated: (1) two tanks were filled with a Biomixture (straw, peat, and Typic Haploperox at the proportion of 2:1:1 v/v) to simulate the biobed system; (2) two tanks were filled with sieved subtropical Typic Haploperox and finally; (3) two thanks were filled with sieved subtropical Typic Hapludults. The days of the wastewater disposal are illustrated in the timeline (Fig. 1). Its corresponding to time applications of commercial products on apple orchards fields, which were conducted accordingly with the good application practices (GAP) to combat *Venturia inaequalis* (Dithane® NT) and *Bonagota cranaodes* (Lorsban® 480 BR). The systems were covered with grass.

The sampling times (days 0, 90, 270, and 420), were also illustrated in the experiment timeline (Fig. 1). Composite samples (~3 kg) were collected in different points (0–20 cm) (Fig. 1) in biobeds and soils and then homogenized. These samples were stored frozen (−20 °C) to avoid pesticide degradation until the ecotoxicological tests were carried out. From this sampling, an amount (30–50 g) of biomixture and soils were separated for chemical determination to verify if there was the degradation of Chlorpyrifos and Mancozeb. This analysis was carried out by the Center of Research and Analysis of Residues and Contaminants (CEPARC) of the Federal University of Santa Maria-RS, Brazil. For Chlorpyrifos, the analysis was performed in Mass Ultra Performance Liquid Chromatography (UPLC-MS/MS). For

**Table 1** Properties of the soils (Typic Haploperox and Typic Hapludults) used to simulate a pesticide disposal overtime

| Properties | Typic Haploperox | Typic Hapludults |
|---|---|---|
| Organic matter (mg $dm^{-3}$) | 0.8 | 0.5 |
| Total organic carbon (mg $dm^{-3}$) | 7.7 | 6.3 |
| pH (water) | 4.9 | 4.8 |
| Cation-exchange capacity ($cmol_c$ $dm^{-3}$) | 10.0 | 8.6 |
| Clay (%) | 30.0 | 47.0 |
| Sand (%) | 24.0 | 18.0 |
| Silt (%) | 46.0 | 35.0 |
| Water hold capacity (WHC) (%) | 78 | 83 |

**Table 2** Physicochemical characteristics of Chlorpyrifos and Mancozeb

| Characteristic | Chlorpyrifos | Mancozeb |
|---|---|---|
| CAS | 2921-88-2 | 8018-01-7 |
| IUPAC name | O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate | Zinc;manganese(2+);N-[2-(sulfidocarbothioylamino) ethyl] carbamodithiate |
| Empirical formula | $C_9H_{11}Cl_3NO_3PS$ | $C_8H_{12}MnN_4S_8Zn$ |
| Molecular mass (g $mol^{-1}$) | 350.58 | 541.1 |
| Relative density (g $cm^{-1}$) | 1.40 | 1.92 |
| Solubility (pH = 7) (mg $L^{-1}$ 20 °C) | 1.05 | 6.20 |
| Log $K_{ow}$ (at 20 °C) | 4.06 | 1.33 |
| Henry's Law constant (25 °C Pa $m^3$ $mol^{-1}$) | 0.478 | $4.4 \times 10^{-9}$ |
| Degradation soil (20 °C aerobic) (days) | 386 | 0.05 |
| Degradation/dissipation field (days) | 27.6 | 15 |

Chemical characteristics and DT50 data were collected from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and IUPAC (https://sitem.herts.ac.uk/aeru/iupac/Reports/)

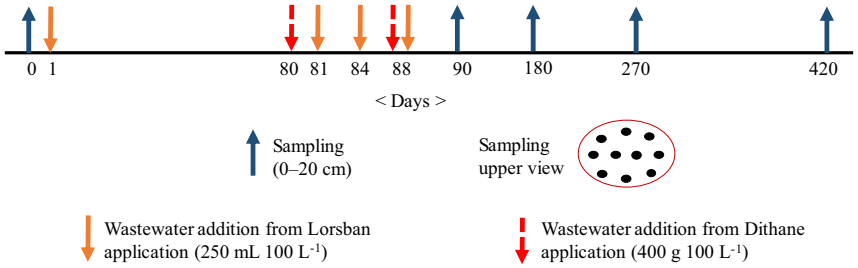


**Fig. 1** Timeline of the experiment in soils and biobeds. Up arrows (↑) indicated the sampling times (day 0, before applications and 90, 270, and 420 days after applications). Down arrows (↓) indicated contamination times with Lorsban® 480 BR and/or Dithane® NT

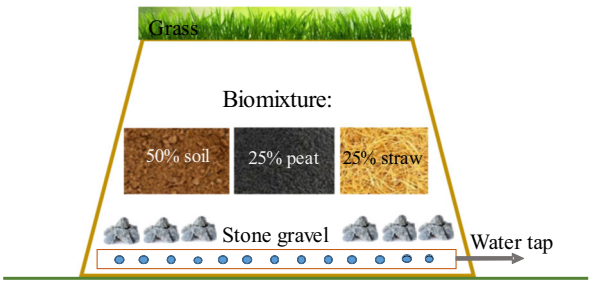


**Fig. 2** Pilot model of biobed filled with biomixture, covered with native grasses

Mancozeb chemical determination, a gas chromatography coupled with mass spectrometry (GC-MS) was used.

To simulate the contamination of an accidental spillage, four tanks (360 L) were also filled with the biomixture. Two of them received 1 L of Lorsban® 480 BR (day 1) to simulate the worst-case scenario of turning a bottle of this product in the biobed system. Besides, the other two systems were used as control (they did not receive any contamination). Sampling was also according to Fig. 1.

The biobeds were maintained outdoor, exposed to natural conditions of temperature and humidity for compound degradation. At the bottom of the tanks, a drainage system was installed to remove the excess of liquid (in case of high-intensity rain), composed of a pipe with holes covered by crushed stone to avoid the entry of solid particles. The biobed system is illustrated in Fig. 2.

## Ecotoxicological tests

Three different species were used to investigate the toxicity of wastewater over time concerning disposal and accidental spillage. The Collembola species used in the laboratory tests was *F. candida* (Collembola: Isotomidae). *F. candida* is a parthenogenetic and edaphic species widely distributed and recommended as test species by ISO guidelines (ISO 2012b). The earthworm was *E. andrei* (Oligochaeta: Lumbricidae)—one of the most studied species for standard ecotoxicological testing, included in ISO and OECD standards, e.g., ISO 11268-2 2 (ISO 2012a) and OECD 222 (OECD 2016). The enchytraeid *E. crypticus* (Oligochaeta: Enchytraeidae) was also used. *E. crypticus* is a test species commonly used in standardized ecotoxicity tests (Kuperman et al. 2004; ISO 2014; Zhang and Van Gestel 2019). This species has sexual reproduction by self-fertilization and possibly also by cross-fertilization (Gonçalves et al. 2016), and it has been largely used in laboratory studies.

To simulate the disposal of pesticide effluents over time, tests with *F. candida* and *E. crypticus* were carried out with biomixture and soils collected at 0, 90, 270, and 420 days. Tests with earthworms were carried at 0, 90, and 270 days. For accidental spillage, reproduction tests with collembolans and enchytraeids were carried out with biomixture collected at 0, 90, 180, 270, and 420 days. Tests with earthworms were carried at 0, 90, and 270 days. For earthworms, the tests at 420 days were not performed due to a lack of material (biomixture and soil).

The species *F. candida*, *E. crypticus*, and *E. andrei* were cultivated in a closed room with a photoperiod of 16/8 h of light/dark and temperature of 20 ± 2 °C, following the protocols ISO 11267 (ISO 2012b), ISO 16387 (ISO 2014), and ISO 11268-2 (ISO 2012a), respectively. The water content of soil samples was adjusted to 50% of their water holding capacity (WHC) at the beginning of the tests. Moisture was adjusted weekly by replacing the lost water. To each test, pH and humidity of biomixture and soils were verified at the beginning and at the end of the experiment.

Tests with *F. candida* were conducted in vessels (120 mL capacity) with ten organisms (10–12 days old) to each replicate ($n = 5$), containing 30 g (wet weight) of test substrate (biomixture or soil). At days 1 and 14, collembolans were fed with 2 mg of dry baker yeast *Saccharomyces cerevisiae*. On day 28, test vessels were filled up with water and some drops of stamp ink, carefully stirred, causing flotation of the organisms and photographed juveniles on the water surface. Counting was carried out using the software ImageJ (Schneider et al. 2012).

For the tests with *E. crypticus*, ten clitellated organisms were transferred to vessel tests (125 mL), containing ~28 g

(wet weight) of contaminated or control substrate ($n = 5$). A few grams of fine oat flour were added once a week as a food supply. At day 28, absolute alcohol (10 mL), some drops of Bengal rose color, and water (10 mL) were added. After 48 h, the number of juveniles was counted using a stereomicroscope (×40).

For earthworms, ten clitellated organisms, weighing 400–600 mg, were transferred to plastic containers (1000 mL capacity) containing 500 g of contaminated soil or control ($n = 4$). Equine dried manure, free of contaminants, was added every 2 weeks as a food supply. Adults were removed at day 28, and juveniles were counted at day 56, using the extraction method by water bath at 60 °C to induce the juveniles to emerge to the surface, which allowed their count.

In addition, all tests had extra negative controls using tropical artificial soil (TAS) (Garcia 2004) to verify the validation criteria proposed by the current guidelines used. TAS was composed of 70% of fine sand (washed and dried), 20% of kaolin clay, and 10% of coconut coir dust. The pH of the soil was adjusted to 6.0 ± 0.5 by adding $CaCO_3$.

### Predicted environmental concentrations (PEC soil) of the pesticides

The time-weighted average concentration for 1 year ($PEC_{year}$) in each natural soil was estimated considering the percentage of interception by apple crops, $DT_{50}$ values, product characteristics and environmental data, according to data available in open databases. The data were assessed using the software ESCAPE (Klein 2015).

The crop interception was measured by the Biologische Bundesanstalt, Bundesortenamt und Chemische Industrie (BBCH) code, which is a decimal code ranging from 0 to 99 to characterize the crop development stage (Meier 2001). Through the BBCH code, it is possible to estimate the fraction of the pesticide dose that was not covered by the crops, thus reaching the soil (fsoil) (EFSA 2007). In Table S1, more information on the variables used to estimated $PEC_{year}$ is available.

### Data analysis

Data from survival and reproduction tests were transformed ($\text{Log}(x + 1)$) to meet the requirements of normality by Shapiro–Wilks test and homogeneity of variances through Bartlett's test. Differences in reproduction (%) to each test species were assessed over time by the *t*-test ($p < 0.05$) comparing sampling times with day 0 (without contamination), which was considered as 100% of reproduction. Results are expressed as the proportion of control reproduction (using the number of juveniles of day 0 as control). The aim was to test if soils and biomix contaminated with residues of pesticides reduced soil fauna reproduction, and how long such effects last. For accidental spillage, at each sampling time, the number of juveniles obtained in the contaminated substrate was compared to those in the control (biomixture without pesticide) through the *t*-test ($p < 0.05$). The aim was to verify in which period control and contaminated substrate did not differ any longer. All statistical analyses were carried out using the software Statistica 7.0 (StatSoft Inc. 2004).

## Results

### Disposal of pesticide effluents over time

All the tests performed accomplished the validation criteria from the international guidelines in the TAS control. Validity criteria for reproduction tests with *F. candida* were achieved, with an adult mortality <20% and number of juveniles >100 per test vessel, not exceeding 30% in the coefficient of variance (ISO 2012b). For *E. crypticus*, adult mortality was <20% and the number of juveniles >25 per test vessel, not exceeding 50% in the coefficient of variance (ISO 2014). For *E. andrei*, adult mortality was <10%, number of juveniles >30 per test vessel, and not exceeded 30% of the coefficient of variation (ISO 2012a).

Concentrations of pesticides in biobed substrates (Biomixture, Typic Haploperox, and Typic Hapludults) over time are shown in Table 3. In general, chemical results

**Table 3** Days after the first wastewater application in biobeds, and concentration of Chlorpyrifos and Mancozeb

| Days after last contamination | Chlorpyrifos (mg a.i. $kg^{-1}$) | | | Mancozeb (mg a.i. $kg^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Biomixture | Typic Haploperox | Typic Hapludults | Biomixture | Typic Haploperox | Typic Hapludults |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 90 | 3.8 | 3.8 | 3.6 | 11.5 ± 5.15 | 6.17 ± 30.9 | 4.38 ± 18.5 |
| 270 | <2[a] | <2[a] | <2[a] | <0.5[b] | <0.5[b] | <0.5[b] |
| 420 | <2[a] | <2[a] | <2[a] | <0.5[b] | <0.5[b] | <0.5[b] |

[a]Lower than LOQ: method quantification limit (2 mg Chlorpyrifos $kg^{-1}$)

[b]Lower than LOQm: method quantification limit (0.5 mg CS2 $kg^{-1}$)

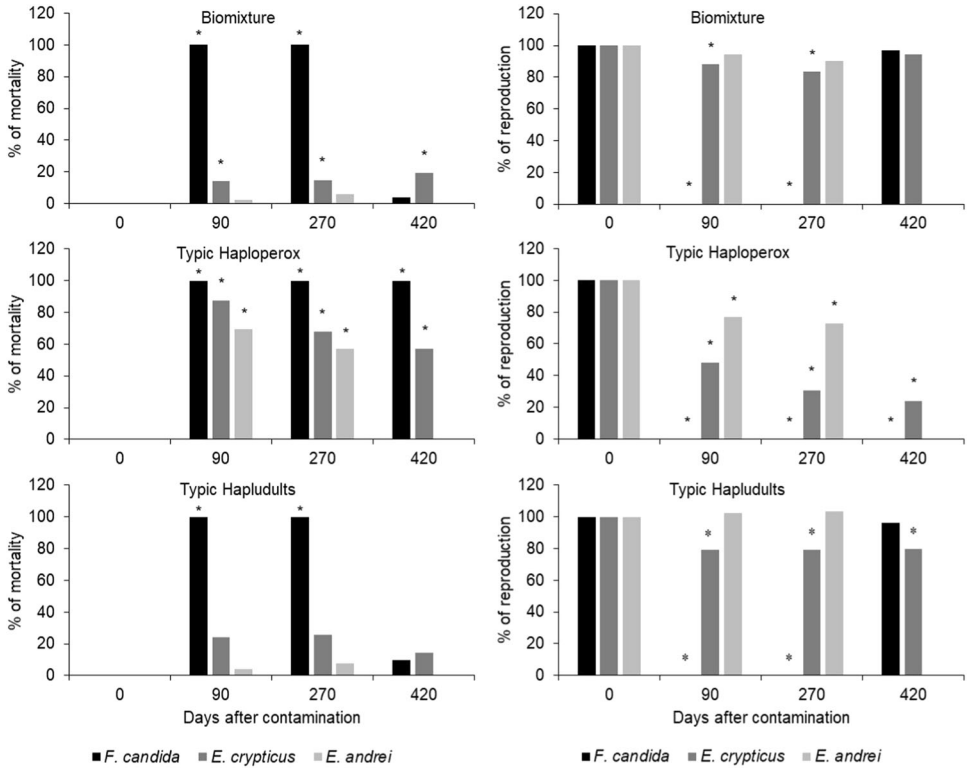


**Fig. 3** Mortality and reproduction of soil invertebrates (*F. candida, E. crypticus*, and *E. andrei*) exposed to Biomixture, Typic Haploperox, and Typic Hapludults after 0, 90, 270, and 420 days of contamination with Chlorpyrifos and Mancozeb. Asterisks indicated significant differences ($p < 0{,}05$) between day 0 and days after pesticide application

showed that for all substrates, both pesticides were reduced over time. The original molecules from Chlorpyrifos and Mancozeb were detected at 90 days, but they were not present after 270 days of disposal in biomixture or natural soils.

In the biomixture, significant deleterious effects ($p < 0.05$) were observed on collembolans reproduction at samples from 90 and 270 days after contamination (without adult survival). However, the sample from 420 days showed no toxicity to this species, indicating the recovery of collembolans and the efficiency of biobeds in reducing the ecotoxicity. The enchytraeid species *E. crypticus* showed to be less sensitive than collembolans to indicate the ecotoxicity of these pesticides. These organisms maintained at least 80% of reproduction in all samples when compared to day 0 (even though mortality was different). For earthworms, non-ecotoxicity was found in survival and reproduction tests (Fig. 3).

In Typic Haploperox, we observed the most pronounced effects. No survival and, consequently, no reproduction of collembolans was observed for all samples after pesticide disposal. Moreover, significant effects on the reproduction of enchytraeids and earthworms were observed for all samples after contamination, due to the low rate of survival. No reduction of toxicity was observed even after 420 days of contamination on this soil for the tested species (Fig. 3).

For Typic Hapludults, results with collembolans resemble those observed in the biomixture substrate, with high toxicity after 90 and 270 days of application (without adult survival), and reduction of ecotoxicity after 420 days. Although survival has not been affected, enchytraeids reproduction showed the same trend of that in biomixture, with a reduction of 20% of the population after pesticide disposal. This trend remained in all samples and times (Fig. 3). As well as in the biomixture, the earthworms were not sensitive to the residues in Typic Hapludults.

**Table 4** Chemical analysis of Chlorpyrifos in the biobed system 90 and 420 days after simulation of accidental spillage (1 liter of commercial product Lorsban® 480 BR)

| Biobed | 90 days (mean ± deviation) | 420 days (mean ± deviation) |
|---|---|---|
| 01 | 370.03 ± 24.23 | 47.6 ± 9.36 |
| 02 | 552.73 ± 23.78 | 15.86 ± 3.21 |

Source: center of research and analysis of residues and contaminants (CEPARC), UFSM, Santa Maria, RS, Brazil

## Contamination of accidental spillage

The results of the chemical analysis pointed to the degradation of the Chlorpyrifos molecule over time (Table 4). At day 420, concentrations were lower ($15.86 \pm 3.21$ mg kg$^{-1}$) in comparison to those in day 90 ($552.73 \pm 23.78$ mg kg$^{-1}$).

The reproduction tests also fulfilled the validation criteria as the experiments of effluents pesticide disposal over time. Although at 90 days, the period of greater contamination of

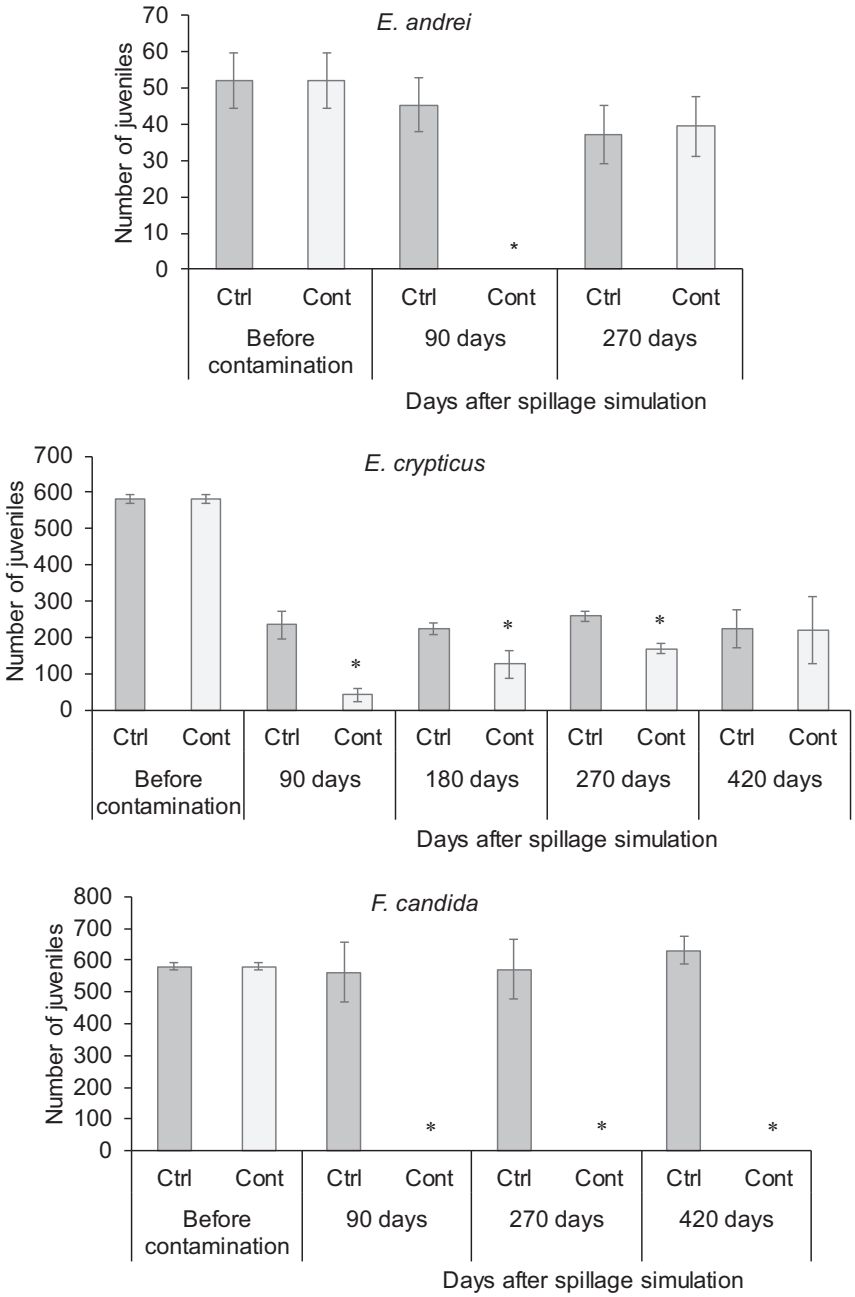


**Fig. 4** Number of juveniles (mean ± standard deviation) of *E. andrei*, *E. crypticus*, and *F. candida* in control (dark gray) and contaminated (light gray) biomixture samples before and after contamination. Asterisks indicate significant differences by the *t*-test ($p < 0.05$)

the biobeds, there was not reproduction of the earthworms, at 270 days, the reproduction rate was already equivalent to the control (Fig. 4). The biomixture contaminated with Chlorpyrifos negatively affected the reproduction of enchytraeids in the three first samplings (90, 180, and 270 days). This was evidenced by the significantly lower reproduction rates ($p < 0.05$) in the contaminated substrate compared with control. However, this effect decreased over time, and there were no more significant differences in reproduction between the contaminated substrate and the control at 420 days (Fig. 4). Collembolans were negatively affected in the biomixture contaminated in all sampling times (90, 270, and 420 days), which was evidenced by the absence of reproduction in all contaminated substrates (Fig. 4).

## Predicted environmental concentrations (PEC soil) of the pesticides

Using the soil data (Table 2), agricultural product information (Table S1), and the average temperature during the experiment contamination (20 °C), PECs of Chlorpyrifos and Mancozeb were estimated for Typic Haploperox and Typic Hapludults (Table 5).

**Table 5** Predicted environmental concentration (PEC soil) estimated to happen after 1 year of pesticide application in an apple orchard

| Soil | Chlorpyrifos | Mancozeb |
|---|---|---|
| Typic Haploperox | 0.5180 mg a.i. $kg^{-1}$ | 0.8533 mg a.i. $kg^{-1}$ |
| Typic Hapludults | 0.5191 mg a.i. $kg^{-1}$ | 0.8601 mg a.i. $kg^{-1}$ |

These values represent an estimative of the worst-case scenario applications according to the current agriculture recommendations

## Discussion

### Disposal of pesticide effluents over time: how are soil faunal organisms affected?

Studies have shown the high toxicity of organophosphates to collembolans (Natal-da-Luz et al. 2012; Santos et al. 2012) in concentrations lower than 1 mg a.i. $kg^{-1}$ in the soil, which could explain the total death of adults (100% of mortality) and consequently, the absence of reproduction at 90 and 270 days in all substrates. Despite of Fang et al. (2009) pointed out that 4 mg a.i. $kg^{-1}$ of Chlorpyrifos in a soil with pH 6.8 could be 83% degraded in 35 days. The literature shows that the half-life ($DT_{50}$) of Chlorpyrifos could be highly variable (14 days to 1 year), depending on environmental conditions such as moisture, temperature, and soil characteristics. Nevertheless, Reproduction data should be carefully analyzed, since in some cases there was a low adult survival rate—or even any adult survival. Consequently, the absence of adults could impact reproduction data.

After degradation, a possible product of Chlorpyrifos hydrolyzes is the 3,5,6,-trichloro-2-pyridinol (TCP), which presents antimicrobial activity and could be persistent to microbiological degradation (Anwar et al. 2009). Furthermore, in specific conditions, organophosphates could oxidize to oxon, a molecule more toxic and generally powerful in acetylcholinesterase inhibition than the precursor compounds (Kralj et al. 2007). This co-metabolite in Typic Haploperox until 420 days could be the reason for the absence of collembolan reproduction, and for the low percentage of earthworms and enchytraeids reproduction when compared to day 0. Even that both soils have slightly differences, the amount of clay and WHC could suggest a stronger retention capacity in Typic Hapludults (clay 40% and WHC 83%) than in Typic Haploperox (clay 30% and WHC 78%).

Another explanation for the difference in the percentage of reproduction between two contaminated soils is that Mancozeb, which is composed mainly by manganese and zinc—80%, could be more available in the presence of other cations, competing by the soil adsorption sites (Matos et al. 2001). Although pH and organic matter are similar, the high number of cations of Typic Haploperox could lead to a lower adsorption than Typic Hapludults, and therefore a greater availability of this pesticide—which could causes a higher toxicity. Soil texture has an important role in soil capacity to retain pollutants (Kramer et al. 2009). It could explain the different effects observed at Typic Haploperox and Typic Hapludults, since more clayey soils usually have greater adsorption capacity (Uddin 2017).

Effects of Chlorpyrifos to soil fauna are widely discussed, as for collembolans and mites (Owojori et al. 2014; Jegede et al. 2017), but especially for earthworms. Silva et al. (2009) assessed the toxicity to *E. andrei* in OECD soil and natural soils at different temperatures. All effect concentrations to 50% of the population ($EC_{50}$) were lower than 8 mg a.i. $kg^{-1}$. In natural soils from Tunisia and Nigeria, $EC_{50}$ values for *F. candida* were even lower than 0.05 mg a.i. $kg^{-1}$ (Kamoun et al. 2018), whereas in a Portuguese sandy soil, $EC_{50}$ was 0.38 mg a.i. $kg^{-1}$ (Santos et al. 2012). Thus, it was already reported a high toxicity of the organophosphates to soil fauna, as well a low toxicity of dithiocarbamates in tests with collembolans and yet, the absence of Mancozeb toxicity to earthworms (García-Santos and Keller-Forrer 2011; Alves et al. 2014). Furthermore, concerning the combined effects of Chlorpyrifos and Mancozeb, Morgado et al. (2016) considered the effects as non-interactive or additive. These authors observed that some environmental conditions, such as soil moisture, affects each pesticide individually and its more relevant to toxicity than the mixture of the two products to isopod *Porcellionides pruinosus*. Considering all available literature and effects observed in the present paper, mostly at 420 days, apparently, the residues of Lorsban® 480 BR could be the toxicity driver.

Therefore, treating the toxicity of the pesticides separately, it can be presumed that, despite the possibility that Mancozeb could have been more available in Typic Haploperox, the drastic reductions in organism reproduction may be associated with residues of Chlorpyrifos contamination. Carniel et al. (2019) found no significant effects of Mancozeb in Typic Hapludults to *F. candida* and *E. andrei* ($EC_{50} > 100$ mg a.i. $kg^{-1}$).

In Brazil, the spray tank washing, as well as the handling of pesticides, are not usually carried out on a waterproof floor with a collector system. The sprayers manuals themselves indicate that the tank washing may occur on the ground, away from rivers and springs, and there is no legislation about this subject. Even though any system is proposed in the legislation, an alternative to decantation and recycling of this residues was already proposed by Rufato et al. (2006). However, there is also a lack of data about its toxicity to soil fauna after the decanting process.

Because of the high dilution, the residues are often considered to be empirically non-toxic to the soil environment. The estimated PEC results present higher values than

the ones founded in chemical analysis, which is expected, since the wastewater does not contain the same amount of the initial preparation. However, this study showed toxicity to soil mesofauna even at 270 and 420 days after soil contamination in Typic Haploperox, which suggests that this residue is potentially harmful to soil and should be treated carefully before soil disposal. To achieve this goal, biobeds seem to be a good alternative, maintaining the tank washing and the handling of pesticides isolated from soil and freshwater. Over the years, the biobeds have proven to be an efficient form of degradation of pesticide residues in some countries (Spliid et al. 2006; Vischetti et al. 2008; Karanasios et al. 2013). These are the first results involving ecotoxicity tests with biobeds in Brazil. However, additional studies should be carried out to assess the efficiency of pesticide degradation with other compounds in this system. The time for degradation and possible adaptations of biobed methodology and biomixture composition needs to be studied for different regions in developing countries.

## How are soil fauna organisms affected by the contamination of accidental spillage?

The degradation of Chlorpyrifos occurs in biotic and abiotic pathways. However, it is already established that the most common degradation for the active ingredient is hydrolytic, which involves the formation of TCP, accelerated under more alkaline conditions (Baskaran et al. 1999). Nonetheless, Brazilian subtropical soils are commonly acidic or, when corrected, they do not have pH higher than 6.0, so that the degradation through the biotic pathway becomes highly relevant at different pH values (Singh et al. 2003).

Since the biomixture is predominantly composed of soil, a lower pH for Brazilian models can have advantages in the decomposition of the compounds, favoring the communities of ligninolytic fungi. These are more-effective microbiological agents that degrade pesticide residues present in biobeds that are already established (Gebler et al. 2015). The straw in biomixture composition promotes the fungal production of enzymes that degrade lignin, such as manganese peroxidase, which also degrades TCP (Coppola et al. 2011). Materials with lignin-degrading microorganisms are a prerequisite for the effective dissipation of Chlorpyrifos and the mineralization of TCP (Chu et al. 2008). These factors can favor not only the accelerated degradation of Chlorpyrifos but also the rapid mineralization of TCP, which prevents the leaching in non-waterproof models of biobeds.

The results point to the sensitivity of earthworms at the moment of greatest contamination (90 days) and later recovery of the reproduction rate (270 days). Earthworms are the most used bioindicator in ecotoxicological tests, and it was already highlighted their sensitivity to organophosphates (IBAMA 1996; Alves et al. 2014). *E. andrei* has different sensitivity in lethal and sub-lethal tests, as pointed by Silva et al. (2009). Researchers concluded the importance of using reproduction tests with earthworms to indicate the ecological risks of pesticides.

Although the registration of pesticides in Brazil requires only the sensitivity of earthworms (Novais et al. 2010), the terrestrial species of the genus *Enchytraeus* could be an adequate alternative to indicate pesticide toxicity. Besides ecologically relevant, these organisms are indicators of good agricultural practices, and they also proved to be sensitive in studies with pesticides (Amorim et al. 2005; Endlweber et al. 2006; Bandow et al. 2013; Pelosi and Römbke 2016; Zortéa et al. 2018).

Enchytraeidae and earthworms, based on limited data sets, do not show a consistent difference in sensitivity (Luan et al. 2005). The advantage of tests with Enchytraeidae than with earthworms is the need for a small amount of sample (180 × 2000 g), besides the duration of the chronic effect test on the reproduction (28 × 56 days, respectively). For tests with biobeds over time, removing a smaller aliquot from the biomixture is interesting to maintain the functionality of the system until the end of the period.

Collembolans can be directly affected by the addition of pesticides, especially insecticides, because of their morphophysiological characteristics similar to Insecta (Ruppert et al. 2005; Zortéa et al. 2018). The high sensitivity of collembolans when exposed to Chlorpyrifos in laboratory tests was observed by Santos et al. (2012), in tests of escape (82%), lethality ($LC_{50}$ 0.13 mg $kg^{-1}$), and reproduction ($EC_{50}$ 0.045 mg $kg^{-1}$) in a Portugal natural soil. This was also demonstrated by Natal-da-Luz et al. (2012), through the application of the insecticide with $EC_{50}$ calculated as 0.288 mg $kg^{-1}$ (0.143–0.432) in sandy soil, with applications directly in the field.

The chemical analysis performed on the last sampling day (420 days) showed Chlorpyrifos concentration of 15 mg a.i. $kg^{-1}$. These high concentrations corroborate with the absence of *F. candida* even after more than 1 year from the Chlorpyrifos application. Results indicated that the time of 420 days was enough to ensure the absence of ecotoxicity for oligochaetes. For collembolans, this time should be longer. The collembolans are an important group of soil fauna, involved in organic material cycling and food webs in the soil ecosystem. A longer time of biomixture permanence in the biobed is required to avoid the risk of Chlorpyrifos to collembolans. The results of the ecotoxicity tests can guide the correct management of the biomixture, indicating when the substrate could be disposed of in soil for composting without implicating a risk to the soil fauna, which will be involved in the processes of decomposition of the organic material.

In Brazil, one of the current pesticide effluent management recommendations is the washing of the equipment and its final disposal directly in the soil. This work stood out the importance of the biomixture as the current cleaning approach to be recommended for the pretreatment of the effluent in biobed before any disposal. The use of biobeds has proven to be an economic, effective, and safe alternative for the disposal of effluents with pesticide residues (Karanasios et al. 2013). Data presented by this experiment demonstrated that the biobeds are also a safer alternative of handling pesticides and can minimize the risks to groups of the soil fauna, as accidental spillages of pesticides can be a pathway of contamination for the organisms (Spadotto et al. 2004).

Some pesticide filling point models with a collection of residues have already been proposed (Gebler and Fialho 2011), and even the process of decantation and reuse (Rufato et al. 2006). However, there is no evidence if this residue could be dangerous to the edaphic soil after undergoing the decantation process. In pesticide sprayer filling points, where products are frequently handled, concentrated commercial products that are diluted into water to form the application solution are used, and, in this scenario, biobeds can be a good alternative to avoid soil contamination.

The risks of product spillages increase with the number of fills, constituting an environmental indicator that cannot be ignored in a pesticide risk analysis (Reichenberger et al. 2007). This activity can cause a point source contamination, producing concentrations equivalent to grams or decigrams per square meter (Zhang et al. 2018), increasing the risk of the pesticide compromising the environmental safety (Gebler and Fialho 2011). Therefore, studies to assess the possibilities of the biobeds use must to consider the efficiency in chemical degradation of compounds, as already proven for Chlorpyrifos by this and other dataset (Tortella et al. 2012). Some studies already showed the biobed effects on microbial communities, with higher recovery after a short period (Tortella et al. 2013). However, biological safety for the disposal of the biomixture needs to be assessed to representative species of soil fauna since they represent an important group with soil functions different than microorganisms. Collembolans and enchytraeids especially proved to be adequate to monitor the efficiency of the biobed system, being sensitive to the contamination by Chlorpyrifos.

## Conclusions

In general, the results demonstrated that biomixture could reduce the toxicity of the spray tank washing effluents with residues of Lorsban® 480 BR (a.i. Chlorpyrifos) and Dithane® NT (a.i. Mancozeb) and accidental spillage of Lorsban® 480 BR (a.i. Chlorpyrifos). Also, the time of disposal of the biomixture contaminated needs to consider the sensitivity of different key groups of soil fauna, as observed in this study. The collembolan species *F. candida* was the most sensitive species to indicate the ecotoxicity of these pesticides when compared to earthworms *E. andrei* and enchytraeids *E. crypticus*. Different level of effects was detected among the tested substrates: biomixture, Typic Haploperox, and Typic Hapludults. The most pronounced effects were observed in Typic Haploperox, and such toxicity remains even after 420 days, indicating that this soil requires more time to pesticide degradation than Typic Hapludults or biomixture. Earthworms were the less sensitive organisms to Chlorpyrifos and Mancozeb residues, which can indicate the inability of these organisms to be used to monitor the ecotoxicity of the biobed system.

The simulation of an accidental spillage of Lorsban® 480 BR (a.i. Chlorpyrifos) in biomixture posed a different risk to the three tested species, where collembolans showed higher sensitivity than earthworms and enchytraeids. The results of organisms exposition to the biomixture on the environmental conditions tested demonstrated that (1) over time, this substrate could reduce or even eliminate the toxicity of Chlorpyrifos to earthworms and Enchytraeidae and (2) safe disposal for collembolans requires a longer time of degradation in the biobed, or an alternative treatment to accelerate the degradation.

Finally, the biobed system or similar should be considered to handle pesticides and washing equipment, once that residues from those crop activities are potentially harmful to soil fauna according to the present study. Also, the results highlighted that future research about biobed efficiency in pesticide degradation should include ecotoxicology tests with standard species and not only chemical analyses. With this information, it will be possible to estimate real risks to soil fauna. One of the main reasons is that not all safe levels of pesticide concentration in soil or water are covered by the environmental legislation in their countries. Still, it is possible to establish acceptable levels of impact for the macrobiota, making it easier to monitor the risk of biobeds locally. Moreover, to reduce uncertainties, further research using different pesticides, soils, and even different materials on biomixtures are suggested. Since pesticides are widely used, different environmental conditions are also important to be observed. Adaptations in time safety to dispose of the biomixture can guarantee that the contaminated substrate only contacts the environment when it no longer poses risks, which can be confirmed by the ecotoxicological tests.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors

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