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Vermicompost Incorporation into Soil Contaminated with Deltamethrin as an Attempt to Reduce the Toxicity Caused by this Pyrethroid: A Structural Study of Soil

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Abstract: The use of vermicompost for remediating soil contaminated with the commercial formulation containing deltamethrin was proposed. Biotoxicity tests with the goal of evaluating the potential remediation degree of the vermicompost after its addition to contaminated soils were performed. The tests lasted 56 days and involved *Eisenia foetida* earthworms. Possible structural changes in humic acids from these soils were evaluated by means of FTIR spectroscopy. The soil contaminated with deltamethrin offers toxicity to the earthworms. However, the vermicompost addition caused great positive impact on soil contamination level, which became quite similar to the reference soil. The vermicompost remedial effect depends on the source material. In this study the vermicompost were from filter cake and orange peel. The results obtained by FTIR pointed out for a weak interaction such as van der Waals type between the pyretroid and humic acids.

Keywords: Soil Remediation, Deltamethrin, Filter Cake Vermicompost, Orange Peel Vermicompost, Toxicological Tests

Introduction

Deltamethrin ((S)-α-cyano-3-phenoxybenzyl (1R, 3R) -3-(2,2-dibromovinyl) -2,2-dimethylcyclopropanecarboxylate) is a pyrethroid that kills insects such as flies and cockroaches, by contact and ingestion. It was synthesized in 1974 and first marketed in 1977. Its action spectrum is very broad and it has been considered the most powerful of synthetic pyrethroids (Santos *et al*., 2011; Toumi *et al*., 2013; Velki and Hackenberger, 2013; Rehman *et al*., 2014).

The low toxicity of pyrethroid insecticides to mammals, birds and limited persistence in soil has encouraged the widespread use of pyrethroids in agriculture and vector control diseases. They are used to protect stored products such as cereals, grains, coffee and dried beans and for the control of diseases such as malaria and Chagas (via vector control) (Farghaly *et al*., 2013).

Despite the low persistence in soil, deltamethrin residue in crops can cause in humans excessive salivation, irregular movements members, tonic and clonic convulsions and increased sensitivity to external stimuli. The pyrethroid impairs the transmission of nerve impulses, which can lead to death (Santos *et al*., 2011; Velki and Hackenberger, 2013).

Bioremediation is a process where organisms (usually plants, microorganisms and earthworms) are used for degrading pollutants in the environment and can be applied *in situ* (de Melo, 2008). The incorporation of vermicompost to the contaminated soilwas used in this study as a kind of bioremediation.

It is known that the addition of vermicompost in soils retains several xenobiotics such as pesticides unavailable them to the environment. Vermicompost contributes to the maintenance of the organic matter cycle, since it is organic matter recycled by earthworms. Besides, it is rich in nutrients and improves the soil structure (Landgraf *et al*., 2005). Filter cake and orange peel, wastes generated in a huge scale in the state of São Paulo, Brazil, provided the raw material for the production of vermicompost. Thus, unwanted waste had an environmentally friendly destination, becoming products with high benefit, able to contribute to minimize problems of environmental contamination.

Humic substances are the main constituents of natural humus and have the ability to interact with organic compounds. They can interact with pesticides in different ways, such as ionic adsorption, hydrogen bond, covalent bond and hydrophobic adsorption, among others (Senesi, 1992). The understanding of the chemical nature, properties and reactivity of humic substances as the main materials that interact with pesticides in soils is important to evaluate the way and extension of their interaction with the system (Senesi, 1992).

Techniques such as Fourier Transform Infrared Spectroscopy (FTIR) help to elucidate the interactions between the functional groups constituent of organic matter and pesticides, for example. The spectra of humic acids, which are the principal constituents to humic substances, are relatively simple, with few absorptions extended bands.

In this study, vermicomposts from filter cake and from orange peel were added to the contaminated soil aiming to its possible bioremediation. For evaluating the remediator effect, biotoxicity tests were performed using earthworms *Eisenia foetida* for 56 days with the goal of evaluating the potential remediation degree of the vermicompost after its addition to the contaminated soils. The results obtained in the toxicity tests were analyzed by the ANOVA and Dunnett test, with assurance of 95%. Therefore, possible structural changes in humic acids from these soil contaminated samples were evaluated by means of FTIR spectroscopy.

Materials and Methods

Wastes constituted from orange peel and filter cake were separately composted in piles of 2.25 m^3 (2.5 m length ×2.5 m width ×1 m height) during 40 days, until the composting process returned to mesophilic phase, as observed by daily temperature measurements. After this period, the so composted orange peel was mixed with cattle manure (2:1 w/w) and vermicomposted for 135 days (until complete degradation of organic matter). The composted filter cake was subjected to the same treatment, by mixing it with cattle manure (3:1 w/w). The residues proportion was calculated to obtain an initial C:N ratio between 20 and 30 for the piles. Sawdust was used as structuring agent and to assist in the aeration of these piles (Pigatin *et al*., 2012).

A red latosol collected in the region of São Carlos, SP, Brazil, was choose for toxicity tests in an area of forest, (21°56'6"S 47°54'16"W) based on prior knowledge of the site with the intent of guarantee the absence of pesticides on the soil.

For better evaluating the effect of the pyretroid on soil, deltamethrin commercial formulation has been used with and without the active ingredient, in order to also evaluate the impact of the dispersant substances contained in the commercial formulations. From now on, deltamethrin refers to the entire commercial formulation and dispersant refers to the dispersants themselves, without the active ingredient. In Brazil, according to the Ministry of Agriculture, Livestock and Food Supply, there are 8 registered products with deltamethrin as the active ingredient for use in 35 crops (MAPA, 2015). For all samples, the formulation concentration used was 25,000 mg L^{-1} (stock solution). This concentration was chosen to mimic the cases of soil contaminated by spillage, namely the contamination events on a large scale.

Sample Preparation

Approximately 500 g of red latosol were placed in plastic containers (15×15×10 cm, with perforated cover), moistened to approximately 40-60% moisture content and intimately mixed with 10.00 mL of the stock solution formulation (in a total concentration of 500 mg kg^{-1} deltamethrin or dispersant).

The obtained vermicomposts: - Filter Cake Vermicompost (FCV) and Orange Peel Vermicompost (OPV) - were added to the contaminated soil with deltamethrin (or dispersant) in order to evaluate their remediation capacity.

Fifteen grams of the vermicomposts were added and mixed up to the contaminated soil, corresponding to 3% of the total mass of the soils. The samples were: soil (reference); soil + dispersant; soil + dispersant + FCV; soil + dispersant + OPV; soil + deltamethrin; soil + deltamethrin + FCV; soil + deltamethrin + OPV. All the so obtained samples were submitted to bio toxicity tests.

Test Organisms

The choice of test organism, the conditions for its maintenance in the laboratory and the requirements for its use in the tests were based on an ISO protocol (IOS, 1993; 1998). Batches of earthworm *Eisenia foetida* were obtained from producers in the city of São Carlos, SP, Brazil. Earth worms were kept in the laboratory in a mixture of reference soil and dry cattle manure (50:50 v/v) in sturdy plastic containers (15×15×10 cm, with rounded bottom perforated lid, with a capacity of 1000 g of dry substrate), at a constant temperature of 20°C with a photoperiod of 16:8 h light: Dark. This mixture was moistened periodically to maintain the moisture content between 40 and 60% of humidity, as cited. The individuals used in the tests were more than one month old with an average weight of 500 mg.

Mortality, Biomass and Reproduction Tests

Mortality, biomass and reproduction tests were performed according to ISO 11268-1 and ISO 11268-2 (Senesi, 1992; Pigatin *et al*., 2012). Every test was performed with five repetitions, each result being the arithmetic mean of these replicates, in vases of 1.00 kg, with 10 adult earthworms. To monitor acute toxicity, earthworms were weighed initially and on days 7 and 14. To monitor biomass, it content was calculated by the percentage difference between the weight obtained at t = 0 and t = 28 days. To monitor the reproduction, the survival earthworms were removed from the vases. The vases, now without adult earthworms, were kept in the same experimental conditions until the 56th day, when cocoons, eggs or young earthworms were counted.

Preparation of the HA Fractions

Soil samples used in all the tests went through the process of extracting humic acids. In total, the samples from the described 6 treatments plus the reference were subjected to a process of extraction and purification of humic acids by differential solubility, according to the methodology proposed by IHSS (Sparks *et al*., 1996) and purified with HCl/HF, followed by dialysis for 7 days (until no chloride ions were determined in dialysis water) and then freeze-dried.

Characterization of the HA Fractions

The HA fractions were characterized by FTIR in accordance with the methodology proposed by Stevenson (Stevenson, 1994). The FTIR spectra were obtained with a Bomem MB-102 spectrometer (Zurich, Switzerland), equipped with an IR source, KBr beam splitter and DTGS KBr detector. For each sample, 32 scans were recorded with a 4 cm^{-1} resolution over a 4,000 to 400 cm^{-1} range on pellets obtained by pressing a mixture of 1 mg of the humic acidand 100 mg of dried KBr.

Statistical Analyses

The tests were carried out in quintuplicate and the results obtained in the toxicity tests were analyzed by the Dunnett test (Zar 2010). This statistical procedure allows the comparison of the dosage applied in relation to a control group. Assuming the nonexistence of a difference between dosages and control group (the null hypothesis), these groups were rejected for a probability equal or lower than 0.05.

Results and Discussion

Vermicomposting is the mineralization process of organic matter executed by earthworms. The earthworms act as microbioreactors leading to various several unit operations that conduce the stabilization of the recent organic matter, transforming it in a more refractory material (Dores-Silva *et al*., 2015). During this process, liberation of some plant nutrients (nitrogen, phosphorus and potassium, among others) occur. The humus produced in the vermicomposting also has a high percentage of colloidal material, thereby improving the soil quality.

Humic acids are able to interact with pesticides, for example, due to the presence of distinct functional groups (carboxyls, hidroxyls, phenols, carbonyls etc.). The addition of humus can decrease the toxicity of the xenobiotic in question, reducing its toxicity in the soil, making it unavailable or may degrade it to other compounds. To understand how this interaction occurs at the microscopic level, FTIR spectroscopy was used. Thus, if the addition of humus can lead to changes in the molecular structure, spectroscopic analysis can evaluate these possible changes during the process.

The FTIR spectra of the humic acids extracted from the soil upon the toxicity experiments involving deltamethrin (or dispersant) showed the same characteristics, with same differences between bands. The bands were attributed according to Stevenson (1994; Silverstein *et al*., 2008; Fialho *et al*., 2010) (Fig. 1).

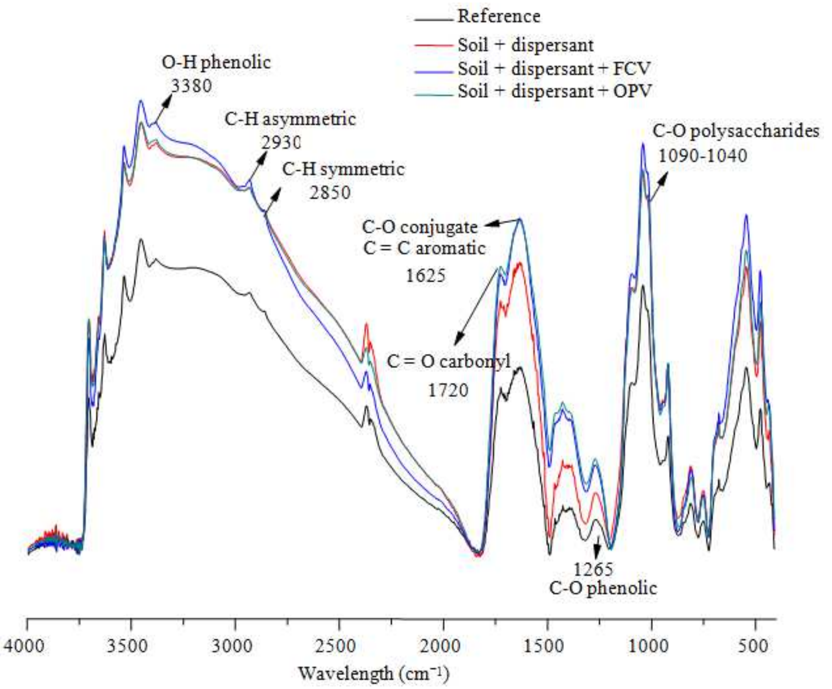
In Fig. 1, the band centered at 3400-3300 cm^{-1} could be assigned to the O-H stretching of the bound water and to some OH groups. Another band at ~3140 cm^{-1} is assigned to N-H stretching of N-containing groups present in the humic acids structure. The bands at 2930-2850 cm^{-1} refer to C-H structures aliphatic stretches (asymmetric and symmetric respectively).

In the region 1720 cm^{-1} appears the band attributed to carbonyl groups unconjugated stretches (C = O) and in 1625 cm^{-1} a band corresponding to conjugated C-O and C = C in aromatic structures. The band centered at 1265 cm^{-1} is attributed to C-O phenol groups stretching and the region 1090-1040 cm^{-1} to polysaccharides stretches.

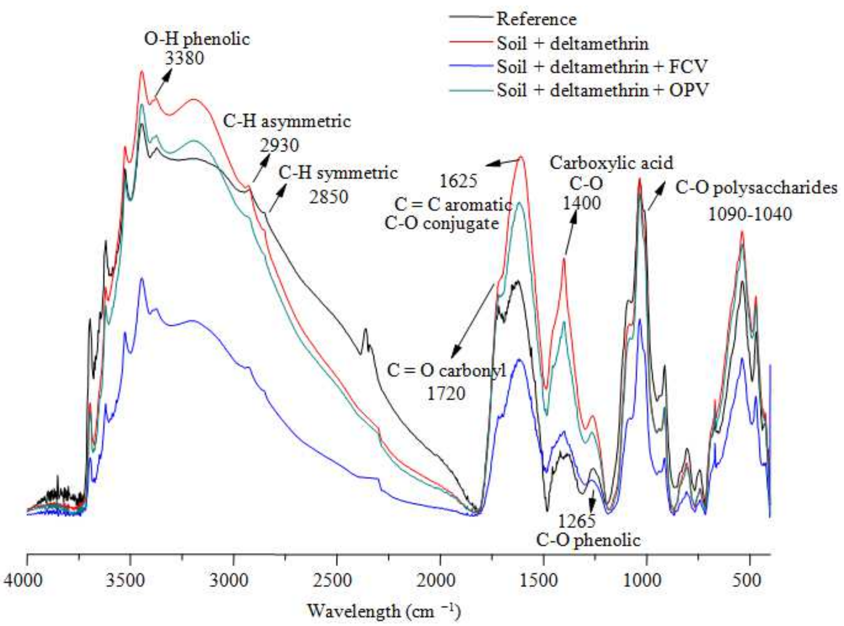
Huang *et al*. (2011; Guerra-López *et al*., 2010) recommend that for a better FTIR spectra interpretation, it is interesting to calculate the second derivative in order to improve their resolution and facilitate the visualization of bands that could be hidden. Thus, Fig. 2 shows the FTIR spectra (second derivative) of humic acids. The bands were assigned in the same way.

For treatment involving the dispersant agent, it is possible to realize the intensity reduction of the bands between 3700-3500 cm^{-1} corresponding to mono substituted aromatic rings stretching, when adding the filter cake or orange peel vermicompost. The band increase in 2350 cm^{-1} refers to C = O carbon dioxide or silicon compounds stretching. The region between 1600-1400 cm^{-1} also exhibited pronounced decrease with the vermicompost addition, which is the region corresponding to C = C aromatic rings, N-H bending and C-H deformations of aliphatic conjugated structures and quinones. There was also an increase in intensity of bands in the region 1200-650 cm^{-1} corresponding to the stretching C-O phenolic groups, or polysaccharides derivatives and C-H vibrations outside the plane. It is noteworthy to remember that humic acids from vermicomposts are the result of less condensed molecules, due to larger number of aliphatic chains. In addition, humic acids from vermicomposts present a large number of O content groups. The bands at 918 cm^{-1} corresponding to vinyl structures, 912 cm^{-1} to O-H outside plane deformations, 802 cm^{-1} to outside the plane $RCH_2 = CHR$ deformation.

For deltamethrin treatment, the bands attribution are similar, highlighting the band centered at 1300 cm^{-1}, characteristic of C-Br stretching. Table 1 and 2 show the specific and common bands for each sample.



(A)



(B)

Fig. 1. FTIR spectra of the soil humic acid from all treatments with the mainly stretched groups: (A) (---) soil (reference); (---) soil + dispersant; (---) soil + dispersant + filter cake vermicompost; (---) soil + dispersant + orange peel vermicompost; (---) (B) soil + deltamethrin; (---) soil + deltamethrin + filter cake vermicompost; (---) soil + deltamethrin + orange peel vermicompost

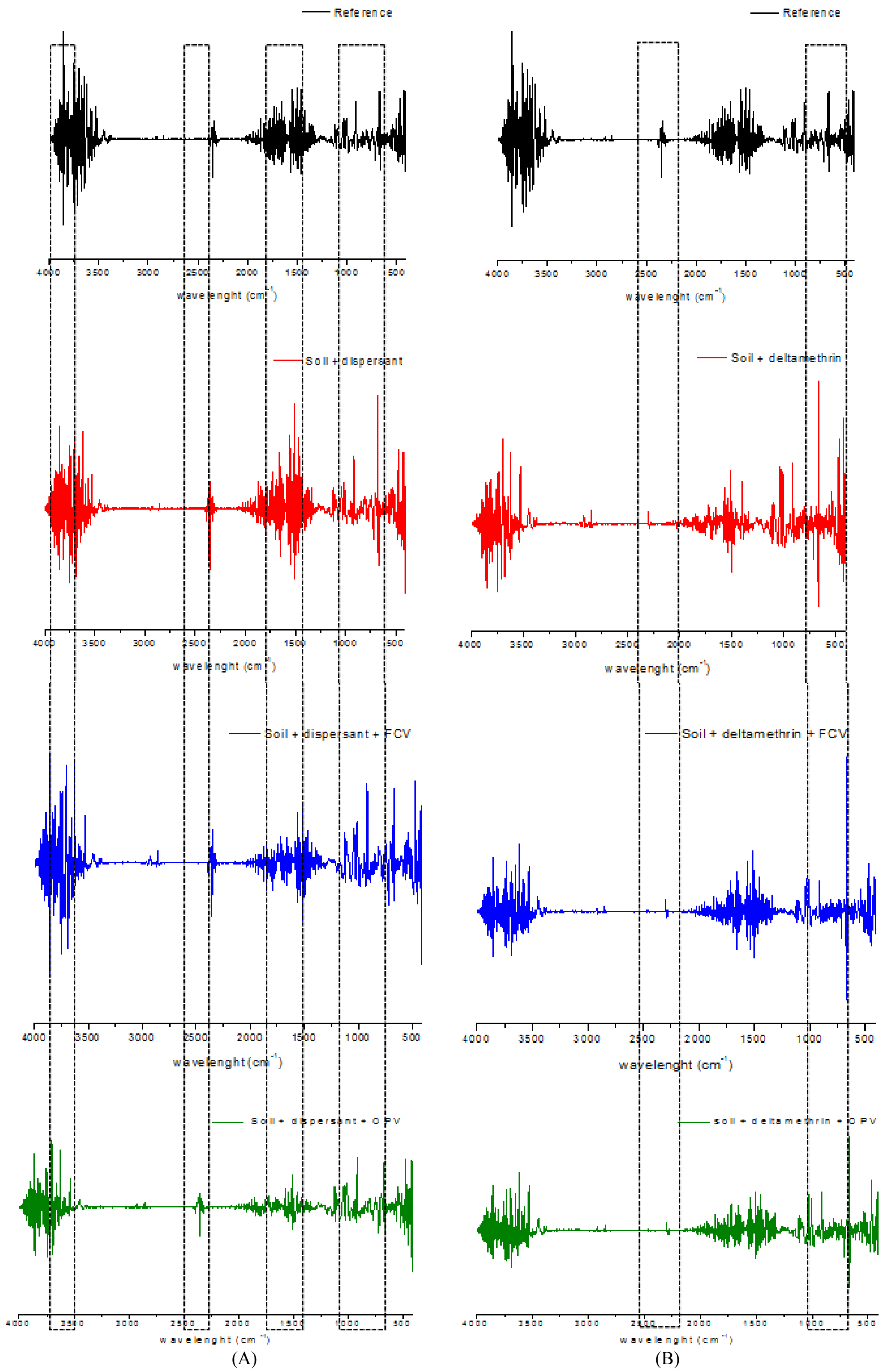


Fig. 2. FTIR second derivative spectra of the soil humic from all treatments: (A) (---) soil (reference); (---) soil + dispersant; (---) soil + dispersant + filter cake vermicompost; (---) soil + dispersant + orange peel vermicompost; (---) (B) soil + deltamethrin; (---) soil + deltamethrin + filter cake vermicompost; (---) soil + deltamethrin + orange peel vermicompost.

Table 1. Major bands in FTIR experiments involving dispersants samples

	Bands (cm^{-1})	New bands
Reference	3861, 3620, 3527, 3448, 2929, 2355, 1265, 1033	
Soil + dispersant	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	1716, 1660, 1630, 1400, 1050, 918, 912, 802, 744
Soil + dispersant + FCV	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	2856, 1716, 1660, 1630, 1400, 1050, 918, 912, 802, 744
Soil + dispersant + OPV	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	2856, 1400, 1050, 918, 912, 802, 744

Table 2. Major bands in FTIR experiments involving commercial formulation of deltamethrin samples

	Bands (cm^{-1})	New bands
Reference	3861, 3620, 3527, 3448, 2929, 2355, 1265, 1033	
Soil + deltamethrin	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	1716, 1660, 1630, 1300, 1050, 918, 912, 802, 744
Soil + deltamethrin + FCV	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	2856, 1716, 1660, 1630, 1300, 1050, 918, 912, 802, 744
Soil + deltamethrin + OPV	3861, 3620, 3527, 3448, 2929, 2355, 1400, 1265, 1033	2856, 1300, 1050, 918, 912, 802, 744

For all mentioned bands, it can be observed that the samples exhibit almost the same characteristics, regardless to vermicompost addition. This is an indicative that there is not a chemical reaction. For the infrared region spectra, changes in the humic acids structure were due to the addition of different compounds (dispersants/deltamethrin and filter cake or orange peel vermicomposts). Based on Dobbss *et al*. (2010), the relationship between the bands centered at 2929 and 1050 cm^{-1} was evaluated. The band at 2929 cm^{-1} is related to non-polar aliphatic groups and the band at 1050 cm^{-1} is related to oxygen polar groups. The relationship between the intensity of these bands is indicative of recalcitrance molecule level and thus the microbial degradation difficulty. In this study, the hydrophobicity index (2929/1050 cm^{-1}) provides important information to assess the remediation potential of the vermicomposts. The greater the intensity of the band at 2929 cm^{-1} compared to 1050 cm^{-1}, greater difficulty provides the molecule to its microbial degradation. The relative intensities of the bands at 2929 and 1050 cm^{-1} are shown in Fig. 3.

The vermicompost addition caused a slight increase in the hydrophobicity index of humic acids samples involving deltamethrin compared to the reference sample. In relation to the experiments involving dispersant, only the sample with addition of FCV showed slight increase. With this result, it can be assumed that the vermicompost had a slight interaction with deltamethrin, making it unavailable to earthworms.

Figure 4 shows the results involving the toxicological tests, which help to better understanding this data. The results of toxicity tests can be used as a reliable indicator of environmental pollution. Data from this study suggested that deltamethrin and its dispersant agent have a lethal effect on earthworms after exposition to these compounds.

The deltamethrin/dispersant addition in the soil caused an increase in earthworm mortality. Under the experimental conditions used here, the mortality had significantly different results from the reference soil, or soil + vermicompost samples.

After FCV and OPV addition, the mortality rate (relative to the reference sample), showed a significant reduction in the case of soils contaminated with dispersant agents. In soil contaminated with deltamethrin, none of the evaluated vermicomposts presented a positive impact on mortality. In the OPV case, mortality was even higher. OPV is more acidic than FCV, so this factor may contribute to this result. It can be assumed that the vermicompost reacted with compounds containing dispersant, inhibiting its toxic effect by complexing them.

In biomass tests, the FCV and OPV increase the earthworm development in the dispersant agent samples. In the deltamethrin samples, this addition did not take place satisfactorily, so it became clear that deltamethrin presents a more toxic effect than the dispersant agent. In the experiments with the presence of the dispersant agent, the vermicompost addition was enough to improve the development of the earthworms, which did not occur in experiments involving deltamethrin.

Concerning the reproduction, the dispersant agent addition to the soil causes a decrease in the eggs number and, consequently, of young earthworms. The FCV addition carries the eggs hatch and thus for the proliferation of young earthworms. The same, although to a lesser degree, is observed after OPV addition.

The soil contaminated with deltamethrin did not present any young earthworms, even after the vermicompost addition. Here, the earthworms using deltamethrin as a food source, which leads to molecule degradation, increasing the hydrophobicity index. This increase is slight due to the high toxicity of deltamethrin, leading to a high mortality observed in the population of earthworms and the loss of biomass observed in the survivors. Deltamethrin has a direct effect on the nervous system of animals, so the 3% vermicompost addition was not enough to overcome this effect (Kumar *et al*., 2016; Soderlund, 2013).

Maitre *el al*. (2012) sustain that biomass changes can be a good indicative of chemical stress, which may link chemical effects to energy dynamics and ultimately inhibit growth. The no earthworm's growth in the deltamethrin samples suggested that the pyretroid has a toxic effect in their organisms. Rathore and Nollet (2012) and Tiwari *et al.* (2016) asserts for the earthworm's strategy for natural survival: Reducing food intake to avoid the toxins, commonly used in earthworms to avoid poisoning heavy metals and organic chemicals.

The hydrophobicity index (Fig. 3) showed a significant difference in samples where there was only the presence of the dispersant, which is related to the lower toxicity of the dispersant compared to deltamethrin. For the two evaluated systems (dispersant agent and deltamethrin), after FCV addition, there is an increase in this index. Already after the OPV addition, this index decreased slightly. This is an indicative that FCV reacts with deltamethrin, making it available to earthworms as a food source. The differences in pH values between the two vermicomposts can contribute for this result. The results obtained in toxicological tests strengthen this hypothesis.

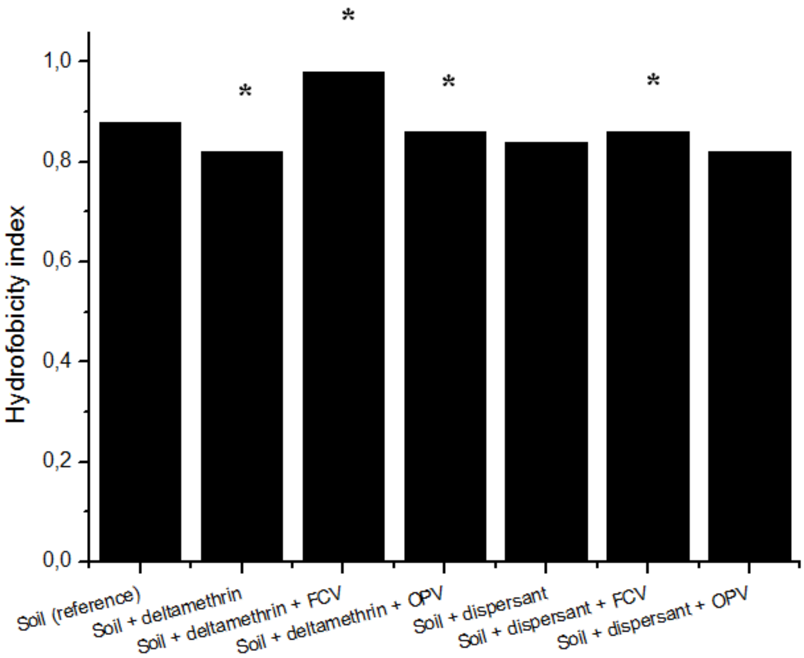
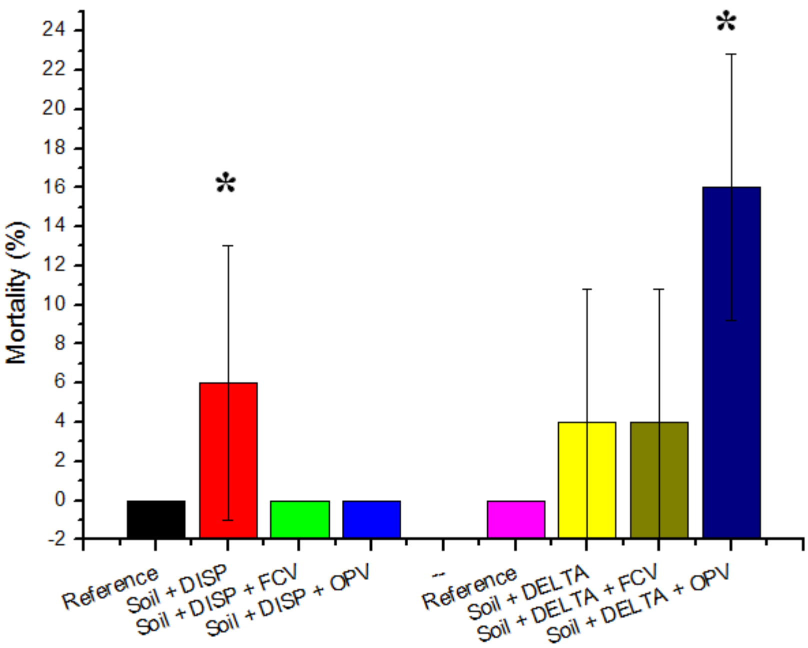
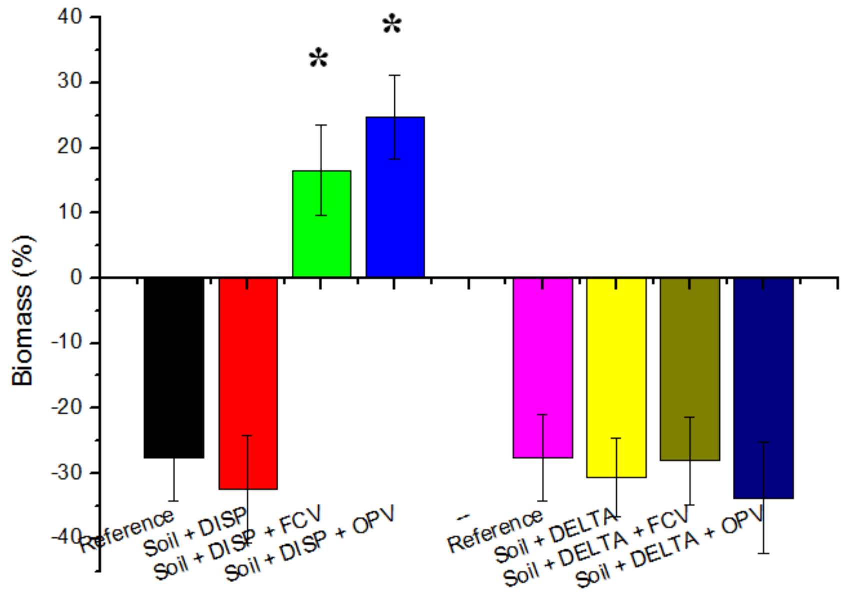


Fig. 3. Hydrofobicity index for humic acid when exposed to commercial formulation of deltamethrin (or only dispersant solution) to a concentration of 500 mg kg^{-1} and Filter Cake Vermicompost (FCV) and Orange Peel Vermicompost (OPV). * = statistically significant difference ($p<0.05$) compared to the reference, according to the Dunnett's test



(A)



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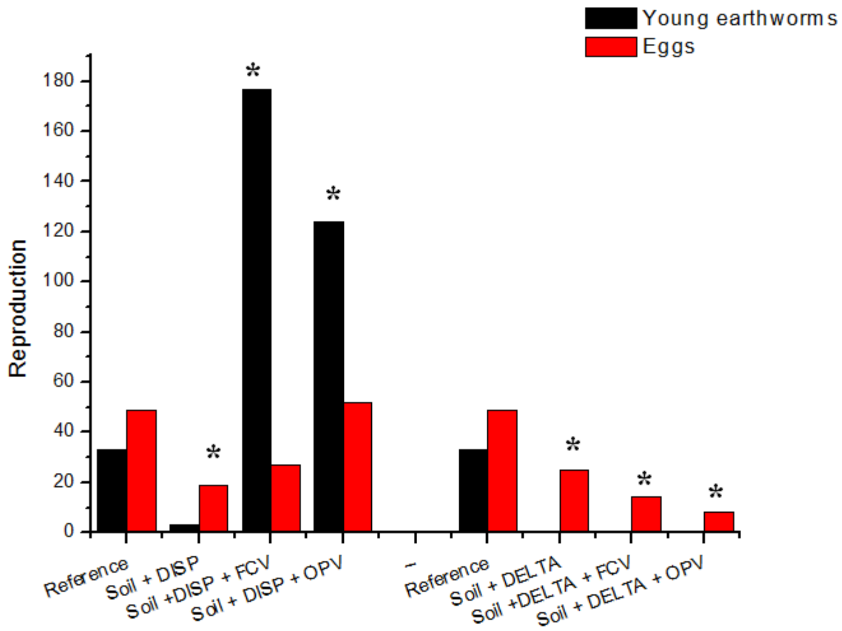

(C)

Fig. 4. Mortality after 14 days (A) biomass after 28 days (B) and reproduction after 56 days (C) average (n = 5) of earthworms (*Eisenia foetida*), when exposed to 500 mg kg^{-1} commercial deltamethrin (or solution dispersant) and Filter Cake Vermicompost (FCV) and Orange Peel Vermicompost (OPV), in natural soil. * = statistically significant difference ($p < 0.05$) compared to the reference, according to the Dunnett's test. Error bars represent standard deviations.

According to the results presented for toxicity tests and FTIR, the interaction between deltamethrin and humic acids can be Van der Waals type according to Fig. 5.

The contaminated soil dispersants offer toxicity to earthworms. However, the vermicompost addition caused major positive impact on the degree of mortality, which has become similar to the reference sample. Since the biomass and reproduction effects of the surviving organisms were similar for both the dispersants and deltamethrin contaminated soil, it can be concluded that the dispersants are also toxic and, in addition, the remedial effect of humus depends on the source material.

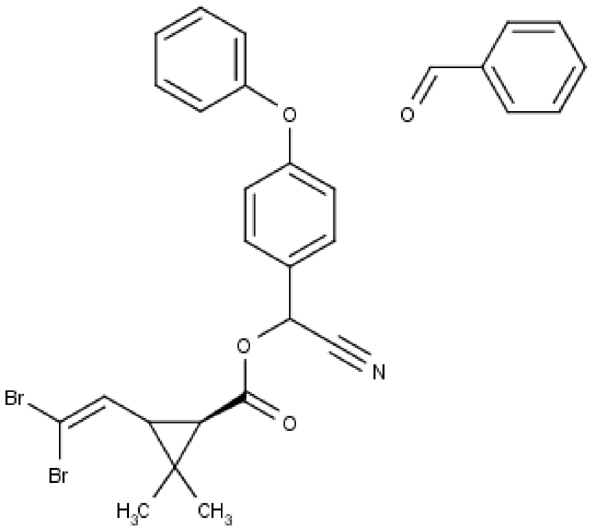

Fig. 5. Scheme of the interaction between the molecule of deltamethrin with carbonyl groups of humic acids.

Conclusion

The great advantage of evaluating the interactions between deltamethrin/dispersant and vermicomposts throughout FTIR spectra is the increase in intensity of some bands in relation to the reference soil. The slight difference in the hydrophobicity degree and the chemical structure of deltamethrin suggest that the interaction between it and the humic acids is the Van der Waals type.

The dispersants containing in deltamethrin commercial formulation presents toxicity to earthworms, but the results for mortality, biomass and reproduction show that the deltamethrin deleterious effect is higher than the dispersant deleterious effect and the addition of 3% of humus was not enough to bring an improvement in developing earthworms (which did not occur in the experiments involving dispersants). The orange peel vermicompost did not show the same results of filter cake vermicompost. It is believed that its higher acidity impairs the survival of earthworms.

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Authors' Contributions

Fernanda Benetti and Lívia B.F. Pigatin: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Michelle M. Kanashiro and Rut Naiara Rodrigues: Participated in all experiments.

Maria O.O. Rezende: Designed the research plan and organized the study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of other authors have read and approved the manuscript and no ethical issues involved.

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