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Bioaccumulation and Elimination of Ivermectin by *Eisenia foetida* (Savigny 1826) Earthworms

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Abstract

Purpose: Bovine and equine faeces are commonly used to produce vermicompost of *Eisenia foetida* earthworms as a soil fertility enhancer. In animal health, ivermectin (IVM) is frequently used for parasite control. However, IVM is eliminated mainly by faeces, which raises environmental concerns. Little is known about the transfer of IVM by the earthworms´ activity. In this work the accumulation of IVM in *E. foetida* worms cultured in vermicompost containing IVM and the ability of the worms to release IVM to a drug-free substrate were evaluated.

Methodology: The acute toxicity test (72 h) of IVM and two bioassays, accumulation (A) and elimination (B), with *E. foetida* earthworms were performed in the current work. In A, the vermicompost produced was a mixture of equine and bovine faeces, the latter added with IVM 3,000 ng/g. Worms and substrates were sampled between 1 and 28 days post treatment (dpt). In B, worms obtained at 28 dpt in A were transferred to a substrate without IVM and sampled between 1 and 14 days later. Samples of worms and substrates were analysed using High Performance Liquid Chromatography (HPLC).

Findings: There was no worm mortality in the toxicity test. In assay A, *E. foetida* worms bioaccumulated up to 26.8% of the IVM present in the substrate. When worms from assay A, were moved to IVM-free substrate, they released 84% of the bio-accumulated IVM during the first day.

Recommendations: This study highlights that IVM bio- accumulated by earthworms and releasing in residue-free substrates represents a contamination risk, especially in farms that are minimising the use of chemical compounds.

Keywords: *Earthworms, Ivermectin, Vermicompost, Accumulation and Elimination, Environmental Risk*

1.0 INTRODUCTION

The relevance of earthworms in improving soil fertility was highlighted thousands of years ago by Aristotle, who called them ´the intestines of the Earth´. This concept was later redefined by Darwin's work in the 19th century (Brown *et al.*, 2003), who called them ´ecosystem engineers´ (Lavelle *et al.* 1997) due to their participation in the construction of new ecological spaces, establishing functional interrelationships with their biotic and abiotic environment (in Rombke *et al.*, 2005).

In this sense, studies conducted by O'Hea *et al.* (2010) proved the synergy between communities of flies and earthworms on the decomposition of faeces, being superior in efficiency compared to the association of other communities of coprophilous organisms.

Cattle faeces provide a great variety of nutrients to the environment; hence, faeces are used by a diverse fauna as means of feeding, development and/or shelter. Both coprophilous and edaphic organisms also participate in the disappearance of dung pats, favouring the liberation of grazing areas and the sustainability of agroecosystems (Valiela 1974; Grønvold *et al.*1996; Iglesias *et al.* 2004; Tixier *et al.* 2015).

The reincorporation of nutrients from livestock faeces into the soil is facilitated by the activities of a fauna whose complexity in taxonomic composition and dynamics is particular to each geographic region. However, many of the antiparasitic drugs used in livestock production are mainly eliminated by faeces, thus, determining considerable drugs' residues in the environment. These residual concentrations are even detected for a considerable and variable time, depending on different factors (Floate 2006; Capleton *et al.* 2006; Boxall *et al.* 2007; Iglesias *et al.*2011, 2022).

Regional studies carried out previously highlighted the impact of ivermectin (IVM) eliminated by treated cattle on the abundance and diversity of arthropods of the coprofauna, as well as the delay in the degradation of the faeces of treated animals (Iglesias *et al.* 2006). Besides, the presence of this drug may involve the loss of biodiversity of an incompletely known community, in addition to complex interactions between organisms in the environment.

The organisms that develop in the faeces and/or in the soil near them participate in the bioaccumulation and/or biotransformation of these compounds. A large part of these substances may be incorporated into the animal food chain, resulting in a factor of transmission of residues (Floate and Fox 1999; Boxall *et al.* 2004; San Miguel *et al.* 2008).

The extensive accumulation of organic waste triggers the proliferation of microorganisms and contaminants that turn watercourses into eutrophics, harming organisms (Garg *et al.* 2005). However, the use of organic wastes to enrich soil fertility is an ancient rural activity. Among organic wastes, manure from production animals is recommended under different strategies and geographical scenarios to fertilise forage crops (Herrero *et al.* 2017). Manure is also an excellent substrate for the cultivation of earthworms to obtain humus (López Gimenez 2000; Garg *et al.* 2005; Lowe *et al.* 2014) and promotes the development and reproduction of them through the biotransformation of manure substrate of different domestic species (Garg *et al.* 2005).

The earthworm species *Eisenia foetida* belongs to the functional group of epigeons. According to the classification proposed by Bouché in 1972 (in Lowe *et al.* 2014) they are functional organisms in superficial soil horizons with organic matter content. It is the most used species in vermiculture due to its excellent use of nutrients and its high reproduction rate. The culture substrate composed by equine and bovine faeces in equal parts has been recommended long time ago (Gagliardi 1984). Thus, the presence of IVM excreted in the animal faeces may be transferred to the *E. foetida.*

However, parasitic disease is often controlled in livestock by the administration of antiparasitic drugs such as IVM, which is mainly eliminated by the faeces of treated animals as an unmodified active drug.

Previous studies evaluated the action of avermectins on *E. foetida*, at the concentrations considered in this study, concluding in the absence of deleterious effects (Sun *et al.* 2005; Carbonell *et al.* 2011). However, an under-studied issue is the IVM accumulation and elimination by earthworms when they are cultivated in manure. This warns of the risk of biomagnification at higher trophic levels (Harris *et al.* 2000), as well as the loss of safety of earthworm humus as a soil improvement resource.

The aim of this work was to evaluate the IVM accumulation in *E. foetida* earthworms cultured in vermicompost containing IVM. Additionally, the ability of worms to release IVM to a drug-free substrate was evaluated.

2.0 METHODOLOGY 2.1 Experimental Site

The processing and conditioning of equine and bovine faeces, its preservation and later observations and measurements were carried out at the Laboratory of Parasitology and Parasitic Diseases, Department of Animal Health and Preventive Medicine (SAMPCISAPA), Faculty of Veterinary Sciences, CIVETAN (UNCPBA-CICPBA-CONICET).

The preparation, extraction and analytical determinations of IVM in samples were carried out at the Pharmacology Laboratory, Department of Physiopathology, CIVETAN (UNCPBA-CICPBA-CONICET).

2.2 Earthworms' Culture

The earthworm culture started eight months prior to the toxicity test, from a population of 120 specimens from a chemicals-free horticultural production.

The substrate consisted of a mixture of bovine and equine faecal matter (1:1), collected from animals without previous anthelmintic treatment. Mixed substrate was prepared periodically and after 48 to 72 h it was added to the worm culture to stimulate the bio-transforming activity and the reproduction of the worms. This fact was verified by the observation of new cocoons on the surface of the culture.

2.3 Chemical Reagents

Both IVM and doramectin (DRM) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents (acetonitrile and methanol) (J.T. Baker®, Center Valley, PA, USA) were

of HPLC grade. Distilled water, subsequently deionized by the purification system (Simplicity®, Millipore, Brazil), was used for all procedures.

For the experimental trials, an injectable formulation of IVM 1% (Bagó®, 100 Series) was used to prepare the IVM stock solution in ethanol (99% purity). Successive dilutions in ethanol were established to achieve IVM concentrations which reflect those are eliminated by faeces after IVM administration by different routes (Herd *et al.* 1996; Lifschitz *et al*. 2000, 2007; Cantón *et al.* 2018).

2.4 Acute Toxicity Test

Prior to development of the acute toxicity test, a humidity test was performed with filter paper, for which 5 vials were prepared with different amounts of water (between 1 and 5.5 ml) and a worm was placed in each of them for 3 hours (Cuevas Díaz *et al.* 2008; Palafox Alejo *et al.* 2012).

Following the protocol 207 of the Organisation for Economic Co-operation and Development (OECD 1984), a 72-h acute toxicity test was performed before starting the IVM bioaccumulation test with *E. foetida* worms.

Two working solutions of IVM in ethanol were prepared: 100 µg/ml and 10 µg/ml. These solutions were used to spike to achieve a final concentration of 3,000 ng/g and 300 ng/g.

The test was performed in 3 x 8 cm glass vials (5 for each concentration) covered on the inside with a piece of filter paper (4 x 18.5 cm).

Two ml of each dilution were poured with micropipette on each piece of filter paper, taking care to distribute it homogeneously. In addition, 3 glass jars were added only with the same volume of ethanol (positive control). After drying under a hood, 3 ml of distilled water were placed in each jar keeping humidity conditions. A worm (average wet weight: 0.5 g) was placed inside each jar. They were all kept at 20°C ± 2° in a dark place. Observations of survival and motility were made between 4 and 72 hours (Figure 1).

Figure 1: Methodology Utilised for the Acute Toxicity Test

2.5 Bioassay of Ivermectin Accumulation in *Eisenia foetida* Earthworms

The bioaccumulation of IVM was evaluated in specimens of *E. foetida* in a substrate of equine and bovine faeces (1:1), the latter being added with IVM (3,000 ng/g). This high exposure would reflect the IVM levels excreted in faeces after the oral, topical or long acting administrations. As the bovine faeces were mixed 1:1 with free-drug equine faeces, the nominal IVM concentration presented to *E. foetida* was 1500 ng/g.

The assay modality was similar to that described by Sun *et al.* (2005), adapting the OECD 317 guideline for faecal matter (FM) as substrate. The substrate added with IVM (group T) and the positive control (group C, ethanol only) were distributed in Petri dishes of 20 cm in diameter (250 g each).

The number of replicates was determined to cover sampling times of both experimental groups. Five plates were prepared for group T and 2 for group C, to which 16 clitellate specimens of *E. foetida* of the same weight were placed on each plate. They were kept at 20±2°C in a dark place and capped with the cover plates. Each day, the substrate was removed and the vitality of the specimens was observed in order to remove those that did not show vital signs.

Sampling of worms and substrate was performed at 1, 3, 7, 14 and 28 days post treatment (dpt); 5 specimens from group T and 2 from group C were collected, carefully washed with distilled water and kept on filter paper with 2.5 ml of distilled water until the intestinal content was eliminated (Figure 2).

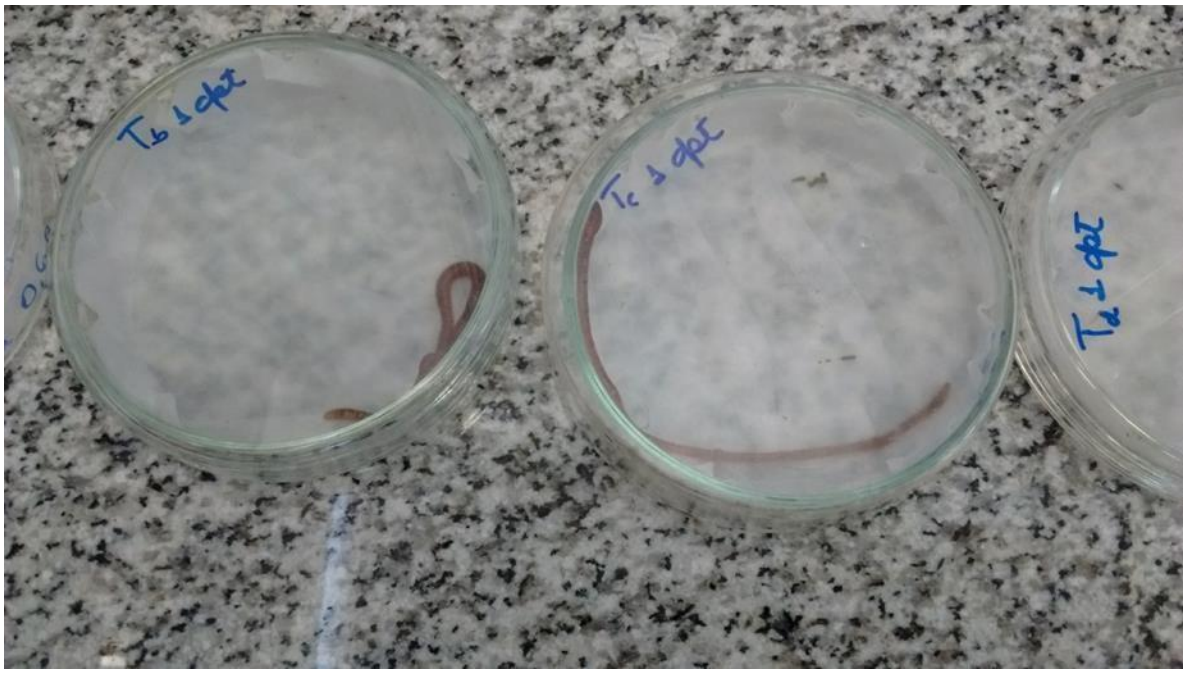

Figure 2: Eisenia foetida Specimens in Petri Dishes: Evacuation of Intestinal Contents

2.6 Analytical Procedure

Ivermectin was quantified from specimens of *E. foetida* and substrate by a high-performance liquid chromatographic (HPLC) with fluorescence detection.

The IVM analytical standards were solubilized in HPLC-grade methanol to obtain a stock solution of 1 mg/ml. From this solution, successive 1:10 dilutions were made in methanol to obtain the different concentrations used, either for direct injection into the chromatographic system or for addition to the blank samples of the matrices evaluated. Once the dilutions were prepared, they were stored at -18°C and protected from light.

The chemical extraction process from the samples was carried out using the technique described by Lifschitz *et al.* (2000).

Doramectin was added as the internal standard (IS) to achieve a concentration of 20 ng/g in worms and 40 ng/g in vermicompost samples. After the IS was added, samples were let stand for 20 min at room temperature before adding 1 ml of acetonitrile and were agitated (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 15 min. The samples were then sonicated in an ultrasonic bath for 10 min (Transsonic 570/H, Laboratory Line Instruments Inc., Melrose Park, IL, USA) and centrifuged for 20 min at 1,300 *g*. The supernatant was transferred to another tube and the extraction procedure was repeated with the pellet.

HPLC water was added to the recovered supernatants and injected into C18 cartridges (Strata, Phenomenex, CA, USA) mounted in a Vacuum Manifold (Merck, USA). Cartridges were preconditioned draining 2 ml of methanol followed by 2 ml of water. Then, cartridges were washed with water (1 ml) and water/methanol (1 ml,4:1). Finally, cartridges were dried for 3 min by increasing the vacuum pressure.

For the elution, 1.5 ml of methanol was added to each cartridge. The eluted samples were evaporated to dryness under a flow of nitrogen at 56°C for 30 min and subsequently derivatized with 100 µl of an N-methyl-imidazole (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:1) and 150 µl of anhydrous trifluoroacetic acid solution (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:2) (De Montigny *et al.* 1990). Once completed the reaction (< 30 sec) an aliquot (100 µl) of the resulting solution was injected directly into the chromatographic equipment (Shimadzu 10 A HPLC System; Shimadzu, Kyoto, Japan).

2.7 Statistical Analysis

The data were analysed using a biexponential model, kinetically defined as an open bicompartmental model, with elimination from the central compartment. The statistical treatment was based on the wet weight data of the different matrices under study.

3.0 FINDINGS 3.1 Acute Toxicity Test

Considering the recommendations of protocol 207 of the OECD (1984), the test was validated on the basis that there was no mortality in the control group. There was also no mortality in those corresponding to the both IVM concentrations evaluated up to 72 h.

However, a reduction in worm´s mobility (slow response to the stimulus) was observed in most of the specimens in both the control and the treated groups after 30 h (Table 1).

Table 1: Acute Toxicity Test in *Eisenia foetida* Earthworms

Acute Toxicity Test													
Time	**Control**			**IVM 300 ng/g**					**IVM 3,000 ng/g**				
	1	**2**	**3**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
4 h	✓	✓	✓ ±	✓	✓	✓	✓	✓	✓ ±	✓	✓ ±	✓ ±	✓
9 h	✓	✓	✓ ±	✓	✓ ±	✓	✓	✓	✓	✓ ±	✓	✓ ±	✓ ±
18 h	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
22 h	✓	✓	✓ ±	✓	✓	✓ ±	✓ ±	✓ ±	✓ ±	✓	✓ ±	✓	✓ ±
30 h	✓ ±	✓ ±	✓ ±	✓ ±	✓ ±	✓ ±	✓	✓ ±	✓ ±	✓	✓ ±	✓ ±	✓ ±
45 h	✓ ±	a	✓ ±	✓ ±	✓	✓ ±	✓ ±	✓ ±	✓ ±	✓	✓ ±	✓ ±	✓ ±
58 h	✓ ±	b	✓ ±	✓ ±	✓ ±	✓ ±	✓ ±	✓ ±	✓ ±	a	✓ ±	✓ ±	✓ ±
72 h	✓ ±	b	✓ ±	✓ ±	✓ ±	✓ ±	✓	✓	✓	b	✓	✓ ±	✓ ±

Endpoints: survival and response to stimulus✓; mobility (±: slow response to stimulus); [a]escaped specimen; [b] unreturned specimen

Two specimens escaped from the test and then recovered with very good mobility, although they were not restored into it.

Among the IVM concentrations evaluated, 3,000 ng/g (0.02 ug/cm^2) was the highest concentration at which no mortality was observed. Based on the results, the concentration of 3,000 ng/g IVM was determined for the development of the bioaccumulation bioassay.

3.2 Accumulation Bioassay

Worms grown in an IVM-added substrate mixture of equine and bovine faeces (1:1) for 28 days showed IVM concentrations that varied increasingly between 9 and 26.8% of the substrate concentrations at the time of sampling, reaching an equilibrium state between 7 and 28 dpt (Table 2) (Figure 3).

Table 2: Accumulation Bioassay: Mean IVM Concentrations in *E. foetida* Earthworms

Days post treatment (dpt)	IVM in substrate (ng/g)[a]	IVM in earthworms (ng/g)[a] (%)[b]
0	2,595.40.±.245.03	-
1	1,201.31.±.108.17	101.08±59.57(9)
3	1,011.08.±.91.04	145.55.±.132.79 (14.4)
7	1,279.15.±.115.18	255.35.±.130.42 (20)
14	1,071.55.±.96.49	240.33.±.106.10 (22.4)
28	848.13.±.76.37	227.10.±.90.64 (26.8)

[a]Wet weight, [b] percentage relative to IVM concentration in substrate; ±: standard deviation

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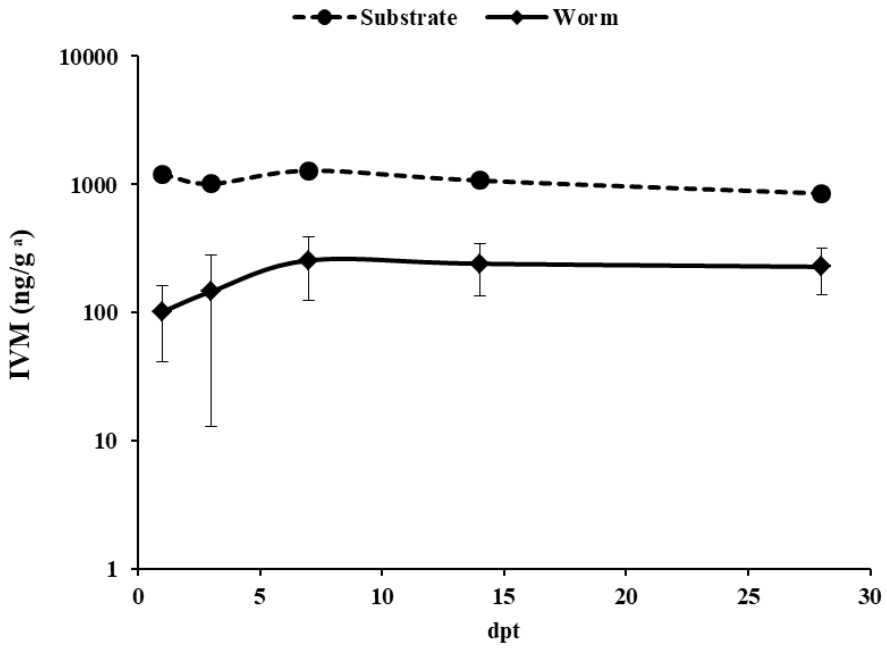

Figure 3: Average Ivermectin (IVM) Concentrations in Substrate ([a] Wet Weight)

Assuming the worm/substrate ratio as a bioaccumulation factor (BAF), it was progressive during the test and it is represented in Figure 4. IVM concentration (wet weight) ratio between earthworm and substrate (W/S) and between substrate and earthworm (S/W) are expressed as a percentage.

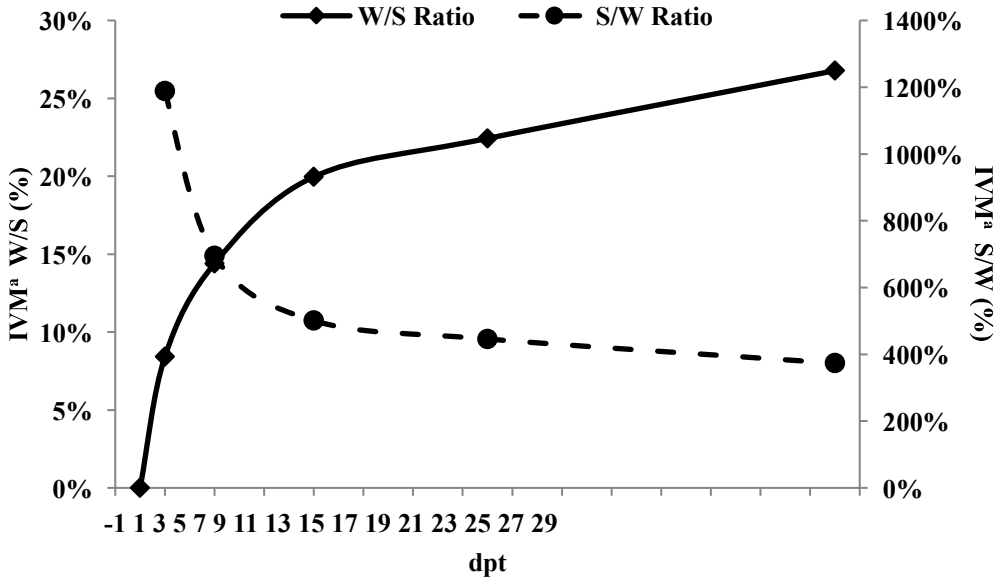

Figure 4: Bioassay of Ivermectin (IVM) Accumulation in Eisenia foetida

3.3 Bioassay of IVM Elimination by *Eisenia foetida* on IVM-Free Substrate

Worms obtained at 28 dpt from the accumulation bioassay were used for the elimination bioassay (considered day 0). Consistent with previous studies (Sun *et al.* 2005), 84% of the IVM detected in the worms was transferred to the IVM-free substrate during the first 24 h and the remaining 16% was gradually recirculated between the worms and the substrate. Since it was not possible to maintain the vitality of the specimens beyond 14 days in both the control and treated groups, only the substrates of both groups were sampled at 21 days. The concentrations detected in the substrate are presented in Table 3.

Table 3: Elimination Bioassay: Mean IVM Concentrations in *Eisenia foetida* Earthworms

Days post treatment (dpt)	IVM in *E. foetida* (ng/g) [a]	IVM removed by *E. foetida* in IVM-free substrate (ng/g)[a]
0	227.10 ± 90.64	0
1	44.36 ± 12.73	5.68±0.61
3	4.88 ± 2.52	12.37±1.12
7	5.72 ± 2.03	9.07±2.30
14	4.38 ± 1.36	6,45±0.60
21	w/s[b]	5.60±0.50

[a]Wet weight; [b] w/s: without earthworm sample;±: standard deviation

Figure 5 shows the ratio of the IVM substrate /worm concentration (S/W) in the elimination bioassay.

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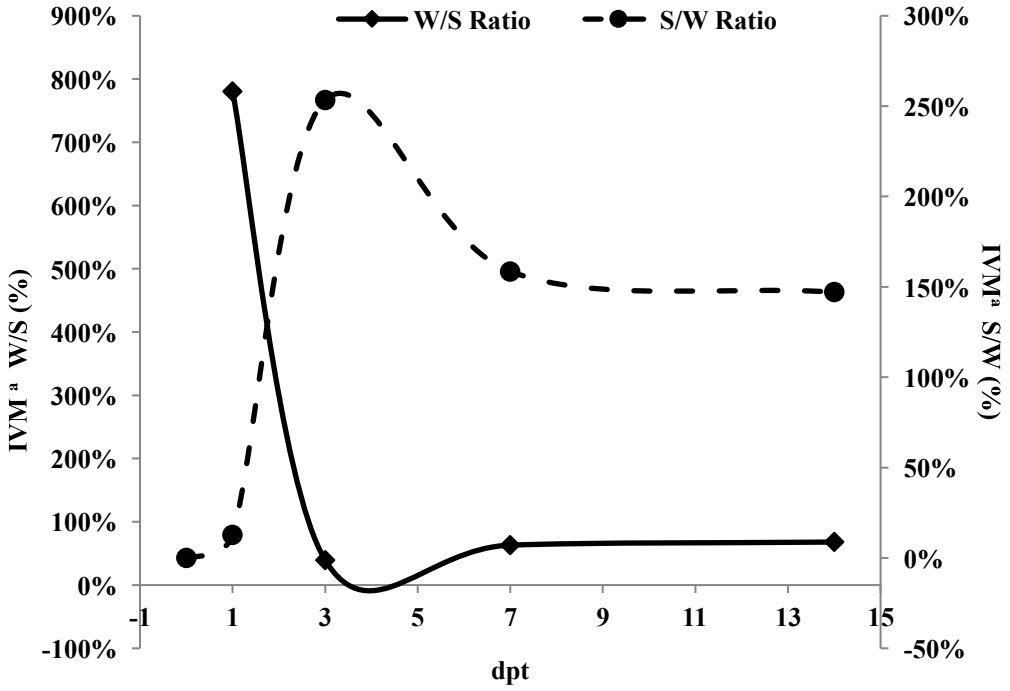

Figure 5: Bioassay of Elimination: Ivermectin (IVM) Worm/Substrate (W/S) and Substrate/Worm (S/W) Ratio (ª Wet Weight)

The accumulation and elimination rates obtained were: ka = 0.16 and ke = 0.02, respectively, showing that *E. foetida* earthworms' bio accumulated IVM when grown in an IVMcontaining substrate for 28 days and released it when transferred to an IVM-free substrate.

4.0 DISCUSSION

Earthworms collaborate in the degradation of manure, speeding up its disappearance from pastures and the incorporation of nutrients to the soil. In addition, earthworms are frequently used in the determination of soil quality, taking into account their ability to accumulate different environmental xenobiotics (Marinussen *et al.* 1997). Earthworms also participate in the bioremediation of contaminated soils (Zapata *et al.* 2016; Barbaran 2017). The effect of these contaminating substances will depend in part on their bioavailability, the degree of biotransformation into substances of lower toxicity and their elimination (Viswanhathan 1994).

Among the earthworm species, *E. foetida* has been the most tested in acute and chronic toxicity studies of various chemical substances since the standardisation of protocols (OECD 1984), being recognized by the OECD 207 protocol in the evaluation of the toxicity of pesticides on this species. Using the same procedures to evaluate the acute toxicity of avermectin B1a (AVM B1a) on *E. foetida*, Sun *et al.* (2005) determined an LC50 of 4.63 ug/cm2 on filter paper and 20.6 mg/kg (average) on artificial soil over a period of 48 h and 2 weeks, respectively. However, the AVM concentrations evaluated by these authors far exceeded those considered in the present study (between 50,000 and 6,300 ng/g, dry weight)

and those that could normally be recorded in soil, regardless of their origin. The use of two IVM concentrations (300 and 3,000 ng/g) in the acute toxicity test, without mortality of the specimens, allowing to plan and to develop the accumulation bioassay at the maximum concentration evaluated. This concentration is detectable in bovine faeces depending on the formulation and route of administration used (Herd *et al.* 1996, Lifschitz *et al.*2007; Cantón *et al.* 2018). Likewise, in terms of toxicity, the survival of the specimens in the acute toxicity test does not rule out the appearance of sublethal effects not evaluated, as may occur with other xenobiotics. In this sense, a set of biochemical alterations can be triggered when specimens of *E. foetida* are exposed to different concentrations of a toxic substance without compromising their survival (Saint-Denis *et al.* 2001). Moreover, Tuerlinckx *et al.* (2015) have revealed the induction of the hormetic effect by IVM manifested in the rate of development and in the activity of the enzyme alkaline phosphatase in that species.

Although it was not the main objective of this work, sublethal alterations in different levels of organisation of specimens of this species may be considered in future studies. In environments contaminated by xenobiotics, organisms can bio-concentrate these substances by contact or bio-accumulate them by ingesting them adsorbed to food particles. These processes can also be biomagnified when they are transferred up the food chain (Franke *et al.* 1994). The value of knowing these factors is that they represent a risk in themselves, regardless of the acute or chronic effect reported by standardised eco toxicity tests (Franke *et al.* 1994).

In the current work, the accumulation and elimination of IVM by *E. foetida* earthworms were evaluated. For the experimental development both, the role of worms in the degradation of bovine manure and the presence of IVM in faeces, as a result of the usual parasite control practices in livestock, were assumed.

Using IVM concentrations in soil that exceeded those considered in these trials, Carbonell *et al.* (2011) reported no mortality of earthworms during the 21-day experimental period. However, the authors noted an inverse relationship between these concentrations and the bioaccumulation factor.

Moreover, even as a non-quantifiable indicator, potential bioaccumulation in organisms can be extrapolated from the physicochemical and structural properties of the molecule in question (Franke *et al.* 1994). The probability of bioaccumulation can be deduced when the octanol/water partition coefficient (log Kow) is greater than 3. This property of IVM, associated with its low water solubility and high affinity for soil and sediment organic matter, is evidence of its tendency to adsorb to organic particles of the substrate. Likewise, the sorption of avermectins to organic carbon is not similar for all compounds of this family of drugs. In particular, IVM involves other sorption mechanisms, such as the formation of complexes with inorganic matter in the soil (Krogh *et al.* 2008).

Certain hydrophobic contaminants can be reversibly bound to organic carbon, thus varying their bioavailability to organisms in these habitats. The presence of cations such as calcium (Ca2+) in soils leads to reduced sorption of IVM (Krogh *et al.* 2008) which would favour the desorption of the compound and, therefore, its bioavailability.

How significant is the bio-concentration/bio-accumulation factor (BC/BACF)?. Franke (1996) postulated the relativity of this concept considering that low BCFs or BAFs can occur with high concentrations of a xenobiotic and, in that case, underestimate the risk and vice versa. In summary, the isolated bio-concentration or bio-accumulation data is not completely efficient for determining environmental or ecotoxicological risk. Rather, the complex web of metabolism, functional and organic damage of the final fate of a xenobiotic. Their ultimate fate will be determined by the properties of the substance, the environment in which exposure occurs and its bioavailability.

Some biological and behavioural aspects of these annelids could be involved in exposure to the presence of IVM in the environment. Namely, the lipid constitution of their membranes, their negative phototaxism, and the close and permanent contact with the substrate. In this respect, in the test conducted, the worm specimens showed increasing accumulation of IVM until the equilibrium state was reached.

In order to detect and quantify IVM in the worms, the specimens were previously rinsed with distilled water. Then, the IVM quantified corresponded to the compound ingested from the substrate and bio-sorbed on membranes, according to the concept defined by Franke *et al.* (1994). In contrast, Sun *et al.* (2005) recorded little accumulation in earthworms cultured with similar initial concentration of AVM B1a in artificial soil, keeping it constant during their trial. Comparatively, in the present study higher IVM concentrations were reached in worms at 14 dpt, than those documented by these authors at 17 days. At this point, it should be considered the nature of the substrate used. As the manure contains a higher percentage of organic matter than the artificial soil, the possibility of sorption of the IVM molecule will be greater due to the properties already described.

In addition to the substrate used, other methodological issues might justify the differences with the data obtained by Sun *et al.* (2005). According to the amounts of substrate used for each experimental group, there would be less availability of substrate per worm (2.25 g/ worm compared to 15.6 g/ worm in this study), which would limit their intake by ingestion. Bioaccumulation is caused by the progressive increase of a substance in an organism or part of it. This event was verified in the assay conducted and is shown in Figure 2. Meanwhile, Spacie and Hamelink (1985) postulated that bioaccumulation can occur only if the rate of chemical uptake by an organism exceeds the rate of elimination, which was also verified in our tests.

Both the accumulation and elimination kinetics were similar to those documented by Sun *et al.* (2005). Although they did not perform quantification of the molecule, the elimination of IVM concentrations occurred in a biphasic mode, in agreement with these authors. Thus, during the first days, the highest percentages were eliminated followed by a slow and sustained release during the remaining time of the experimental period.

In a study carried out prior to these bioassays, it was found that IVM concentrations in soil were transferred to pasture species grown in the same soil (Iglesias *et al.* 2022). Thus, the compound obtained by worm activity in bovine faeces may be a vehicle for transferring IVM to plants growing on amended substrates.

In this sense, the application of organic amendments, which are often used by small horticulturists and family farms, could result in a product that is not suitable in the organic and chemical-free concept.

5.0 CONCLUSION AND RECOMMENDATION 5.1 Conclusion

In this work, IVM concentrations were detected and quantified in earthworms *Eisenia foetida* cultured for 28 days in vermicompost with added bovine faeces containing IVM.

Although the observation and recording of new capsules or cocoons was not contemplated, the survival of the specimens of this species was not affected by the presence of IVM at the concentrations and times tested.

As has been shown, *E. foetida* specimens bio-accumulated IVM. However, the subsequent release of IVM from earthworms in IVM-free substrate alerts to the potential risk of contamination. This finding is of interest in the context of organic productions and in those that tend to reduce the use of these compounds.

E. foetida proved to be an appropriate organism to monitor the behaviour of IVM residues used in livestock activities as well as the subsequent use of livestock faeces in composting and nutrient reuse systems.

In the meantime, potential effects of bio-sorbed IVM concentrations in the environment could occur at longer assessment times, showing the source of bio-magnification. This highlights the need for further comprehensive studies and pharmaco-epidemiology trials.

5.2 Recommendations

The use of vermicompost as a soil amendment is a growing trend among horticultural and ornamental growers who minimise the use of chemicals. In these areas, the source of the worms and manure must be known, as well as the time elapsed since the administration of ivermectin to the animals in production.

In addition, in the field of animal health, emphasis should be placed on the rational use of chemicals justified by epidemiological and regional studies.

Long-term knowledge will also need to be integrated with future research to provide information integral to that obtained in this study.

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Data availability- the data generated during and/or analyzed during the current research are available from the author (Lucía Iglesias) on reasonable request.

Declarations

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