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An Environmental Concern: Uptake of Ivermectin from Growing Substrate to Plant Species

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Abstract: ***Ivermectin (IVM) is an antiparasitic drug used worldwide. However, its high level of faecal elimination, its transfer to the soil, and the use of manure for soil amendment, represents a potential environmental risk. Two trials were conducted to evaluate the uptake of IVM to: 1) a crop of ryegrass (Lolium multiflorum) and clover (Trifolium repens) growing for 120 days post treatment (dpt) in IVM-spiked soil at 3, 000 (High group, HG) and 90 ng/g (Low group, LG); 2) a crop of radish (Raphanus sativus) and lettuce (Lactuca sativa) growing for 60 dpt in a mix of soil and 10% IVM-spiked manure at 3, 000 ng/g. Soil, soil-manure mix and plants were sampled starting at 15 dpt and at different intervals. IVM concentration (IVMc) was quantified by HPLC. In the HG, IVMc in soil decreased from 2, 154 to 225 ng/g; in ryegrass ranged between 378.65-21.74 ng/g. Strikingly, clover development was delayed; hence, sampling begun 30 dpt. IVMc in clover ranged between 94.09-4.56 ng/g. Significant differences were detected between species (p=0.0374). In the LG, IVMc varied between 22.26-1.02 ng/g in ryegrass and between 10-1.02 ng/g in clover, differences being statistically not significant between species (p=0.8301). IVM was detected in both horticultural species at significant levels (p>0.05) in all sampling times and IVMc was between 10-5 ng/g in radish and 17.70-6.55 ng/g in lettuce. In the substrate IVMc decreased from 1, 311 to 116 ng/g. Consequently, IVM in soil and in composted substrate is transferred to plants during its growth and could be incorporated into the food chain of both livestock and humans.***

Keywords: ivermectin, soil, horticultural plants, pasture species, substrate transfer

1.Introduction

The intensive use of veterinary drugs for disease control in animal health makes several of them a growing contamination threat leading to consternation in animal, public and environmental health. Residual compounds can be uptaken by plants and potentially enter to the food chain. Although many of these drugs have been used routinely for several decades, the presence of their residues in vegetation has not been fully evaluated. Therefore; the situation represented by different molecules for veterinary use in relation to their destination, which includes a wide scope of different substrates, such as soil, microbiota, mesofauna, and vegetation, is even more disconcerting.

Drugs used in animal and human treatments persist as parent drug for a long time in both manure and bio-solids, understanding these as the solid, semi-solid and liquid residues resulting from the treatment of domestic waste water. Unlike waste water, which goes through different types of degradation treatment to avoid the presence of pathogens, nutrients and heavy metals, manure is stacked, composted or reserved in ponds without any type of treatment. This leads to a higher concentration of drug residues and, consequently, to a higher biochemical oxygen demand, which further prevents degradation [1], thus favouring its environmental persistence. Frequently, both bio-solids and manure are spilled on ploughable land as fertilizers [2] [3] [4].

Two productive scenarios promote the use of bovine manure: the intensive agricultural activity and, the accumulation and persistence of dung pats in pastures. The former scenario generated a nutritional deterioration of the soil due to the reduction of organic matter, loss of the physicochemical structure and the nutritional demand of developing plants. The latter, lessened the grazing area causing more productive losses. It is also known that the amendments with manure of different species provide nutrients to the substrate in horticultural and ornamental crops [5] [6].

Ivermectin is one of the most widely used therapeutic drugs for the control of parasites in animal health. However, there is more information on the environmental residues of other chemical compounds such as antibiotics [7] [8] [9]. As to IVM, most of these studies used culture media or were performed under laboratory conditions. These facts arise the question about the fate of this compound when its field concentrations are due to common veterinary practices, concentrations at which IVM environmental persistence has been demonstrated [10] [11].

Certain topics are still under study, such as the final destination of those veterinary drugs that are eliminated by faeces and depends on the conformation and use of the soil or substrate and the vegetation present. In this sense, monitoring drug entry into vegetation and potential bioaccumulation will be a contribution to promote the reduction of metabolites or the parent drug in bio-solids and manure before being applied to farmland.

It is common to observe grasses growing around dung pats; however, cattle generally avoid their ingestion [12]. Although the phytotoxicity of different veterinary drugs of conventional use has been studied [13], and some works consider important their effects on plant development [14] [15] the possibility that plants convey ivermectin originated from veterinary treatments to other links of the trophic network is worrying.

Taking into account the situation generated by the veterinary use of ivermectin, it is appropriate to explore its behaviour in pasture species or its activity in substrates

obtained when mixing manure and soil to optimize horticultural production.

The aim of this work was to determine the uptake of ivermectin present in the cultivation substrate by pasture and horticultural species under simulated field conditions.

2.Materials and Methods

Experimental Place

The germination test was conducted at the Laboratory of Parasitology of the Faculty of Veterinary Sciences (FCV), UNCPBA, Tandil, Argentina. Trials with crops were carried out under simulated field conditions in an experimental area of the FCV and chemical analyses were done at the Laboratory of Pharmacology (FCV, UNCPBA).

Seed Species

Seeds of pasture species, *Lolium multiflorum* and *Trifolium repens*, and seeds of horticultural species, *Raphanus sativus* and *Lactuca sativa*, were provided by Agencia de Extensión Tandil, Instituto Nacional de Tecnología Agropecuaria (INTA), and Programa Pro-Huerta of the same institution.

Soil and Faecal Samples

A fenced plot of the experimental area at the University Campus was selected. Plots without grazing animals were covered by a consociated pasture (*Trifolium repens*, *T. pratense*, *Lolium perenne* and *Dactylis glomerata* species). The soil type was characterized as typical Argiudol and its organic matter percentage was 5.42 (Walkley and Black method, Soil Analysis Laboratory, Faculty of Agronomy, FA-UNCPBA, Azul, Argentina). Before sampling, the vegetation coverage was removed and the soil layer (0-20 cm depth) was extracted travelling in zigzag transect. Once in the laboratory, the soil samples were crumbled, homogenized and reserved until utilization.

Bovine faecal matter (FM) used for the trials was obtained from Holstein cattle, of approximately two years old and 300 kg of live weight, belonging to a commercial dairy farm with parasitic control and free of drugs, which guaranteed the absence of previously administered antiparasitic residues.

The daily records of temperature and precipitation during the experimental period, between March 17 and July 13, were provided by the Institute of Plains Hydrology (IHLLA), using the instruments available at the meteorological station (Li-1200S, Li-Cor Inc. Lincoln, Nebraska) located at the University Campus (UNCPBA). Data are presented in Figure 1.

Seed Germination Test

An *in vitro* germination test was performed to evaluate the viability of seeds. This aspect has great significance for crop yield improvement and quality. A seed is considered germinated when the length or the radicle reaches 3 mm. Then, the methodology was adjusted according to the OECD guideline 208 [16] and the technical description reported by Sobrero and Ronco [17].

Filter paper disks (12 cm diameter) were moistened with 2 ml of distilled water and were placed on Petri dishes. Three replicates per species were prepared and 20 seeds were placed in each dish (Figure 2), maintained at 24°C, moistened and covered with dark polyethylene film for 72 h, during which the germination percentage was evaluated.

Experimental Culture Pasture species

IVM addition was accomplished by diluting the necessary amount of IVM in a solution of ethanol (10%) in water. Ivermectin was added to the soil where pasture species were cultivated at two levels: 3, 000 ng/g, a high-exposure representing a faecal elimination scenario (topical, oral or long-acting formulations) and 90 ng/g, the average concentration registered in the soil underlying dung pats [11].

Three wooden containers (60x45x40 cm) were filled with 20 kg of soil from the experimental area (0-20 cm depth). Soil was previously homogenized and plant remains were removed. The soil containers were filled with soil added with IVM to reach a concentration of 3, 000 (T3000) or 90 ng/g (T90) and one of them was added only with ethanol (Control). The containers were kept at room temperature for 24 h to evaporate the ethanol. Then, three samples were obtained at different points to determine the initial concentration of IVM. Samples were maintained at-20°C until analysis by HPLC. Simultaneously, the physicochemical characterization of the different cultivation substrates (control soil, control bovine FM, control mixture FM/soil and bovine FM added with IVM 3000 ng/g) was carried out at the Soil Analysis Laboratory, Faculty of Agronomy (FA-UNCPBA), Azul, Argentina (Table 1).

The sowing was done by rows, one species in each half of the container. For each crop half, 5 g of clover seeds and 7 g of ryegrass seeds were weighed (C, T90 and T3000). Prior to germination, the crops were covered to avoid desiccation and disturbance or preying by birds. Later, they were arranged at the experimental plot under simulated field conditions. Every day, all crops were watered with the same amount of water and their development was recorded.

Whole seedlings and substrates were sampled at 15, 21, 30, 45, 60 and 120 days after seeding, i.e., post-treatment (dpt). Each seedling was carefully cleaned under a stereo microscope with the help of a fine brush moistened in distilled water. After removing the water excess with a paper towel, they were identified, wrapped in tissue paper and stored at -20°C until analysis by HPLC.

Horticultural species

Three wooden containers (60x45x40 cm) were used, one of them served as control (C), for the cultivation of both horticultural species, radish and lettuce. The other two containers were filled with IVM-added substrate and sown

each of them with only one of the studied species (Tl, Tr). Each container was filled with 16 kg of soil from the experimental plot (0-20 cm depth), free of plant material and homogenized, over which a 2-cm layer of bovine FM mixture added with IVM was placed. This mixture was elaborated with bovine faeces spiked with IVM (3, 000 ng/g), subsequently dried and processed in a blender with soil in a ratio of 1: 10, as recommended by the Soil Analysis Laboratory (personal communication). Additionally, the control substrate (C) was made of bovine faeces without IVM and subjected to the same elaboration process. Physicochemical properties of substrates are shown in Table 2.

Before sowing, to allow the total evaporation of the ethanol, substrates remained at room temperature for 24 h. Then, to determine the initial concentration of IVM, three samples were obtained at different points in each container, which were kept at-20°C until analysis by HPLC. Subsequently, the sowing was carried out in rows (1.8 g of radish seeds and 1.6 g of lettuce seeds). The containers were placed in the experimental plot under field conditions. Prior to germination, and until plants reached sampling development, the crops were covered with a shadow mesh to avoid desiccation or being disturbed by birds.

Sampling of whole seedlings and substrate (0-2 cm depth) was performed after the appearance of secondary leaves at 15, 21, 30, 45 and 60 days, considered as post-treatment days. With the same methodology for the pasture species, each plant was cleaned, excess water was removed with tissue paper and the plants identified, wrapped in tissue paper and kept at-20°C until analysis by HPLC.

Chemicals

Standards of IVM, doramectin (DRM), abamectin (ABM) were provided by Sigma-Aldrich, St. Louis, MO, USA. Acetonitrile and methanol were all HPLC grade. For all procedures, distilled and deionized water was used (Simplicity®, Millipore, Brasil). To carry out the addition of IVM in the different experimental tests, a 1% IVM commercial formulation (Bagomectina®, Serie 100), and diluted (1: 10) in analytical ethanol, was used.

HPLC Analysis

Considering that in previous tests the section of aerial and underground parts for the analytical procedure and determination of IVM concentrations did not show significant differences, in the present bioassay whole plants were analysed.

The extraction phase was carried out according to the methodology described by Lifschitz *et al [*18]. Samples consisting of 0.5 g of FM or soil, 0.25 g of culture substrate and 1 g of plants were added with 40 ng/g of internal standard (DRM). After standing for 20 min, 1 ml of acetonitrile was added, shaken for 15 min (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA), sonicated for 10 min (Transsonic 570/H, Laboratory Line Instruments Inc., Melrose Park, IL, USA) and centrifuged during 20 min at 1300 *g*. The supernatant was transferred to another tube and the extraction procedure was repeated with the solid residue. The both recovered supernatants were injected into C18 cartridges (Strata, Phenomenex, CA, USA) mounted in a Vacuum Manifold (Merck, USA) which were conditioned with 2 ml of methanol and 2 ml of water and, then, the supernatants of each sample injected and washed with distilled water (1 ml) and 1 ml of water: methanol (4: 1). For elution, 1.5 ml of methanol was passed through each cartridge. Eluted samples were evaporated with nitrogen at 56°C for 30 min prior to derivatization with 100 µl of a solution of N-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1: 1) and 150 µl of trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1: 1.5) [19]. An alicuot (100 µl) was directly injected to the chromatographic HPLC system (Shimadzu 10 A HPLC System; Shimadzu, Kyoto, Japan).

Statistical Analysis

The data obtained were analysed for normality (Kolgomorov-Smirnov and Shapiro-Wilk) and statistically compared using the Mann Whitney or Student´s t test, accordingly. Data was fitted to a biexponential model. Statistical analysis was based on wet weight of all matrices.

3.Results

Culture of Pasture Species

Soil substrate added with IVM 3, 000 ng/g
The development of clover species up to the sampling size was delayed for 30 dpt (Figure 3). IVM concentrations, in this species, from 30 to 120 dpt varied between 94.09 ng/g and 4.56 ng/g, while, in ryegrass samples, IVM concentrations, between 15 and 120 dpt, were between 378.65 and 21.74 ng/g.

The IVM concentrations detected in ryegrass specimens were significantly higher than those detected in clover samples (p=0.0374) (Figure 4).

Table 3 shows the IVM plant/substrate concentrations rates in ryegrass and clover species at different sampling times post-treatment.

Soil substrate added with IVM 90 ng/g

Ivermectin concentrations detected in ryegrass, between 15 and 120 dpt, ranged from 22.26 to 1.02 ng/g and, in clover, between 10 and 1.02 ng/g, being differences between both plant species statistically not significant (p=0.8301). Data are shown in Figure 5 and the IVM plant/substrate concentrations rates are presented in Table 4.

Culture of Horticultural Species

Culture in IVM-added mixed substrate soil/FM (3, 000 ng/g) of both, *Raphanus sativus* and *Lactuca sativa*, was carried out for 60 days. Ivermectin concentrations were detected in all specimens sampled between 15 and 60 dpt. There were no significant differences between the IVM concentrations detected in radish and lettuce (p>0.05)

during the experimental period (Figure 6). Meanwhile, the concentrations detected in substrate averaged between 1, 311 and 116 ng/g. The IVM plant/substrate concentrations rates for both species are presented in Table 5.

4.Discussion

The results obtained in the bioassays with pasture and horticultural species show that from the cultivation substrate, whether soil or a mixture of soil with bovine faeces, IVM is transferred to the plants and can be detected during the plant development up to 60 – 120 days after emergence. This transfer, from artificial dung pats elaborated with IVM-added bovine faeces to the underlying soil and nearby vegetation, was previously corroborated in simulated field conditions [11]. In the present work, cultures with pasture and horticultural species were exposed to the environment during the experimental period.

Biosolids are mostly used as a source of nutrients and organic carbon in agriculture, landscaping and domestic crops (USEPA, 2006). The practice of spreading manure or spilling liquid manure on pasture is quite old and still common [15]. The compost made with manure from different species and at different latitudes continues to be evaluated for use as amendment [20] [21]. However, some products used in animal health, and present in this substrate, can affect the development of plants [22] [23] [7].

The factors that determine the bioavailability of a compound in organisms in a given environment are as varied as the diversity of physicochemical, biological and phenological conditions that characterize them. The mobility of pharmaceutical products, facilitated by the network action of these factors, still generates contradictions [24]. The kinetic studies carried out by Rath *et al. [*25] under controlled laboratory conditions, demonstrated the mobility behaviour in seven types of subtropical soil when four antimicrobials and three families of antiparasites for veterinary use were evaluated. Thus, the present work supports the relevance of determining the concentrations of these compounds in the soil as well as the possible effects of microbial processes and the effects on crops under field conditions as stated by Jjemba [1]. Furthermore, the environmental persistence of significant ivermectin concentrations was observed in our region [10] [26].

When evaluating the development of pasture species in soil added with the highest IVM concentration (3, 000 ng/g), a delay in germination and growth of clover was observed, which prevented sampling for up to 21 dpt.

During germination and the early days of seedling's development, the physiological processes taking place are easily affected by toxic substances that can be detrimental to survival and normal development, thus this period is a stage of great sensitivity to adverse external factors [17]. In recent years, several *in vitro* studies reported the ecotoxic effects of antiparasitic drugs [14] [15]Sobrero and Ronco [17] highlighted the reversibility of the germination inhibition as a function of the continuous exposition. These authors evaluated the phytotoxicity of complex environmental samples and volatile compounds. Moreover, they suggested to consider the development of hypocotyl and radicle as indicators of sublethal effects.

These authors referred to the 120-hour acute toxicity tests, whereas the data recorded in this work show a scenario similar to the natural one, in which the development of this species, like that of ryegrass, was evaluated for 120 days.

On the other hand, the grass species, *L. multiflorum*, presented higher concentrations of IVM in relation to the legume species, *T. repens*. Some specific properties determine the uptake of non-ionizable hydrophobic compounds from the substrate. Among them, there is a positive correlation between the lipid content of the ryegrass root and the bioconcentration factor and between the latter and the transpiration rate for chemicals of anionic, cationic or neutral nature [27]. Overall, the accumulation of compounds derived from pharmaceutical and personal care products is higher in the roots, compared to the leaves, branches and fruits in decreasing order [28].

When the plant/substrate IVM concentration rate was determined throughout the experimental period, it was observed that in the culture with IVM 90 ng/g the samples of both species exceeded the ratio of 1 in two of the samplings carried out (Table 4). This result would be representative of the bioaccumulation factor in the plants from the substrate.

In accordance with Horvat *et al. [*29], and extrapolating to a real situation in a production system, these findings may be relevant if the daily consumption of an animal is considered. Certainly, the animals' consumption behaviour, both in grasslands or near bodies of water, could lead to the development of strains of parasites resistant to these anthelmintic compounds. It will be necessary to conduct a study to determine the potential long-term risk. This requirement will not only be with a chemical compound, but with the combination of different groups, taking into account that current antiparasitic control programmes use a mixture of drugs from different chemical families.

The horticultural species were selected taking into account the consumption of the different structures (leaf and succulent root, respectively), being both frequently cultivated in family and community gardens. As in pasture species, in both lettuce (*L. sativa*) and radish (*R. sativus*) IVM was transferred from the culture substrate, i.e., bovine faeces-soil mixture, concentrations being detected during the 60 days of environmental exposure. Differences in IVM concentrations between both horticultural species were statistically not significant. Likewise, the IVM plant/substrate concentration rate, interpreted as a bioaccumulation factor, did not indicate this effect (Table 5).

Monitoring for the presence of residual amounts of xenobiotics intended for animal health is carried out in human food of animal origin. However, given the observations reported in the present work, veterinary drugs

can reach humans due to consumption of vegetables cropped from lands presenting high concentrations of IVM. This raises concern due to the absence or, at least, scarcity of regulatory standards about this matter, especially for pharmaceutical products used in animal health. Since the 1980´s, the occurrence of pharmaceutical and personal care products identified as traces of environmental pollutants represents one of the contaminants that generate adverse effects for humans, wildlife and natural ecosystems.

In the meantime, it will be necessary to evaluate the potential risk of consumption, taking into account its frequency in eating habits, the new diets based on ideals of animal protectionism, the ways of consumption, as well as the average daily intake of vegetable leaves or other parts of the plant. Wu *et al. [*30] estimated human exposure based on four factors: concentration of chemical compound, average daily intake, time of exposure and body weight. Likewise, recent trends in organic chemical-free crops should not be unrelated to these findings. Meanwhile, in the aforementioned works, the IVM was not considered as a compound to be evaluated.

Recent trials evaluated the IVM effect on *Arabidopsis thaliana* species, the first plant which genome was completely sequenced, resulting, for that reason, widely used as a model for plant biology research, in addition to its recognized soil remedial function. Using an acute toxicity test of up to 72 h, Syslová *et al. [*31]reported the action of IVM affecting the regulation of genes of this species involved in the metabolism of drugs, the response to stress and pathogens. This evaluation was carried out in the root and foliar section, identifying six metabolic products of phase I and parent drug, respectively. Although the presence of IVM metabolites was not evaluated in our bioassays, the concentrations detected during the 60 days in both vegetable species suggest the persistence of the molecule in different edible parts in a culture with amendment of IVM-added bovine manure throughout the harvest and consumption period.

5.Conclusions

This study evaluated the IVM transference from substrate to plants in an experimental design under simulated field conditions We observed an IVM concentration-dependent effect indicative of drug phytotoxicity in the development of clover up to 30 dpt. This hindered sampling of this species at 15 and 21 dpt.

The concentrations of IVM detected in the grass species (*Lolium multiflorum*) were significantly higher than those in the legume ones (*Trifolium repens*). This observation shows that each pasture species plays a different role in the exposure to and potential absorption of ivermectin in grazing animals.

Concentrations of IVM were detected in all samples of vegetables species (*Lactuca sativa* and *Raphanus sativus*) throughout the development period. This time coincided with that of harvesting and consumption of both, lettuce and radish.

In addition, a great variability of IVM concentrations at the different sampling times was observed in culture substrates during the experimental period of all assays, i.e., in both soil assays (3, 000 and 90 ng/g) and in the mixture of bovine faeces/soil (3, 000 ng/g).

These findings determine a need for monitoring the fate and persistence of this frequently used antiparasitic drug. In regions where environmental regulatory standards are scarce, local studies should be prioritized. These should include the kinetic behaviour of chemical compound, the frequency of its use, as well as the environmental conditions and fate of manure.

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Figures

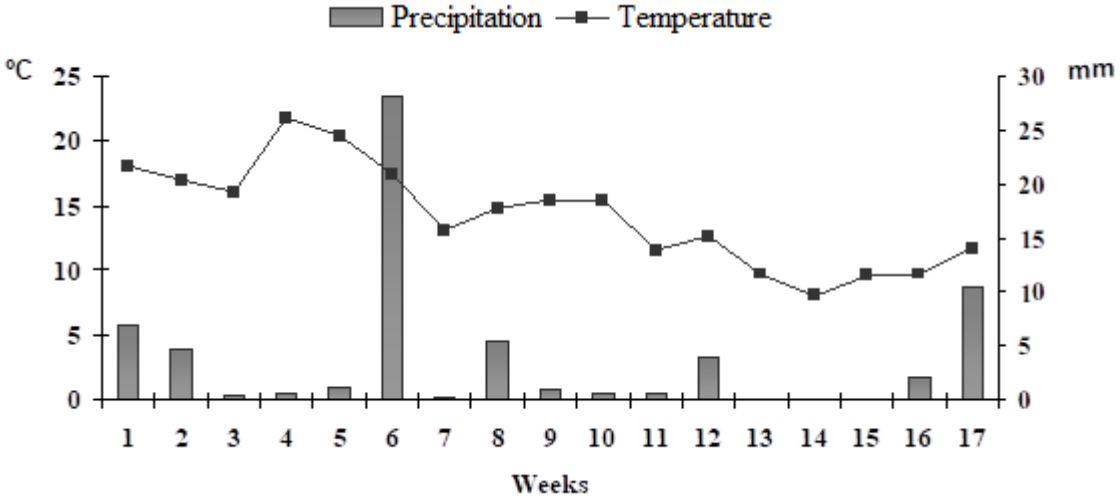

Figure 1: Average temperature (C) and weekly precipitation (mm) recorded during the experimental period. (March 17-July 13)

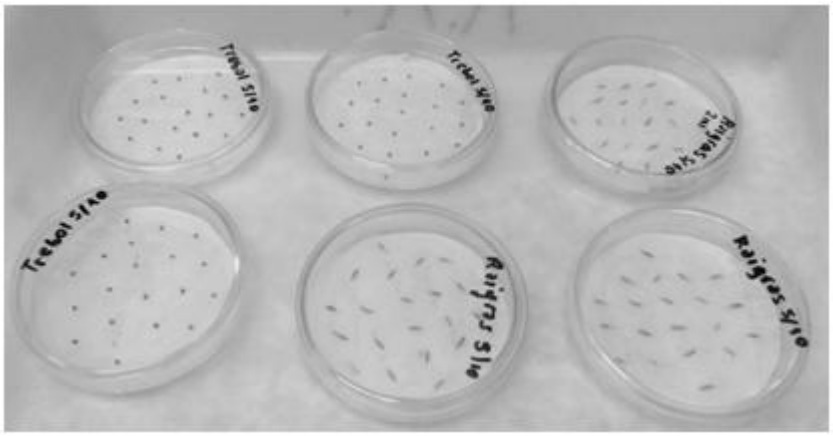

Figure 2: Germination test of rye grass and clover seeds carried out before the experimental culture with pasture and horticultural species. The same procedure was carried out with the seeds of lettuce and radish (OECD 208 guidelines).

c

Figure 3: Differential development at 21 days post treatment (dpt) of pasture species, *L. multiflorum (*rye grass) to the left of each image, and *T. repens (*clover) to the right of each one. (a) Control group (C) culture, without ivermectin (IVM) addition; (b) IVM 90 ng/g group culture; (c) IVM 3000 ng/g culture.

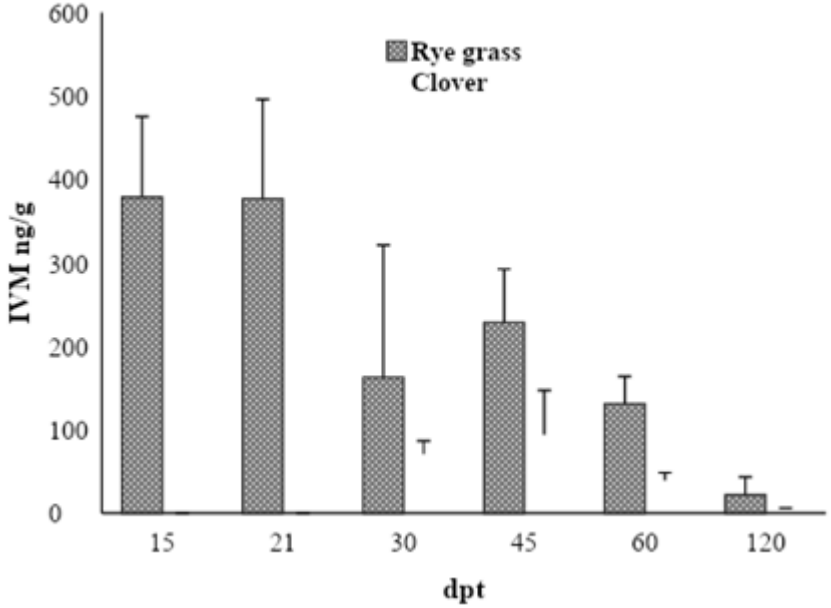

Figure 4: Ivermectin concentrations (IVM) detected in the pasture species grown in regional soil (0-20 cm depth) added (IVM 3000ng/g) and sampled between 15 and 120 days post treatment (dpt) (rye grass) and between 30 and 120 dpt (clover)

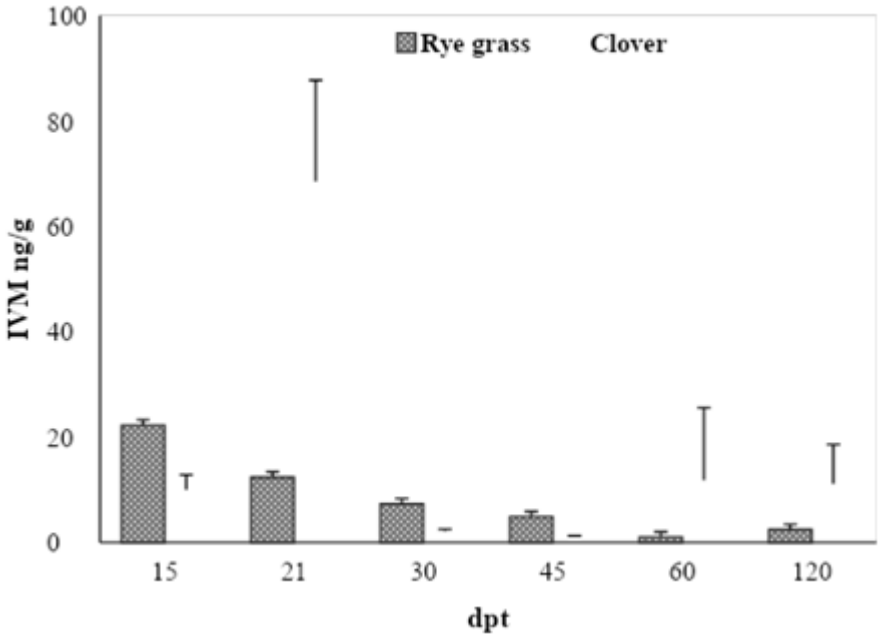

Figure 5: Ivermectin concentrations (IVM) detected in the pasture species (rye grass, clover) grown in regional soil (0-20 cm depth, added with IVM 90 ng/g) and sampled between 15 and 120 days post treatment (dpt).

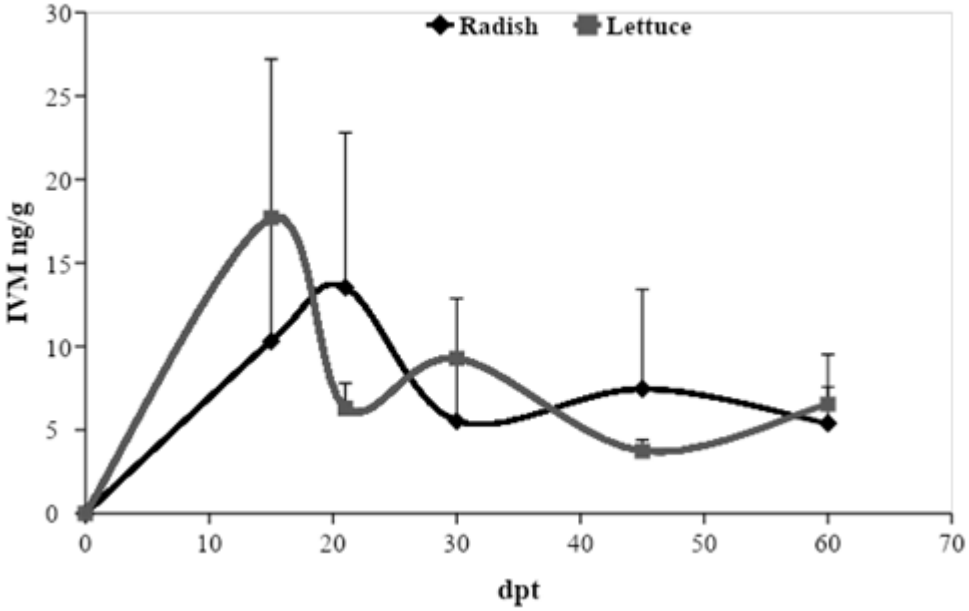

Figure 6: Ivermectin concentrations (IVM) detected in lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) grown in substrate mixture of added bovine faeces (IVM 3000ng/g) and regional soil (1: 10), sampled until 60 days post treatment (dpt).

Tables

Table 1: Edaphological properties of soil samples from the experimental area (0-20 cm depth) of Universitary Campus, FCV-UNCPBA, Tandil, Argentina). Soil Analysis Laboratory, Faculty of Agronomy (FA-UNCPBA), Azul, Argentina

Soil type	*%OMa (*)*	*pHb*	*BRc*	*Texture %*			*Textural Class*
				Sand	**Clay**	**Silt**	
Typical Argiudol	5.42	6.4	0.080	40.55	39.81	19.64	Clay loam

a OM: organic matter (Walkley & Black); **b (**1: 2, 5) in water; **c** BR: basal breathing (mg C-CO2 g-1day-1)

Table 2: Analytical characterization of vegetable culture substrates (control soil, faeces control, faeces/soil mixture, added faeces (IVM 3, 000 ng/g)

Designation of origin	*pH (1: 2.5) in water*	*EC (µS/cm) **	*N of Amonium (mg N kg-1)*	*Organic Matter* +	*N of Nitrates (mg N kg-1)*
Control Soil (0-20cm)	6.01 Weakly acidic	400	Below the detection limit	4.55 Well provided	104.05 Well provided
Control Faeces	7.92 Moderately alkaline	578	129.34	65.22 Well provided	12.5 Low
Control Mixture faeces/soil (1: 10)	6.54 Very weakly acidic	658	86,.23	7.06 Well provided	26.15 Low
IVM added faeces	8.02 Moderately alkaline	500	123.19	66.39 Well provided	39.71 Low to moderate

*EC electrical conductivity (salinity estimator), + Walkley & Black.

Table 3: Plant/substrate relation (regional soil added with IVM 3, 000 ng/g) of samples of pasture species sampled between 15 and 120 days post treatment (dpt) (rye grass) and between 30 and 120 dpt (clover)

Days post treatment (dpt)	*IVM substrate (ng/g) **	*Plant/substrate relation*	
		Rye grass/Substrate	*Clover/Substrat e*
15	1, 157 ± 174.36	0.33	w/s
21	1, 401 ± 105.34	0.27	w/s
30	412 ± 0.10	0.40	0.17
45	412 ± 153.22	0.55	0.23
60	293 ± 229.23	0.45	0.13
120	258 ± 34.56	0.08	0.02

* Wet weight; w/s: without sample

Table 4: Plant/substrate relation (regional soil, added with IVM 90 ng/g) of samples of pasture species (rye grass and clover) sampled between 15 and 120 days post treatment (dpt)

Days post treatment (dpt)	*IVM substrate (ng/g) **	*Plant/substrate relation*	
		Rye grass/Substrate	*Clover/Substrate*
15	14.79 ± 1.67	1.50	0.68
21	27.67 ± 4.70	0.45	2.48
30	3.17 ± 0.16	2.31	0.69
45	4.95 ± 1.46	0.99	0.21
60	5.32 ± 3.17	0.19	2.22
120	9.59 ± 9.95	0.25	1.16

* Wet weight

Table 5: Plant/substrate relation of samples of horticultural species grown in a mixture of added bovine faeces (IVM 3, 000 ng/g) /regional soil (1: 10) substrate and sampled between 15 and 60 days post treatment (dpt)

Days post treatment (dpt)	*IVM substrate (ng/g) **	*Plant/substrate relation*	
		Radish/Substrat e	*Lettuce/Substrat e*
15	1, 053 ± 257.85	0.01	0.02
21	309 ± 117.55	0.04	0.02
30	225 ± 32.54	0.02	0.04
45	326 ± 334.65	0.02	0.01
60	580 ± 123.82	0.01	0.01

* Wet weight

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