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Toxicity of the antimicrobial oxytetracycline to soil organisms in a multi-species-soil system (MS·3) and influence of manure co-addition

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Abstract

The effects of oxytetracycline (OTC) on soil organisms have been studied using a multi-species-soil system (MS·3). Oxytetracycline concentrations of 0.01 mg/kg, 1 mg/kg and 100 mg/kg soil were added to the 20 cm top arable soil layer, with and without horse/cow manure (0.15 g organic N/kg soil) co-addition. No mortality was observed for *Eisenia foetida* S. but significant effects on soil microbial enzymatic activities (phosphatase, dehydrogenase) were observed. The effects on soil microorganism were observed earlier but then recovered in systems with manure co-addition. More important, OTC related plant inhibition was observed in the manured but not in the non-manured systems. Oxytetracycline reached 0.19 and 1.85 mg/l in the leachate of the soil spiked with 1 and 100 mg OTC/kg, respectively and 0.05 and 1.14 mg/l for the same OTC concentrations in the manured systems. The results confirm that manure can modify both the fate and the effects of OTC and that the multi-species-soil systems can reproduce the conditions for a realistic effect estimation of veterinary medicines.

Keywords: Pharmaceuticals; Antibiotics; Oxytetracycline; Soil microcosms

1. Introduction

The use of animal manure as organic fertilizer is a sustainable practice when the nutrient balance is properly addressed [1]. However, drugs used in veterinary medicine and as growth promoters in livestock are excreted by animals and emitted into the soil with their manure [2]. Monitoring programs have confirmed the presence of pharmaceuticals in different environmental compartments [3–5]. For example, antibiotics such as tetracyclines have been found in farmland fertilized with slurry [6,7]. The traditional human health concerns related to spreading antibiotic resistance genes [8] have been expanded for considering also ecosystem risks. Environmental risk assessment protocols for veterinary medicines have been developed in Europe [9] and harmonized at the international level [10]. These evaluations require scenarios, models and data. A multi-species-soil system (MS·3) for arable land has been developed and included in proposals for testing strategies of veterinary medicines [11]. The MS·3 offers a cost/effective experimental approach to measure simultaneously the fate and effects of agrochemicals under controlled laboratory conditions [12,13].

Oxytetracycline (OTC) is a broad-spectrum bacteriostatic antibiotic used for the treatment of a wide variety of infections [14]. It has been widely used as feed additive for therapy of systemic bacterial infections in farmed fish [15], as a growth stimulator in livestock [16] and as prophylactic treatment of bacterial diseases in plants [17]. Its log K_{ow} of 1.22 ± 0.75 [18] reflects its hydrophilic properties and the potential excretion through urine. Tetracyclines are excreted mostly in faeces and urine of treated animals as the parent compound, representing 50–80% of the applied dose [19].

This paper studies the fate and effects of OTC in soil systems using a multispecies soil system (MS·3) designed to mimic arable land. Despite its low K_{ow} oxytetracycline sorbs

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strongly on manure [20]. Therefore, the incluence of manure co-addition was also investigated.

2. Materials and methods

2.1. Origin and characteristics of the soil

Agricultural soil was collected from the top 10 cm layer near Madrid (Spain). The soil had not received manure or pesticide applications in during the last decade. Soil was sieved through a 5 mm mesh in situ, transported to the laboratory, and kept aerated in a cool and dark location for 2 weeks. The soil was characterized for basic pedological descriptors such as sand (77.3%), silt (10.8%) and clay (11.8%) content; and physical-chemical properties: pH (7.9), residual humidity (1.94%), density (1.37 g/ml), maximum water holding capacity (20.11%), NH_4^+ content (0.58 mg/100 g dry soil), total organic carbon (0.25%), total organic matter (0.43%), biomass (22.98 mg C/100 g dry soil), extractable P (12 mg/kg), extractable K (200 mg/kg) and oxidizable C (0.43%), following British Society of Soil Science (BSSS) methods (http://www.soils.org.uk).

2.2. Multi-species-soil system (MS·3)

The design of multi-species-soil systems has been described elsewhere [12,13]. Briefly, the MS·3 were columns of 8 kg of sieved soil assembled as a 20 cm depth agricultural soil core in 20 cm diameter PVC cylinders coupled to a leachate collector system. The soil provided an active microbial community and soil macro-organisms (plants and terrestrial invertebrates) were added to the system. Each sampling day, the soil column was removed from the cylinder and divided into two layers, from 0 to 10 cm depth and from 10 to 20 cm depth for further sub sampling. Earthworms, germinated seeds and plants were then collected for further analysis.

2.3. Test compound and manure amendment

Oxytetracycline dihydrate (OTC) technical product (CAS No. 79-57-2) was provided by Huashu Pharmaceutical Co. Shijiazhuang, China. Oxytetracycline was dissolved in MilliQ water and applied immediately to 8 kg of soil at 40% MWC, at amounts calculated for achieving final concentrations of 0.01, 1 and 100 mg/kg soil ww. Soil columns were then saturated with a final volume of 2.2 l natural spring-water (pH 7.6, alkalinity 3.25 mg Ca CO_3/l, hardness 408 mg Ca CO_3/l, electric conductivity 0.434 mS/cm at 20 °C). In a parallel experiment, 12.5 g/kg soil of 50% horse 50% cow manure was also added to the soil, giving a final fertilization rate of 0.15 g organic N/kg soil.

Effects were assessed after 7 and 21 days of exposure. Each concentration, including the OTC-free control, and time point was assayed in triplicate; giving a total of 48 columns.

2.4. Soil invertebrates and plants

Ten adult (with *clitelum*) *Eisenia foetida* S. individuals of similar weight (250 ± 50 mg) reared in the laboratory were added to each column. Mortality was recorded after 7 and 21 days. Certified seeds from wheat *Triticum aestivum* L., rape *Brassica napus* L. and spring vetch *Vicia sativa* L. were supplied by the Spanish office of plant varieties. Ten seeds from each species were sowed in each column. Emergence of seedlings, stalk elongation and biomass production (only aerial part) were recorded after 7 and 21 days.

2.5. Exposure experimental conditions

Controlled light/dark cycles 10/14 h (8000 lux, light intensity) were established. The MS·3 systems were watered with natural spring water three times per week simulating 1000 mm rainfall/year. After 7 or 21 days, the MS·3 columns were opened. Earthworms were collected for survival assessment. Plant aerial parts were collected from the upper end of the soil core and were sorted by species. Stalk length was recorded. Then, plants were allowed to dry in filter paper envelopes at room temperature (20 ± 2 °C). Dry weight (aerial part biomass) was recorded. Homogenized soil samples from two different depths, 0–10 and 10–20 cm, were collected for chemical analysis and microbial activity assays.

2.6. Analysis of leachates and soils

Leachates were collected at day 1 and weekly at days 7, 14 and 21 in amber bottles. Samples were kept refrigerated at 4 °C for further chemical analysis. Quantitation of OTC in leachate (25 ml) and soil (10 g) samples was performed using solid-phase extraction cartridges followed by RPHPLC–DAD (reverse phase high performance liquid chromatography–diode array detection, Waters 2695–2696, Milford, USA). Soils samples were homogenized in 25 ml buffer pH 5 (250 ml Na_2PO_4 0.1 M, 400 ml $C_6H_8O_7{\cdot}H_2O$ 0.1 M, 1.5 g EDTA) sonicated and centrifuged. Soil supernatants and water samples were concentrated in SPE cartridges (Oasis HLB 3cc, Waters Co, Milford, USA), conditioned with 5 ml CH_3OH and 5 ml MilliQ water, and analyzed by reverse phase HPLC (Waters 2695–2696, symmetry C185 μm, 4.6 mm × 250 mm column; mobile phase NaH_2PO_4 0.1M (53%), CH_3OH (35%), CH_3CN (12%), *N*,*N*′-dimethyloctylamin (3%); flow rate of 1.0 ml/min; diode array scan at $\lambda = 210$–500 nm). The detection limits were 0.025 mg/kg for soil and 0.005 mg/l for leacheates. OTC quantitation was performed by external standard with a calibration curve ranging from 1.0 to 10 μg/ml. Oxytetracycline recovery was 90% in water samples fortified with 100 mg OTC/l and 16% in soil samples fortified with 10 mg OTC/l spiked just before the analysis. The low recovery from soil samples was related to the ionic binding to divalent metal ions forming metal complexes [20], therefore, measured concentrations were not corrected for recovery.

2.7. Microbial activities: respiration

The effect of OTC on soil microbial respiration (as glucose-induced CO_2 production rate) was determined in the native soil before setting up the MS·3 columns. Soil samples spiked with 1, 10, 100 and 1000 mg OTC/kg soil ww were incubated at 30 °C and CO_2 production measured on days 0, 7, 14 and 28, using BacTrac 4300 (SY-Lab, GmbH, Purkersdorf, Austria). The measurement was based on impedance increments of an aqueous solution of KOH 0.2% w/v. Respiration rates were calculated in the linear phase of the respiration curves.

2.8. Microbial activities: phosphatase and dehydrogenase

Phosphatase and dehydrogenase activities were determined in the native soil before setting up the columns and in the MS·3 top (0–10 cm) and bottom (10–20 cm) soil layers on days 7 and 21. Each layer was thoroughly homogenized and triplicate samples were collected and analyzed. Dehydrogenase (DH) activity was measured by incubation with a tetrazolium substrate for 24 h at 27 °C and colorimetric quantification of the reaction product TPF, as described in [21]. Phosphatase activity was determined by spectrofluorometry at $\lambda_{ex} = 360$ nm and $\lambda_{em} = 465$ nm according to Freeman et al. [22].

2.9. Data analysis

Multi factor analysis of variance (Software Statgraphics plus Version 4.1) with a Multiple Range Test based on the Fisher's least significant difference (LSD) procedure were used for detecting differences within treatments (sampling day, OTC concentration, manure amendment) at a 95% ($p < 0.05$) significance level.

3. Results

3.1. Chemical analysis of OTC in soil and leachate

Although watering was identical for every column, the volumes of the collected leachate showed some variability among columns and among collection weeks. The OTC concentrations measured in soil and leachate are shown in Table 1. Oxytetracycline was not detected in leachate from control columns or the 0.01 mg OTC/kg soil treatment. Detectable concentrations of OTC appeared in the leachates from the medium and high treatments. The maximum OTC concentrations were detected on day 1; values decreased for samples collected during the first week, and were only detected in the highest treatment (100 mg OTC/kg soil) on weeks 2 and 3. Concentrations in leachates from manured systems were lower than those from non-manured systems. Soil OTC concentrations on days 7 and 21 were similar in the OTC (0.54 and 1.84 mg OTC/kg soil) and OTC plus manure (0.56 and 1.73 mg OTC/kg soil) soils exposed to 1 and 100 mg OTC/kg soil. Mass balance showed OTC recoveries

Table 1
Measured concentrations of oxytetracycline (OTC) in leachates and soil from the MS·3 columns (mean ± S.D. of $n = 6$ samples)

Initial mg OTC/kg soil	Leachate sampling week	ppm OTC recovered			Total OTC recovered (soil + leachate) (mg)
		Leachate	Soil (0–10 cm) ($N = 6$)	Soil (10–20 cm) ($N = 6$)	
Non-manured					
1	0	0.19	0.54 ± 0.02	0.55 ± 0.02	4.3
	1	0.04			
	2	n.d.			
	3	n.d.			
100	0	1.85	1.84 ± 0.12	2 ± 0.08	15.9
	1	0.37			
	2	0.07			
	3	0.07			
Manured with horse/cowdung					
1	0	0.05	0.56 ± 0.02	0.54 ± 0.02	4.42
	1	0.02			
	2	Non detected			
	3	Non detected			
100	0	1.14	1.73 ± 0.16	1.86 ± 0.44	14.44
	1	0.56			
	2	0.08			
	3	0.06			

OTC concentrations in control and in the MS·3 treated with 0.01 mg OTC/kg soil were below the detection limits (0.025 mg/kg soil; 0.005 mg/l leacheates) in all cases and are not included.

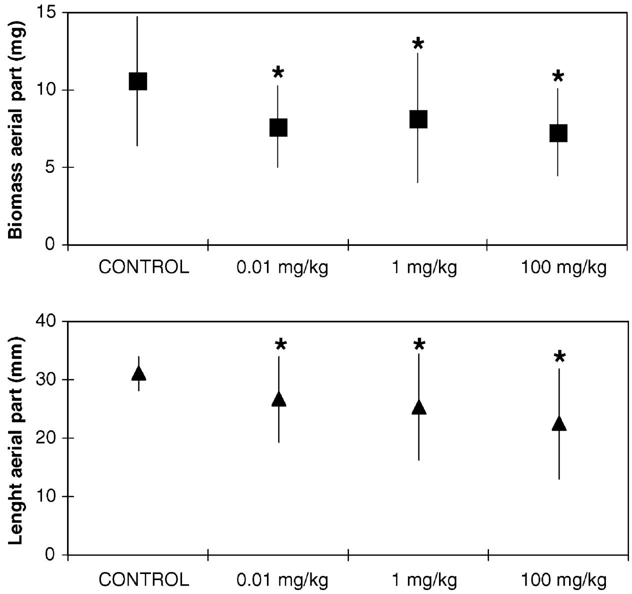

Fig. 1. Effects of oxytetracycline on plant elongation and growth in MS·3 columns exposed to 0, 0.01, 1 and 100 mg OTC/kg soil and manured with 12.5 g/kg soil of 50% horse 50% cow manure (0.15 g organic N/kg soil) on day 7; squares: biomass of the aerial parts of *Brassica napus* on day 21; triangles: length of aerial part of *Triticum aestivum* on day 21. Asterisk denotes a statistically significant difference vs. control ($p < 0.05$).

of 51–57% from soils exposed to 1 mg/kg and 1–2% from soils exposed to 100 mg OTC/kg.

3.2. Effects on Eisenia foetida S. effects on plants

No significant earthworm (*Eisenia foetida S.*) mortality was observed after 7 or 21 days at any OTC concentration.

A decrease in seed germination was sporadically observed, but no dose/response effects were observed. Stalk elongation and biomass results are presented in Table 2. In manure-free systems no inhibitory effects on plants were observed. In fact, significant increases in elongation and biomass of *Triticum aestivum* L. at 0.01 mg OTC/kg soil and an increase of *Vicia sativa* L. biomass at 100 mg OTC/kg soil were observed. However, manure co-addition changed this pattern, provoking significant reductions in *Triticum aestivum* L. and *Brassica napus* L. elongation; and *Brassica napus* L. biomass at the medium and high and even sporadically at the low concentrations (Fig. 1).

3.3. Effects on soil microbial activities

Respiration rates as mg CO_2/h 100 g dry soil are presented in Fig. 2. Respiration inhibitions of 16–25% and 28–38% were observed in soils spiked with 100 and 1000 mg OTC/kg soil.

The evolution of the enzymatic activities in the OTC-free controls is presented in Figs. 3 and 4; an increase in dehydrogenase activity at day 7 and a decrease in phosphatase activity at day 21 were the most relevant effects. The addition of ma-

Table 2
Effects of oxytetracycline (OTC) on terrestrial plants in non-manured and manured multi-species-soil systems (MS·3-arable land)

Endpoint	Plant species	Non-manured				Manured with horse:cow dung			
		MS·3 control 7 days	MS·3 OTC 7 days	MS·3 control 21 days	MS·3 OTC 21 days	MS·3 control 7 days	MS·3 OTC 7 days	MS·3 control 21 days	MS·3 OTC 21 days
Biomass production (mg)	*Triticum aestivum*	7.2 ± 3.9	10.1 ± 3.2 (at 0.01 ppm)	25.5 ± 11.0	n.s.	5.8 ± 3.2	n.s.	31.8 ± 8.7	n.s.
	Brassica napus	5.7 ± 2.5	n.s.	8.8 ± 3.4	n.s.	7.5 ± 5.2	n.s.	10.6 ± 4.1	7.7 ± 2.6 (at 0.01 ppm)
	Vicia sativa	11.8 ± 6.1	n.s	33.7 ± 16.8	46.6 ± 19.0 (at 100 ppm)	10.4 ± 6.6	7.5 ± 4.3 (at 100 ppm)	28.6 ± 8.9	n.s.
Stalk elongation (cm)	*Triticum aestivum*	7.2 ± 4.1	23.6 ± 2.2 (at 0.01 ppm)	25.8 ± 6.1	n.s.	6.4 ± 3.9	8.1 ± 2.9 (at 0.01 ppm)	31.1 ± 2.9	26.7 ± 7.3 (at 0.01 ppm)
	Brassica napus	4.6 ± 3.3	n.s.	9.8 ± 2.3	n.s.	7.1 ± 2.8	3.3 ± 1.7 (at 0.01 ppm)	9.9 ± 2.6	8.3 ± 3.0 (at 1 ppm)
	Vicia sativa	9.5 ± 4.2	n.s.	43.1 ± 12.1	n.s.	6.3 ± 4.6	n.s.	39.5 ± 6.8	n.s.

Mean ± standard deviations are shown. Three concentrations 0.01, 1 and 100 mg OTC/kg soil (ppm) were tested; the value presented in the table corresponds to the lowest OTC concentration tested at which significant ($p < 0.05$) differences vs. controls were observed (the concentration is shown in parenthesis). The n.s. indicates no significant differences at any concentration.

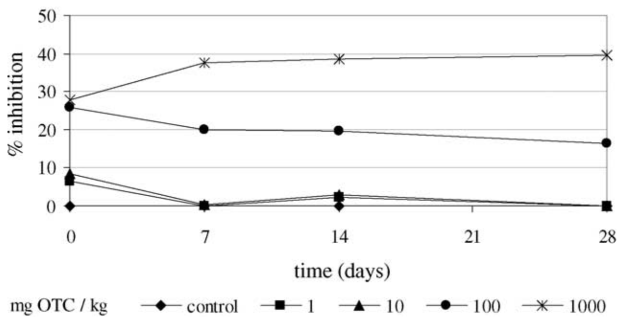

Fig. 2. Effects of oxytetracycline on the microbial respiration (measured as CO_2 production) of the soil used for setting the systems.

nure did not changed this pattern but the differences were less pronounced.

The effect of OTC on dehydrogenase and phophatase activities is shown in Figs. 5–7; due to technical problems the phosphatase activity of non-manured MS·3 was not measured on day 7. In the initial assessment of the soil used for setting the systems, a dose-response curve 24 h after application with and EC50 value around 100 mg OTC/kg soil was observed for the dehydrogenase activity; a non dose-related inhibition was

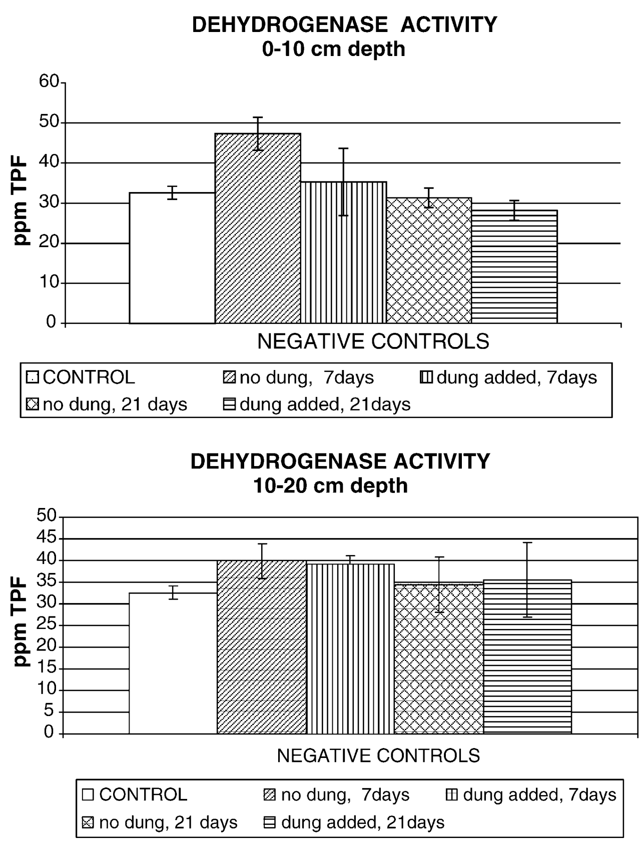

Fig. 3. Dehydrogenase activity in the soil used for setting the systems (control) and in the OTC-free control MS·3 columns, included those manured with horse:cow dung (dung added) at two depths: 0–10 and 10–20 cm, and two sampling days: 7 and 21 days. Results are shown as mean ± S.D. of $n = 3$ samples.

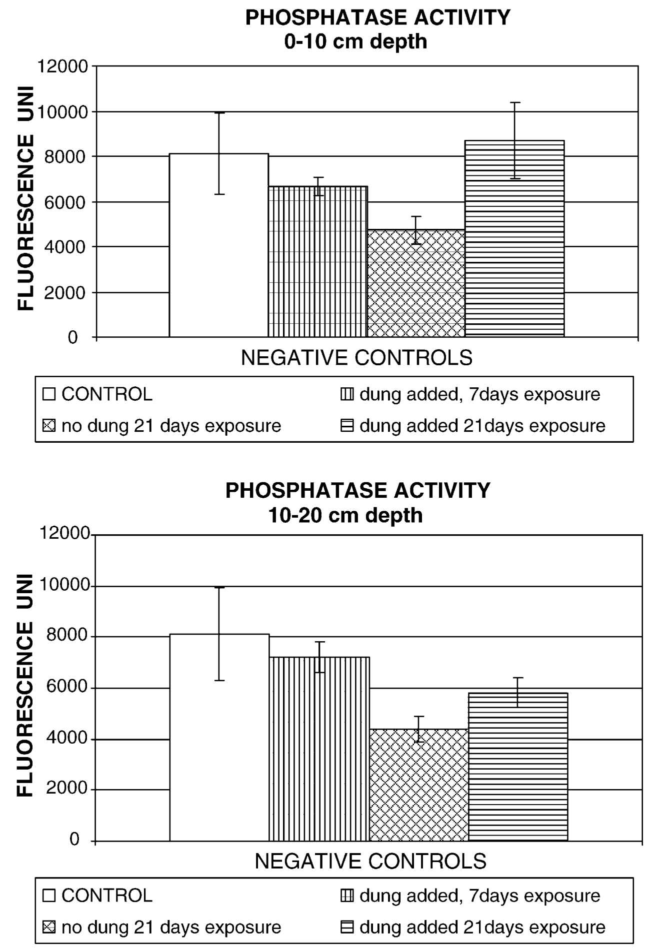

Fig. 4. Phosphatase activity in natural soil (control) and in control soils sampled from MS·3 columns, included those manured with horse:cow dung (dung added) at two depths: 0–10 cm and 10–20 cm, and two sampling days: 7 and 21 days. Results are shown as mean ± S.D. of $n = 3$ samples. *Note*: Due to technical problems the non-manured columns were not analyzed on day 7.

observed for phosphatase. In manure free-systems, an initial induction of DH at the low and medium concentrations was observed on day 7, and a clear inhibition at the highest concentration on day 21. Phosphatase activity showed a potential dose-related reduction trend but no significant differences were observed at any of the tested concentrations.

In manured system the inhibition of DH at the highest dose was observed on day 7 but recovered on day 21. A reduction in phosphatase activity on day 21 in the upper soil layer was observed at all tested doses.

4. Discussion

Veterinary pharmaceuticals are widely used worldwide and, through agricultural practices, they are released in the environment. Only recently, concern for their potential environmental risk has raised, followed by regulatory implementations [9,10,23], and scientific revisions, e.g. [24].

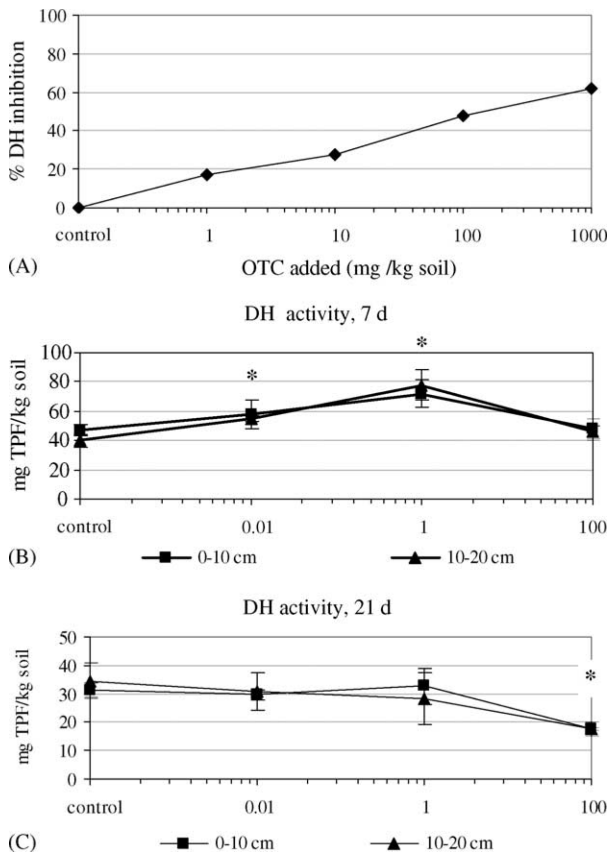

Fig. 5. Dehydrogenase activity in non-manured soils: (A) initial activity in the soil used for setting the systems after 24 h exposure to increasing OTC concentrations; (B) activity in MS·3 columns exposed to 0, 0.01, 1 and 100 mg OTC/kg soil on day 7; and (C) activity on day 21 days; square: activity at 0–10 cm depth layer, triangle: activity at 10–20 cm depth layer. Asterisk denotes a statistically significant difference vs. control ($p < 0.05$).

Tetracyclines have been identified in soils as the results of manure applications [7]. Oxytetracycline in particular has a half-life in manure of about 30 days and can be detected in aged manure [25]. Despite its low Kow, oxytetracycline sorbs strongly in soil, with Kd values between 417 in sand soil and 1026 in sandy loam soil and no significant desorption [26]. The process is assumed to be related to ionic binding with metal complexes formed between soil, metal ion and oxytetracycline [20] and is very rapid, been complete in few minutes [27]. The low OTC recovery observed for soil samples is explained by this rapid sorption; very strong extraction procedures are required for improving the recovery [28].

The binding also reduces OTC mobility and leaching potential [29]. OTC was not detected in leachates from laboratory experiments [26]; but it was observed in semifield studies associated to preferential flow [30]. In our study, OTC was detected in the leachate from columns treated at 1 mg/kg during the first week and in the leachate from columns treated with 100 mg/kg during the whole experiment. During the experimental design of the MS·3 it was confirmed that these systems cover the preferential flow movement associated to worm channels (Alonso et al., submitted for publication).

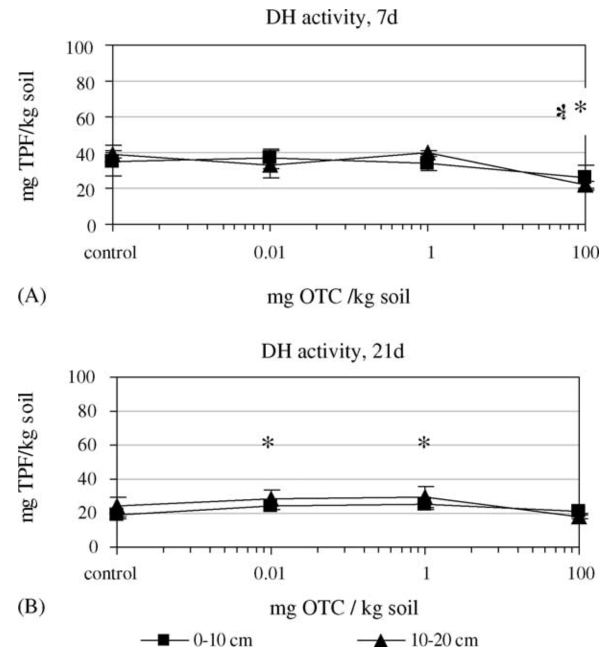

Fig. 6. Dehydrogenase activity in manured soils: (A) activity in MS·3 columns exposed to 0, 0.01, 1 and 100 mg OTC/kg soil and manured with 12.5 g/kg soil of 50% horse 50% cow manure (0.15 g organic N/kg soil) on day 7; and (B) activity on day 21. Square: activity at 0–10 cm depth layer, triangle: activity at 10–20 cm depth layer. Asterisk denotes a statistically significant difference vs. control ($p < 0.05$).

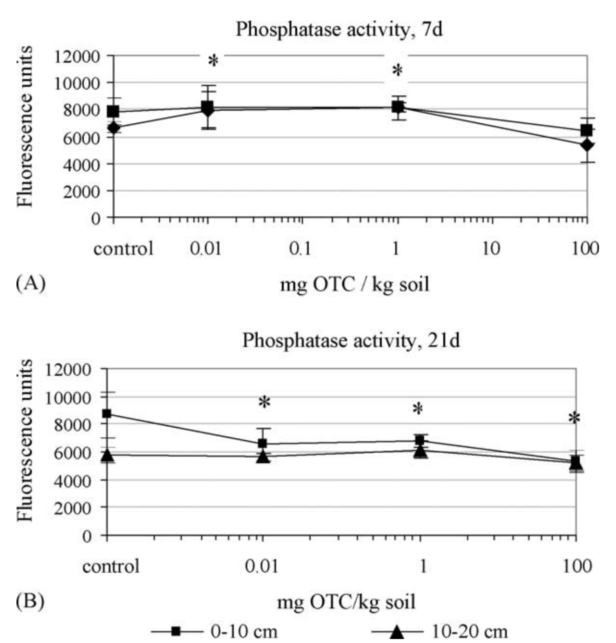

Fig. 7. Phosphatase activity in manured soils: (A) activity in MS·3 columns exposed to 0, 0.01, 1 and 100 mg OTC/kg soil and manured with 12.5 g/kg soil of 50% horse 50% cow manure (0.15 g organic N/kg soil) on day 7; and (B) activity on day 21; square: activity at 0–10 cm depth layer, triangle: activity at 10–20 cm depth layer. Asterisk denotes a statistically significant difference vs. control ($p < 0.05$).

Manure co-addition reduced the leaching potential of OTC under the studied conditions. These results confirm recent investigations suggesting that manure can significantly affect the fate of pharmaceuticals in soil [12,31]; an aspect that is currently not considered in regulatory risk assessment protocols. The results also confirm the suitability of the multi species soil system MS·3 as a proper tool for assessing fate properties of veterinary pharmaceuticals under realistic conditions, including the co-application of manure, as previously suggested [11].

No mortality for adult *Eisenia foetida* S. was observed. This result is in agreement with observations on other soil invertebrates including collembola, enchytraeids and annelida [14] with LOEC values >5000 mg/kg soil for OTC.

Results for plant germination, elongation and biomass showed variability in the sensitivity of the different tested species. Oxytetracycline concentrations of 0.01 mg/kg could promote *Triticum aestivum* L. elongation when observed at day 7 but this effect disappeared at longer time (21 days) observation. Batchelder [32] also observed *Triticum* growth promotion after exposure to OTC. Toxicity only appeared at concentrations above 160 mg/kg, which are higher than those employed in this study.

Antibiotics exert their action against microbial populations. Therefore, this ecological receptor received a larger attention in our experimental design. Functional endpoints based on soil respiration and soil enzymatic activities were used. The initial experiments suggested a low sensitivity of carbon respiration, requiring concentrations of 100–1000 mg OTC/kg soil for observing significant effects, with a maximum inhibition of about 40%. The observed inhibition was lower than that recently reported by Vaclavik et al. for a sandy loam soil with 1.6% organic carbon [33]. Good dose-effect responses were observed for DH activity, with an EC_{50} close to 100 mg/kg. Therefore, the enzymatic activities where employed in the MS·3. Dehydrogenase activity was also the most sensitive parameter in the MS·3. Significant effects at 100 mg/kg, but below the 50% trigger, were observed at day 21. The addition of manure did not modify the baseline enzymatic activities. In fact, the activities measured in the manured OTC-free MS·3s where closer to the initial soil activities than those measured in the manure-free controls. Manure co-addition also modified the responses to OTC, the inhibitory effects where already observed at 7 days, and recovered at the end of the experiment.

Oxytetracycline is very effective (toxic) against several groups of microorganisms while other are resistant. In general, functional endpoints are considered the ecologically relevant parameters for assessing the effects of chemicals on soil populations [34,35]; however, its use when testing selective antimicrobials requires further considerations. Our results indicated that despite the clear antimicrobial activity of oxytetracycline, significant effects on soil microbial functional endpoints appear exclusively at very high concentrations. These results are in agreement with the reported minimum inhibitory concentrations (MICs) of OTC solutions for soil bacteria [19], which were 0.25 mg/l for sensitive and 32 mg/l for resistant strains; and confirmed recently published results [33]. The effect of a disturbance on microbial community function depends on its duration, recovery potential and sensitivity of the different groups of organisms [35,36]. Other possibilities such as pollution-induced community tolerance (PICT) methods have been suggested for assessing selective antimicrobials [37]; although the sensitivity of PICT and enzymatic activities measured in the MS·3 were similar for the antimicrobial sulfachloropyridazine [13]. Veterinary drugs and/or their metabolites are excreted by urine and faeces and the environmental concern is associated to the spread of manure from treated animals on agricultural land as fertilizer. Few previous studies had confirmed the effect of manure co-addition on the fate of veterinary medicines [12,31], while the influence of manure on the effect side had only been hypothesized [33]. This study confirms both aspects, and offers a higher-tier laboratory tool for a cost/effective assessment. The information produced by a battery of bioassays and leaching studies is combined into a single test conducted at three fixed nominal concentrations. Exposure follows realistic estimations, which can be adapted to local conditions (irrigation, temperature, soil characteristics, etc.) when required.

The tested concentrations were selected following current European regulatory triggers, and can be adapted to other requirements if required. The low concentration corresponds to the exposure trigger of 10 μg/kg [9] and is included to confirm the relevance of this value, which in fact has been augmented to 100 μg/kg during the international harmonization [10]. The highest concentration offers a safety factor of at least 100 for most cases, as the soil concentrations of veterinary pharmaceuticals rarely exceed the 1 mg/kg level [24]. For OTC, data from Halling-Sørensen [2] estimated OTC PEC_{soil} of maximum 286 μg/kg soil in a beef cattle field worst-case scenario and <1000 μg/kg soil in a fattening pigs manure application scenario. The manure free MS·3 assay suggested that the highest concentration tested, 100 mg/kg, only produced slightly effects on some microbial enzymatic activities, without reaching the 50% inhibition trigger. These results are in agreement with reported data on the toxicity of OTC for plants, soil fauna and soil microbial respiration, and suggest a low risk of the antibiotic for the soil community.

However, the manure co-addition modified the toxicity pattern and identified significant inhibitory effects on plants at concentrations of 1 and 10 mg OTC/kg and even occasionally at 0.01 mg OTC/kg. These sublethal effects were species-specific and better observed at the latest time point (21 days), and probably, are not related to direct toxicity but to influences in nutrient availability. The relevance of these findings requires further investigations. Nevertheless, the results suggest that standard risk assessment procedures based on direct toxicity of the veterinary medicines on traditionally used soil species and endpoints could underestimate the actual risk. It should be considered that the drug will reach the soil as the result of a fertilization process, and potential reductions in plant growth, even if indirect and associated to nutrient availability, manure degradation, etc., are relevant and should be identified. The study has been done with a typical Spanish agricultural soil, with a very low organic matter content, and particularly for indirect effects, the extrapolation of observed results to other soil conditions is not clear.

5. Conclusions

The results observed for OTC confirmed the suitability of MS·3 to produce a cost/benefit assessment of the effects of veterinary drugs on soil systems, and the need for covering the effect of manure as previously postulated.

The expected concentrations of oxytetracycline in soils due to manure amendments is not expected to produce significant direct effects on soil organisms. However, indirect effects on plant growth associated to the co-addition of the antimicrobial and manure cannot be disregarded.

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