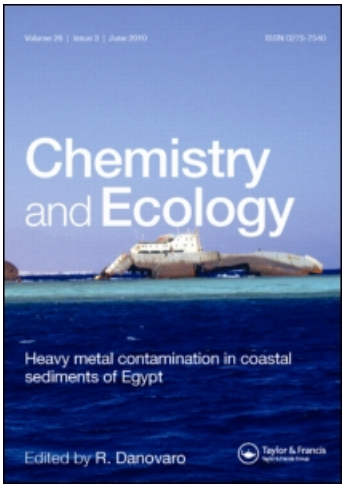

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Degradation of monensin on soils: influence of organic matter and water content

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Different kinds of pharmaceutical compounds (PhC) are used in veterinary medicine. Intensive bovine production involves the use of growth promoters that reach the environment through animal excreta. The most widely used growth promoter in Argentina is monensin, an ionophore antibiotic. In this work, the influence of soil organic matter and soil water content on the degradation of monensin was analysed. Results obtained show that both parameters are in direct relation with the degradation of monensin in soils.

Keywords: degradation; monensin; soil; organic matter; water content; growth promoter

1. Introduction

Environmental pollution by organic compounds has been extensively studied over the last 30 years. The transport and fate of pesticides and surfactants in the environment have been widely analysed [1–5]. However, pharmaceutical compounds (PhCs) in environmental matrices are one of the most recent research areas being considered as an international hotspot [6]. Only few and isolated reports can be found in the literature before 1990. Though the main effect of some PhCs in the biological target is well known, the authors had difficulty finding published literature about the concentration of these compounds in the environment and their possible effect on the biota [7–12].

Different analytical methods for the determination of PhCs in the environment have been developed. However, in the last decade, high-performance liquid chromatography (HPLC) coupled with electrospray ionisation tandem mass espectrometry (ESI/MS/MS) has become the analytical technique of choice for the determination of polar environmental pollutants, and is especially suitable for environmental analysis because of its selectivity and sensitivity [13,14].

The type of production system in any part of the world is generally defined by the production cost and the profit obtained from that product. Meat production systems in Argentina were traditionally extensive, based on the use of natural or implanted pastures as a feeding source, with low

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animal density and cattle grazing on large areas. In the last 15 years, the growth in world food demand and the improvement of the cost-profit relation of soybean production caused many areas previously used for animal feeding purposes to be turned over to soybean crops instead. Nonetheless, during this period, bovine stock was not modified in a significant way, suggesting an increase in intensive bovine production systems. Expansion of soybean crops produced a decrease in the available grazing area and an increase in the number of CAFOs (concentrated animal feeding operations). These intensive systems are characterised by a high animal density (15–40 m^2 per animal) [15]. Cattle is confined and fed concentrated diets based on a high proportion of grains (corn, sorghum, oats, wheat, soybean, sunflower), a minimal amount of forage, and a mineral supplement containing salts of copper, calcium, cobalt, manganese, zinc, sodium, iron and growth promoters. These growth promoters are usually antibiotics added to food in sub-therapeutic doses. Most of them belong to the ionophore family. Ionophore antibiotics are polyethers produced by different *Streptomyces* strains and exhibit antibacterial and coccidiostatic activity [16].

Feed is the major cost for the farmer; thus, the success of intensive production systems will depend on a shorter fattening process. For this reason, the use of growth promoters is widespread, as they improve feeding efficiency. This allows the animal to reach slaughter weight faster, diminishing their time on the farm.

In previous works in our laboratory, identification of the most important pharmaceuticals used in intensive breeding systems was achieved. The most widely used growth promotor in Argentina is monensin. Its use has grown exponentially in the last 15 years. Monensin (Figure 1) is an ionophore polyether antibiotic produced by *Streptomyces cinnamoniensis* [17]. Growth promotion with sodium monensin was phased out in the European Union in January 2006 [18]. The total dose of monensin as a growth promoter in cattle is 300 mg/animal/day. Since the oral absorption is 50% [16], 150 mg/animal/day is excreted in the manure without any modification. In the year 2001, 267,900 animals were fed for at least 3 months in feedlots, prior to slaughter in the province of Buenos Aires. Therefore, it may be estimated that about 3.6 tons of monensin over a surface of 307,571 km^2 reaches the environment every year [15]. Preliminary studies on the sorption of monensin to solid matrices were performed in our laboratory in order to assess the risk of contamination of the water bodies geographically close to intensive cattle production systems. The results observed suggest a strong dependence of the monensin behaviour in solid matrices on their nature, more specifically, on their organic matter content, because an increase in organic matter involves an increase in the variety and amount of interactions between the drug and the matrix [19].

The aim of this study was to evaluate the degradation of monensin on soils at different water content to complement sorption studies.

Since sorption on soils with low organic matter content appeared to be lower, the potential risk of contamination of water bodies should be greater in the Salado River lower drainage basin, where intensive bovine production systems have increased in the last few years. In that area, soils are poor in organic matter and groundwater is shallow. The results described in this paper

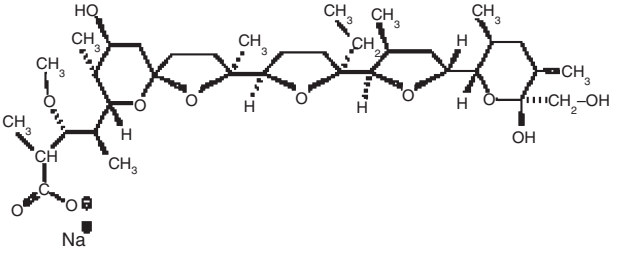

Figure 1. Monensin structure.

contribute to a better knowledge of monensin environmental behaviour in different soils and water content conditions.

2. Experimental

2.1. *Materials*

Monensin sodium salt (90% purity) and the derivatising agent vainillin were purchased from Sigma–Aldrich (Seelze, Germany). Analytical grade methanol, dichloromethane and sulfuric acid were obtained from Sigma–Aldrich. Stock solution was prepared by weighing and dissolving 5 mg of monensin in 100 mL of methanol. Calibration standards were prepared by a serial dilution of the stock solution with methanol.

2.2. *Solid matrices*

Two soils were sampled in the soil surface layers (0–15 cm). Soil A was collected from the Salado River basin (Roque Perez County). This soil is a typic Argentinian Hapludolls of the Salado Basin. One of the biggest feedlots of the Buenos Aires province (Senasa Sep–08) is located in Roque Pérez County, where shallow groundwater is found at a depth of between 3 and 10 metres [20]. Soil B was collected from an urban park area (the campus of the School of Veterinary Medicine, University of Buenos Aires), far away from animal breeding zones. Soil A is a loam type with lower organic matter and clay content than soil B, a clay loam type soil.

Soil samples were air dried, ground and passed through a 2 mm sieve and stored in closed containers at room temperature (20–25 °C). Analysis was performed within one week of collection.

Soils were analysed for extractable phosphorus (Bray–Kurtz 1 method), pH (1:2.5 soil:water ratio), organic carbon (Walkley–Black method), organic nitrogen (micro-Kjeldhal) and clay content (Bouyoucous method) by standardised methods [21,22]. The results of the soil analyses are shown in Table 1.

Soils were spiked with monensin and homogenised (24 μg/g final concentration). The soil (20 g dry wt) was weighed into 100 mL glass recipients, and ion free water was added to achieve 80% field capacity. Soils were placed in a dark cabinet at 22–25 °C and in aerobic medium. The cabinet floor was covered with water in order to maintain the moisture of the soils. All the samples were weighed daily and water was added if necessary to maintain the 80% field capacity moisture level. Finally, extractions at 0, 7, 14, 21, 33, 40, 47 and 54 days were made. All the tests were conducted in triplicate. Experiments with soil B were also performed with extraction times of 0, 70, 188 and 212 hours.

In another experiment, samples were spiked with monensin in order to assess the relevance of soil water content in the persistence of the antibiotic. Air dried soil A (200 g, 11.3% of field capacity) was spiked with 3.2 mg of monensin (16 ppm). Samples (20 g) were weighed and water was added to achieve 80% and 100% of field capacity, respectively. The samples with 80% and 100% of field capacity were placed in a dark cabinet with a layer of water on the floor in order

Table 1. Soil analysis.

	OM %	OC %	ON %	C/N	P (ppm)	pH	EC (dS m^{-1})	Clay %	Silt %	Sand %	Soil type
Soil A	3.80	1.90	0.22	8.64	6.94	6.2	0.38	15	46.7	38.3	Loam
Soil B	9.38	4.69	0.36	13.03	6.23	7.1	0.72	33	46	22	Clay loam

Note: OM, organic matter; OC, organic carbon; ON, organic nitrogen; C/N, carbon/nitrogen ratio; P, phosphorus; EC, electric conductivity.

to maintain their moisture. The samples with 11.3% field capacity (without water addition) were placed in a different cabinet, without water. All the cabinets were kept at 20–25 °C. After 7 days, all samples were extracted and analysed. Determinations were performed in triplicate; the relative error was <1.0% for them all.

2.3. *Extraction procedure and monensin analysis*

The Folch method [23] was adapted for the extraction. This method was employed to extract monensin in feed mineral supplement with almost quantitative recovery. Briefly, the spiked samples were suspended in methanol:water (90:10 v/v), mixed (Waring Blender) for 15–30 min and filtered. pH was adjusted to 7.5–8.0 with 0.5% NaOH and extracted with dichloromethane (3 × 20 mL). Extracts were dried and redissolved in methanol (20 mL) for analysis. Matrices without the addition of the drug were used as blanks. In previous work in our laboratory this method showed a direct relationship between the organic matter content in the sample and the amount of drug extracted [19].

Monensin was analysed spectrophotometrically after derivatisation with vainillin [24]. The extracts (or standard monensin) were mixed with vainillin reagent in a 9:1 ratio and heated at 60 °C for 25 min. After cooling at room temperature, samples were read at 518 nm (JASCO 7850).

3. Results and discussion

The sorption and the persistence of a particular organic xenobiotic in the environment are essential to evaluate their potential contamination effect. The latter parameter is normally expressed as the degradation half-life. The first study on the degradation of monensin in the environment was performed in 1984 [25]. Although it was the pioneer study into monensin degradation, the extraction procedure and monensin analysis is not clearly explained in the paper. In laboratory studies with manure-amended soils, a half-life of 13.5 days was estimated for monensin, whereas in field studies, shorter half-lives of 3.3 and 3.8 days were observed in manure-amended and unamended soils, respectively [26]. In another report, a half-life in soil of 2 days was estimated with slight differences in two soils with organic matter of 1.7% and 4.4% respectively and at field capacity [27]. In the present work, degradation experiences were performed with two soils of different characteristics (Table 1). The results of monensin concentration (mean of three determinations) at each extraction time, together with its coefficients of variance, are shown in Table 2. Considering the interactions of the compound with the components of the soil, results are expressed as the fraction remaining from the monensin concentration determined at time 0 (soil A: 22.07 μg/g, soil

Table 2. Monensin degradation in soils A and B.

Soil A				Soil B			
Time (days)	Monensin (μg/g)	CV (%)	Fraction remaining	Time (hours)	Monensin (μg/g)	CV (%)	Fraction remaining
0	22.07	7.47	100	0	14.77	0.29	100
7	18.36	3.26	83.2	70	12.56	2.76	85.0
14	16.27	0.67	73.7	140	5.80	2.62	39.3
21	10.17	0.64	46.1	188	1.67	2.59	11.3
33	8.00	0.70	36.2	212	1.13	5.73	7.7
40	6.09	2.14	27.6				
47	6.03	1.79	27.3				
54	4.19	3.61	19.0				

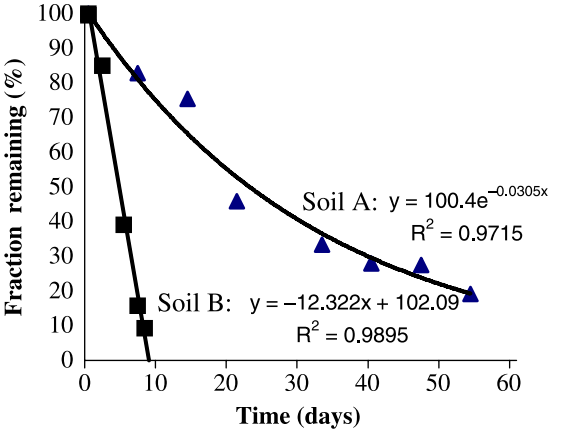

Figure 2. Monensin degradation in soils A and B.

B:14.77 μg/g). Reduction of monensin concentration is slow in soil A (3.8% of organic matter content), and seems to follow a first order decay function (R^2: 0.9715): $C = C_0 e^{-kt}$, where C is the monensin concentration at time (t), C_0 is the initial monensin concentration and k is the monensin degradation rate constant. Using this model, the half-life was calculated as 22.7 days (Figure 2). On the other hand, soil B, with higher organic matter content (9.38%), showed a steady degradation of the compound. After 7 days, the amount of monensin present in the matrix was 16%. For this reason, a second experiment was designed in order to determine the half-life time of monensin in soil B. The extractions were performed at shorter times, in order to observe the reduction of the concentration of monensin in the soil. In this case, the fast decay of monensin to values lower than the limit of quantification prevented the correct determination of the half-life, which was estimated at 4.2 days, following a linear regression model (R^2: 0.9895) (Figure 2).

The results described above suggest a correlation between degradation of monensin and organic matter content. No previous reports of half-life higher than 14 days in laboratory experiments with soils or field studies with unamended soils have been found in the literature. Soil A, characteristic of the lower Salado River basin, in the Flooding Pampa, is a silty soil, with low organic matter, while soil B is a soil with a higher content of organic matter and humic substances, as its higher C/N ratio [28] shows (Table 1). This fact can explain the difference in degradation observed, since the occurrence of microorganisms in soils is related to soil carbon and nitrogen. Without added substrate or amendment, soil organisms generally metabolise at low rates. Soil humus represents a source of organic nutrients available for microorganisms [9]. On the other hand, both soils also differ in clay content. Most nutrients are associated with clay or silt particles, which also retain soil moisture efficiently. Therefore soil B offers a more favourable habitat for organisms.

Soil moisture content varies considerably in any soil, and soil organisms must adapt to a wide range of soil moisture contents. Biodegradation is the breakdown of organic compounds through microbial activity. Biodegradable organic compounds serve as a substrate for microbes, and their bioavailability, which is one important aspect of the biodegradation, depends largely on water.

The influence of soil water content on monensin degradation was analysed in three conditions (air dried soil, 80% and 100% field capacity) using soil A, where degradation processes are slower. After 7 days, samples were analysed for monensin content. The results obtained clearly show the relevance of soil water content in monensin persistence (Figure 3).

In the air dried soil (11.3% field capacity) no degradation was observed, while at 100% field capacity, 25% of the compound was degraded, with all other abiotic parameters (temperature, pH, soil texture) having the same values.

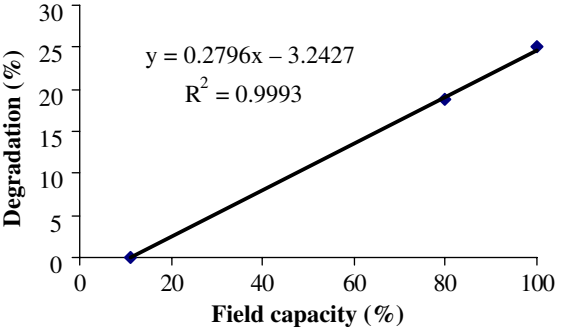

Figure 3. Monensin degradation in soil A after 7 days vs. field capacity.

The field capacity in soils with a mean annual precipitation between 900–1000 mm could remain as high as 80% during the rainy season. According to the results shown in Figure 3, the degradation profile of monensin in soil A will be deeply modified (>15%) during this part of the year. In contrast, during times of drought the surface layer of soil can achieve the condition of air dried soil, when no degradation was observed after 7 days.

4. Conclusions

Knowledge of the facts that affect the fate and transport of the ionophore monensin in the Province of Buenos Aires (Argentina) is essential to assess the risk of contamination of the water bodies geographically close to intensive cattle production systems. Sorption to soils and persistence in the environment are two key characteristics of any pollutant. Retention of monensin shows a strong dependence on the organic matter content of solid matrices. The results obtained in the present paper show that persistence is also related to the organic matter content of the soil. An increase of 150% in organic carbon content leads to a 5.5-fold diminution in monensin half-life. Water soil content is another factor that strongly influences the degradation rate of this ionophore antibiotic, when other abiotic parameters remain constant. An increase of 20% in the water content leads to an increase of 33% in monensin degradation time. The analysis of organic matter and water content of soils is of importance in assessing the potential risk of contamination by monensin.

The results described above and the different half-life values of monensin found in the literature suggest that organic matter content, water and microbial flora are the most important parameters in the degradation of monensin in the soil.

Further studies are required to fully understand the influence of these parameters and how their modification may maximise the degradation rate of monensin.

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