Fate and impact of the antibiotic ciprofloxacin in soils from integrated terrestrial microcosms submitted to pig slurry amendment

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❖ Introduction

Fluoroquinolones are a major class of antibiotics worldwide used for human and veterinary medecine. They enter the environment through the spreading of sewage sludge or manure onto agricultural soil. The effects and fate of ciprofloxacin, a main antibiotic of the class, are poorly known within the soil ecosystem.

❖ Objectives

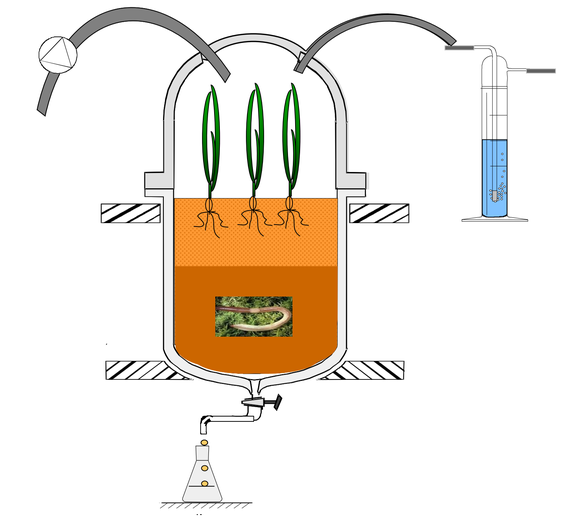
The present study was performed in integrated terrestrial microscoms including soil-dwelling organisms in order to provide new insights upon the possible ecotoxicity of ciprofloxacin onto the soil ecosystem.

❖ Materials & Methods

We used integrated microcosms filled with 2kg of a luvisol collected in the upper layer of an experimental filed in Versailles. Four experimental treatments were prepared : non-amended control soil, soil amended with pig slurry spiked with low and high amounts of ciprofloxacin, and soil spread with an acetonic solution or antibiotic. The pig slurry was first spiked with [2-^{14}C]-ciprofloxacin for studying the fate of the antibiotic, or unlabeled antibiotic to assess its effect. Only the upper layer of soil (1 kg) received the antibiotic.
The integrated microcosms were incubated for 168 days under 16h light at 20°C and 8h darkness at 18°C, to assess the fate of ciprofloxacin in soil.
-After 56 days, three **seeds of wheat were sowed** in each microcosm.
-After 70 days, **water was spread** onto the soil to mimic 20_mm rainfalls and to allow recovery of leachates.
-After 84 days of incubation, four **mature earthworms** (*Aporrectodea caliginosa* Savigny 1826 and *Apporectodea longa* Savigni 1826) **were introduced** in each microcosm.
The duration of experiments to assess the effects of ciprofloxacin on microbial communities was only 28 days.

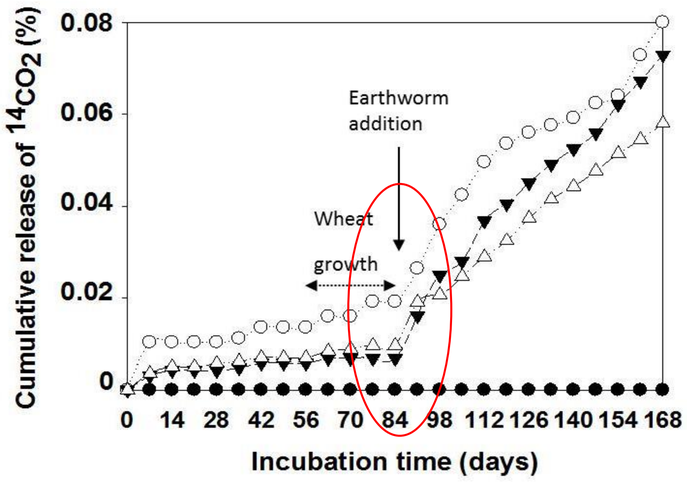
The $^{14}CO_2$ evolved from the soil because of biological activity was continuously trapped in NaOH solutions.
Soil cores were extracted to determine extractable and bound 14C in the two layers of soils at To, and after 84 and 168 days of incubation.
Wheat seedlings were harvested and dried after a 28-day period of growth.
The radioactivity was measured in all liquid fractions by liquid scintillation counting, and in solid fraction after combustion.
Enzymatic activities were determined using spectrophotometric methods.

Design of the integrated microcosms



❖ Results & Discussion

- Mineralization of [2-^{14}C]-ciprofloxacin in soils



Control soil (□), soil amended with slurry spiked with 25 (O) and 250 (▼) µg/kg dry soil of antibiotic, and soil spread with the high amount of antibiotic (Δ) during the 168 days of incubation.

► **Earthworms increase the mineralization of ^{14}C-ciprofloxacin**

- Distribution and transfer of ^{14}C in the microcosms

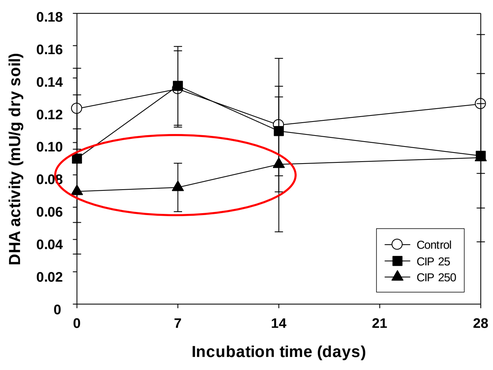
Mass-balance analysis in the compartments of our microcosms filled with soil amended with slurry spiked with the high amounts of antibiotic, or soil spread with the high amount of antibiotic

Compartment	% of initial labelled carbon applied					
	At the beginning		After 84 days		After 168 days	
	spiked	spread	spiked	spread	spiked	spread
Mineralized	0	0	0.007	0.010	0.073	0.058
Upper soil layer						
-non-extracted	78.021	77.378	79.060	85.312	51.709	47.988
- extracted	21.527	21.488	0.560	0.570	n.d.	n.d.
Lower soil layer						
-non extracted	n.m.	n.m.	8.602	13.556	38.399	43.178
- extracted	n.m.	n.m.	n.d.	n.d.	n.d.	n.d.
Leachates (70 days)	n.m.	n.m.	0.014	0.005	n.m.	n.m.
Wheat seedlings (56-84 days)	n.m.	n.m.	<0.001	0.002	n.m.	n.m.
Total labelled carbon measured	99.548	98.866	88.236	99.445	90.608	91.166

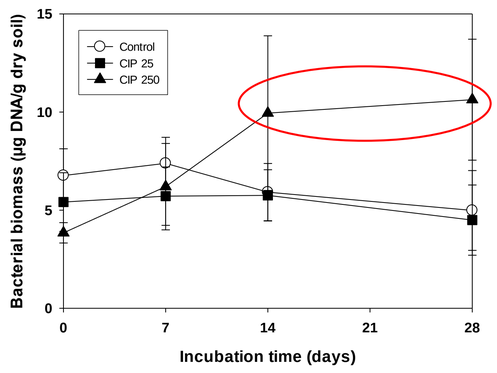
n.d. not detected; n.m. not measured

► **Earthworm bioturbation changes distribution of^{14}C-ciprofloxacin in soil layers**
Ciprofloxacin is weakly transferred to leachates and higher plants

- Effect of ciprofloxacin on soil microbial communities



Effect of ciprofloxacin on soil dehydrogenase activity. Antibiotic amounts were 25 and 250 ng/kg dry soil.



Effect of ciprofloxacin on bacterial biomass. Antibiotic amounts were 25 and 250 ng/kg dry soil.

► **Ciprofloxacin decreased dehydrogenase activity and slightly increased bacterial biomass**
Other enzymatic activities, as well as soil fungal biomass, were not affected by the antibiotic

❖ Conclusions

1.**Ciprofloxacin** introduced in the Luvisol through spiked pig slurry or direct spreading **is very weakly mineralized**, **but is mainly stabilized** as non-extractable residues

2.**It is also weakly transferred** to leachates or higher plants

3.**Earthworms modified the fate of the antibiotic**, as well as its distribution in the soil

4.**Weak effects have been noticed** on the **soil microbial communities**

References: Mougin C, Cheviron N, Repincay C, Hedde M, Hernandez-Raquet G, 2013. Environmental Chemistry Letters (on line). DOI: 10.1007/s10311-012-0385-z / Igel-Egalon A, Cheviron N, Hedde M, Hernandez-Raquet G and Mougin C, 2012. Environmental Toxicology 27, 129-136.

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