Impact of antibiotics from pig manure on soil microorganisms and earthworms

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IMPACT OF ANTIBIOTICS FROM PIG MANURE ON SOIL MICROORGANISMS

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Rationale / objectives

- Faeces of livestock, including pig manure, are contaminated by antibiotics.
- When reaching agricultural soils after manure application as amendment, these chemicals may impact the soil microorganisms.

► **Our objectives are to assess i) the impact of selected antibiotics on the enzymatic production by the basidiomycete *Trametes versicolor* in liquid cultures, ii) the impact of selected antibiotics on microorganisms in soil microcosms.**

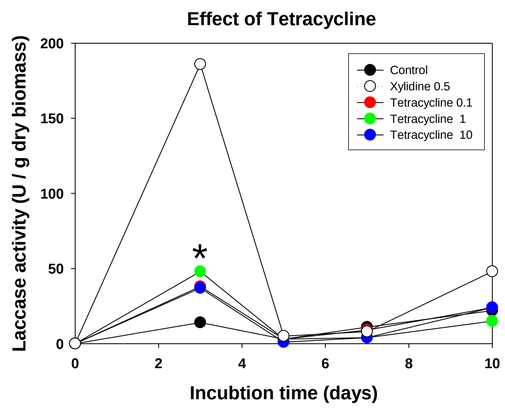
Impact of antibiotics on enzymatic activities in *T. versicolor*

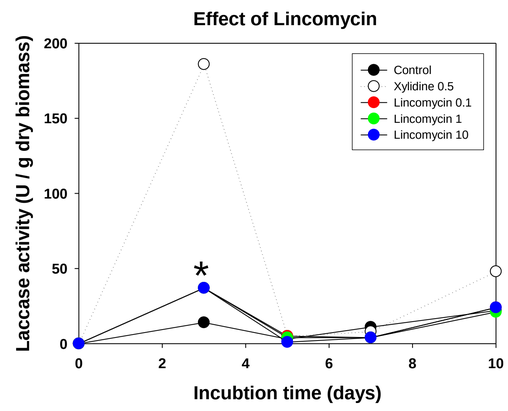
Its is necessary to understand the molecular basis governing the enzymatic secretion in order to develop biomarkers for ecotoxcity assesssment in soils. Fungal liquid cultures allowing high contaminant bioavailability appear as efficient tools for that purpose.

Tetracycline, Lincomycin, Sulfadiazine and Ciprofloxacin are antibiotics often used in farms. Their effects have been studied on the activities of extracellular oxidases (laccases, manganese-dependent peroxidases) and hydrolases (β-glucosidase, acidic phosphatase) in liquid cultures of *T. versicolor*. Each compound was provided alone at 0.1, 1 and 10 mg/L in the culture medium. For laccase activity, xylidine at 0.5 mg/L was used as a positive control. The effects were assessed during 10 days of exposure at 25°C.

►Among these activities, only these of laccases have been slightly induced, but significantly*, after 3 days of fungal exposure to Tetracycline or Lincomycin. No dose-response relationships appeared in the case of Lincomycin exposure.

►Although these activities have been previously shown to be modified after fungal exposure to various environmental contaminants (metals, PAHs, pesticides…), they do not behave as relevant biomarkers of exposure to antibiotics.





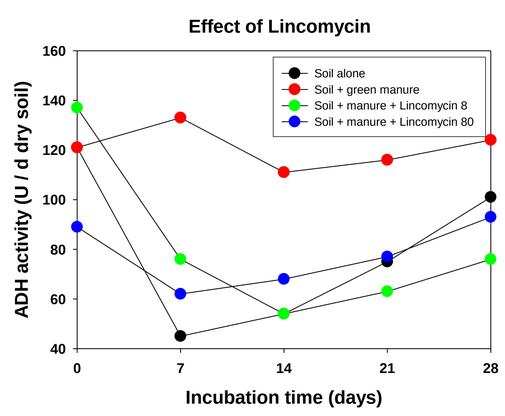
Impact of antibiotics on soil microbial communities

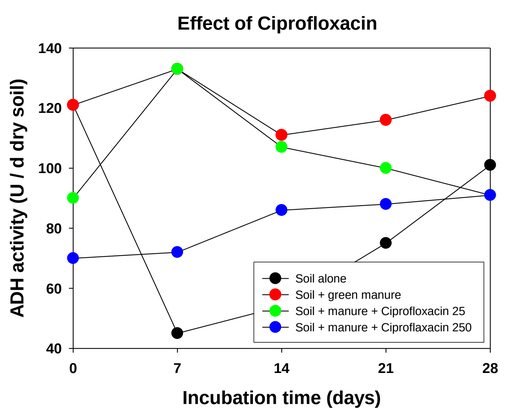
Soil microcosms provide integrated systems for the assessment of possible adverse effects of xenobiotics on microbial communities. Both functional and structural parameters are taken into account in the study.

Lincomycin and Ciprofloxacin are two antibiotics widely quantified in French pig manure samples. Their effects have been studied in soil (a silt loam) microcosms supplemented by green or spiked manure samples, each applied at 30T/ha. Final concentrations, of agronomic relevance, were 8 and 80 µg/kg dry soil for Lincomycin, 25 and 250 ng/kg dry soil for Ciprofloxacin. Modification of soil functional biodiversity was assessed by enzyme activity measurements (laccase, dehydrogenase, β-glucosidase, acidic phosphatase) and litter-bag assays (wheat straw degradation), during 28 days of exposure at 18°C.

►Among enzymatic activities, only that of dehydrogenase (ADH) was affected by manure spreading. Il was increased in the presence of green manure with respect to untreated soil.
Then, it was decreased after spreading of Lincomycin-containing manure by comparison with green manure alone. In that case, the stimulating effect of the amendment was suppressed.
The lowest amount of Ciproflaxin was without effect during the first two-weeks. By contrast, ADH activity seemed inhibited by the higher amount of antibiotics.
Laccase activity was never detected in the soil samples.

►No effects have been evidenced using the litter-bag assay.

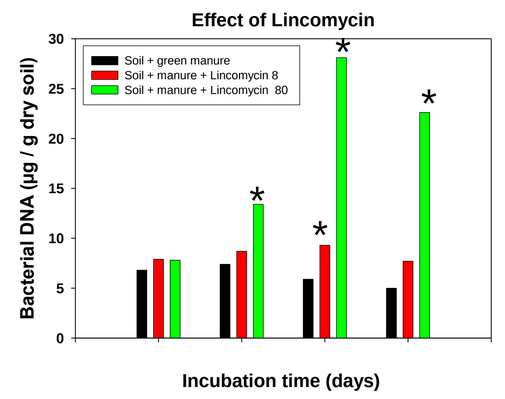


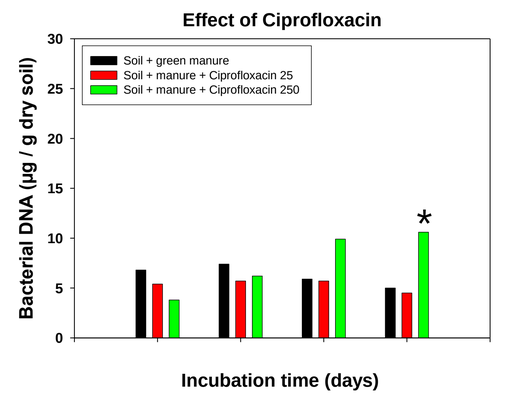


In these experiments, bacterial and fungal biomasses have been determined after DNA extraction from soil samples and Q-PCR using specific primers.

►Bacterial biomass was affected by spiked-manure spreading. Il was significantly* increased in the presence of the highest amount of Lincomycin whatever the time of exposure, and to a lesser extent in the presence of the highest amount of Ciprofloxacin, but only at day 28.

►Lincomycin and Ciprofloxacin, whatever their concentrations, did not modify fungal biomass in soil microcosms.





Conclusions

1) In fungal liquid cultures, only the laccase of *T. versicolor* was induced after exposure to Tetracycline or Lincomycin.
2) In soil microcosms, only the dehydrogenase activity was affected by the presence of Lincomicin or Ciprofloxacin in pig manure.
3) In soil microcosms, only the bacterial biomass was affected by the presence of Lincomicin or Ciprofloxacin in pig manure.
►We were not able to evidence drastic ecotoxic effects of antibiotics on soil microorganisms when the chemicals were applied with respect to realistic concentrations on an agronomic point of vue.

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