Chloramphenicol can occur in crops naturally

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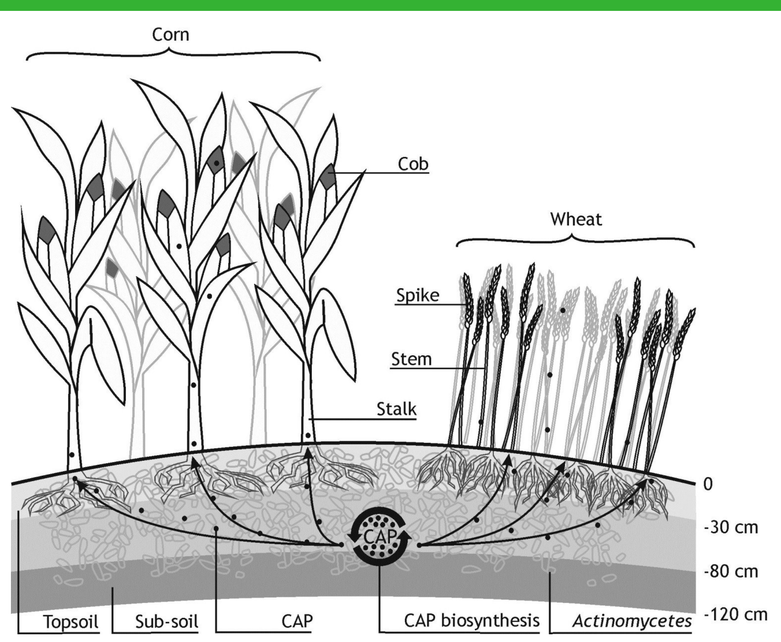
Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic that is banned for use in food-producing animals. During the last decade, findings of CAP residues in animal products have had a major impact on international trade. Recently CAP was detected in straw and animal feed. CAP is produced for commercial use by chemical synthesis but it is also biosynthesized by soil-dwelling *Actinomycetes*.

We studied the hypothesis that CAP can be transferred to arable crops after biosynthesis by *Streptomyces venezuelae* in soil. Crops are processed to animal feed and used as stall bedding and thus, if the hypothesis is accepted, this can explain unexpected non-compliant CAP findings in feed and products of animal origin.

Three conditions have to be fulfilled to confirm the posed hypothesis: **(A)** CAP should be sufficiently stable in soil to enable transfer, **(B)** soil bacteria must be able to produce CAP in soil and **(C)** CAP should be transferred from the soil to the above ground biomass.

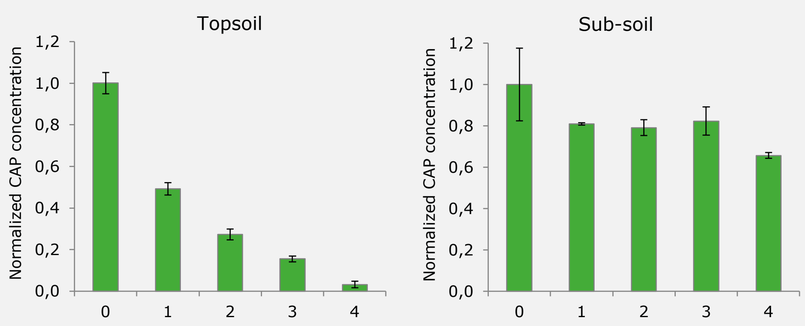


Tested hypothesis showing CAP biosyntesis in soil and the subsequent uptake by wheat and corn.

Results & discussion

A. Stability in soil

CAP was added to 2 g aliquots of topsoil and subsoil and placed at room temperature. At t = 1, 2 and 3 days two aliquots of each soil were stored at -70 °C. At day 4 the CAP concentration in all aliquots was determined using a validated LC-MS/MS method.

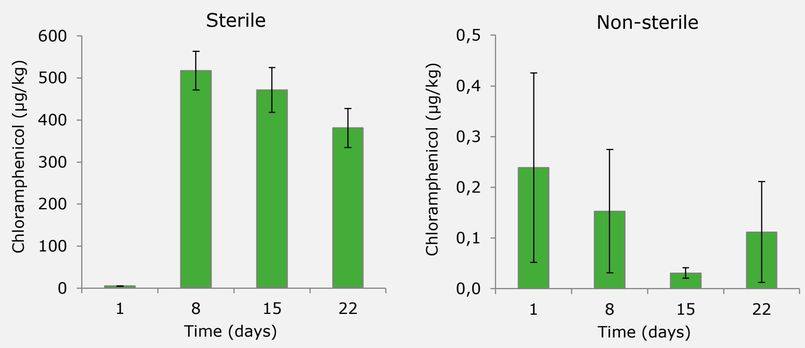


Normalized CAP concentrations in topsoil and sub-soil during incubation at room temperature.

- CAP degradation depends on soil composition; bacterial activity and organic content are the main factors.
- CAP is rapidly degraded in topsoil with a half-life of approximately one day.

B. Production in soil

Sterilized and non-sterilized topsoil were inoculated with *Streptomyces venezuelae* at $2*10^6$ CFU g^{-1} soil in duplicate and incubated at 28 °C. At day 1, 8, 15 and 22 the CAP concentration was determined using a validated LC-MS/MS method.

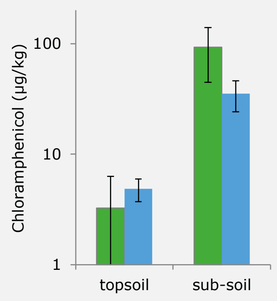


CAP concentrations in inoculated ($2*10^6$ CFU/g) sterile and non-sterile topsoil during incubation at 28 °C.

- Substantial CAP production in the sterile soil samples.
- Low CAP production in non-sterile soil: (competing) microbial flora triggers a physiological response.
- The collapse of the CAP concentration is explained by the degradation of CAP in topsoil.
- CAP is produced continuously during the experiment.

C. Transfer to crops

Wheat and corn were planted in topsoil and sub-soil. After germination and a 2 week growth phase, CAP was weekly added via 100 mL solution gifts (total of 7.5 mg CAP) via a plastic cylinder installed in the pot. After harvest the stems, spikes, stalks and cobs were analyzed separately using a validated LC-MS/MS method.



Total free CAP in (●) corn and (●) wheat harvested on topsoil and sub-soil.

Transfer experiment in the final growing stage.

- The CAP concentration in the wheat stems and the corn stalks was significantly higher than that in the wheat spikes and maize cobs.
- <0.5% of the total administered amount of CAP was recovered in the crops as free CAP.
- CAP transfer is related to the bioavailability of the drug and thus highly depends on the experimental setup.

Calculations

- For wheat, the production of in total 8 µg CAP/kg soil during the growing period is needed to yield 0.1 µg/kg CAP in wheat assuming a plant density of 200 plants/m^2 and an average wheat plant mass of 16 g.
- For corn this is 2 µg CAP/kg soil assuming a plant density of 10 plants/m^2 and an average corn plant mass of 185 g.

Conclusions

- CAP is degraded in soil but the degradation kinetics allow uptake.
- CAP can be produced by bacteria in soil in reasonable amounts.
- CAP transfer from soil to crops depends on the bioavailability of CAP.
- Low-ppb concentrations of CAP in crops can be explained by the natural production of CAP by soil bacteria and the subsequent uptake of the drug.

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