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Occurrence of Chloramphenicol in Crops through Natural Production by Bacteria in Soil

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ABSTRACT: Due to the unexpected findings of the banned antibiotic chloramphenicol in products of animal origin, feed, and straw, the hypothesis was studied that the drug is naturally present in soil, through production by soil bacteria, and subsequently can be transferred to crops. First, the stability of chloramphenicol in soil was studied. The fate of chloramphenicol highly depends on soil type and showed a half-life of approximately one day in nonsterile topsoil. It was found to be more stable in subsoil and sterile soils. Second, the production of chloramphenicol in soil was studied, and it was confirmed that *Streptomyces venezuelae* can produce chloramphenicol at appreciable amounts in nonsterile soil. Third, a transfer study was carried out using wheat and maize grown on three different soils that were weekly exposed to aqueous chloramphenicol solutions at different levels. Chloramphenicol was taken up by crops as determined by chiral liquid chromatography coupled to tandem mass spectrometric analysis, and the levels in crops were found to be bioavailability related. It was concluded that chloramphenicol residues can occur naturally in crops as a result of the production of chloramphenicol by soil bacteria in their natural environment and subsequent uptake by crops.

KEYWORDS: *chloramphenicol, natural occurrence, soil, crops, LC-MS, animal feed, residue analysis*

INTRODUCTION

Chloramphencol, **1** (Figure 1), is a broad-spectrum antibiotic that has been used in all major food-producing animals.

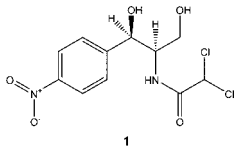

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Figure 1. Structural formula of *RR-p*-chloramphenicol.

Traditionally, chloramphenicol is produced for commercial use by chemical synthesis,[1] but it is biosynthesized by the soil organism *Streptomyces venezuelae* and several other *Actinomycetes*[2] as well. The drug has been evaluated by a number of organizations,[3−5] most recently in 2005 by the Joint Expert Committee on Food Additives at its 62nd meeting.[1] Chloramphenicol is a suspected carcinogen, and due to its linkage with the development of aplastic anemia in humans,[1] the drug is banned for use in food-producing animals in the European Union (EU)[6] and in many other countries, including the United States, Canada, Australia, Japan, and China. A minimum required performance limit (MRPL) of 0.3 μg/kg was set by the European Commission for analytical methods to be used in testing for chloramphenicol in products of animal origin.[7]

In recent years, findings of chloramphenicol residues in food products have had a major impact on international trade.[8] In 2012 noncompliant findings of chloramphenicol in the European Union were related to casings, meat products, and feed.[9] In 2010, the detection of chloramphenicol in plants and soil of mainly Mongolian origin[10] was reported. High levels of chloramphenicol were found in plant material, whereas chloramphenicol was detected in only a small number of soil samples. A first monitoring of chloramphenicol in European straw (n = 21) resulted in 57% positive samples with concentrations mainly below 1 μg/kg, but the maximum level was as high as 11 μg/kg.[11] A more extensive follow-up study (n = 104) carried out in our laboratories showed 37 positives (36%), of which seven were above 0.3 μg/kg and a single result was above 1 μg/kg: 6.8 μg/kg. All samples contained the antimicrobially active isomer of chloramphenicol,[12] and no correlation between the chloramphenicol concentration and the origin or type of straw was found.

Various hypotheses have been proposed to explain these results such as the illegal use of the drug in animal production and the production of chloramphenicol by soil bacteria naturally present in the environment.[13] Due to the variety of positive samples and the fact that recent findings of chloramphenicol in several products produced in different countries could not be explained by the use of the drug, additional investigations were urgent. A hypothesis, which to our knowledge has never been supported by solid scientific evidence, is the potential accumulation by arable crops of chloramphenicol naturally produced in soil by biosynthesis by

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Actinomycetes. If proven correct, the chloramphenicol-containing crops are processed to animal feed or used as stall bedding, and consequently animals might ingest these products, which may result in noncompliant chloramphenicol findings in products of animal origin.

Two conditions should be fulfilled to confirm the posed hypothesis: soil bacteria must be able to produce chloramphenicol in soil under natural conditions and crops should be able to accumulate chloramphenicol from the soil in the aboveground biomass. The adsorption, stability, and production rate of chloramphenicol in soil was studied long ago under laboratory conditions.[13,14] After inoculation of sterile soils by chloramphenicol-producing bacteria, chloramphenicol concentrations of up to 1.1 mg/kg were found. Upon addition of an additional carbon source, chloramphenicol production even reached 25 mg/kg after 18−31 days of incubation.[13] In nonsterile soils, however, chloramphenicol production was not detected. In that specific study, the detection limit of the applied method was 50 μg/kg, and thus low, but relevant levels of chloramphenicol that may have been present could not have been detected at that time.[15] Transfer studies confirmed the presence of detectable levels of veterinary drugs in plants, among which are tetracyclines,[16,17] trimethoprim,[18] sulphonamides,[17−19] anticoccidials,[20] and florfenicol.[21] A monitoring study, which focused on the detection of veterinary drugs in manure, groundwater, soil, and plants, confirmed the presence of low levels of chloramphenicol in plants and soil (<1 μg/kg).[22]

To verify the posed hypothesis, a series of three experiments were performed using state-of-the-art techniques. First, the stability of the antibiotic in soil was studied under sterile and nonsterile conditions. Second, the net production of chloramphenicol by *S. venezuelae* in sterile and nonsterile soil was investigated, and third the active uptake of free chloramphenicol by wheat and maize was quantitated in a controlled greenhouse experiment. Wheat and maize were selected because these are the major crops used as stall bedding and/or animal feed constituent. Results from these three experiments are combined to gain insight regarding the hypothesis that chloramphenicol contamination in crops can be explained by the natural production of chloramphenicol by soil bacteria.

MATERIAL AND METHODS

Reagents. ULC grade water and acetonitrile and HPLC grade methanol and ethyl acetate were obtained from Biosolve (Valkenswaard, The Netherlands). Ammonium formate, formic acid, acetic acid, and 25% ammonia were obtained from Merck (Darmstadt, Germany). Milli-Q water was prepared using a Milli-Q system at a resistivity of at least 18.2 MΩ/cm (Millipore, Billerica, MA, USA). The reference standard of *RR-p*-chloramphenicol (≥98% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA), and the internal standard *RR-p*-chloramphenicol-d_5 (>99% purity) was obtained from Witega (Berlin, Germany). Stock solutions were prepared in MeOH at 100 mg/L, and all dilutions were prepared in milli-Q water.

Transfer Study. *Soil Used in the Transfer Study.* The soil used in this study was a sandy soil (Gleyic Podzol, FAO) used for regular agriculture and originates from the Droevendaal experimental farm of Wageningen University. Fresh soil was collected prior to the experiment on May 10, 2012, from two depth layers, i.e., the 0−30 cm layer (topsoil) and the 80−120 cm layer (subsoil). Two hundred killigrams of both soil types was transferred to the laboratory, where it was homogenized and sieved (<2 mm using stainless steel sieve). The natural moisture content was determined by weight loss at 105 °C. To obtain a range in organic matter a third soil was created by mixing dried topsoil with an equivalent amount of subsoil. This resulted in a series of soils with similar mineralogical properties and minor differences in pH. The latter varied from 5.0 (subsoil) to 5.2 (topsoil), as determined in a 1:10 (soil:solution) extract using 0.01 M $CaCl_2$. The main variable of relevance is the soil organic matter (SOM) content, which was determined in all soils by loss on ignition (LOI) at 550 °C. The SOM content ranged from 0.8% in the subsoil, to 2.1% in the mixed soil, to 3.2% in the topsoil, the latter being representative for normal arable sandy soils in The Netherlands.

Pot Experiment to Determine Chloramphenicol Transfer. Approximately 6.5 kg of air-dried top soil, mixed soil, and subsoil was used in 8 L ceramic pots. To obtain the desired moisture content at the start of the experiment, 370 mL of distilled water was added to each pot, which is equivalent to 80% of the water-holding capacity for this soil type as determined experimentally. During the growth of the crops, the moisture content in the pot was maintained at 80% of the water-holding capacity by weight loss and correction for the total biomass present in the pot. Plants were watered daily during the growing season using normal tap water. In order to keep the growing conditions in all pots equal, a starting dose of N, P, K, and Mg fertilizer was initially mixed with the soil. In total 1500 mg of N (25% as 2 M $Ca(NO_3)_2$ and 75% as NH_4NO_3), 327 mg of P (NaH_2PO_4), 241 mg of Mg ($MgSO_4$), and 1245 mg of K (KCl) were added to 6.5 kg of soil to overcome nutrient deficiencies. During the growth of the crops, aliquots of 50 mL of a nutrient solution based on the same ratio of N, P, K, and Mg as listed here were added depending on the growing status of the plant. Two crops, maize (var. LG 30-208) and wheat (var. Lavett), three soil types, subsoil, mixed soil, and topsoil, and three chloramphenicol treatment levels, blank, low, and high, were used in the growing study, each carried out in duplicate. This resulted in a total of 36 pots. In each pot 10 maize or 20 wheat seeds were planted. To avoid any salt damage, the seeds were placed in a layer of 0.5 cm of soil that was not amended with fertilizer. After mixing the bulk soil with the required amount of fertilizer, filling the pots with soil, and installing the seeds in the top 0.5 cm layer of unamended soil, the 36 pots were transferred to the Nergena greenhouse facilities (Wageningen UR) on July 18. The temperature and moisture in the greenhouse were kept constant at 20 °C and 80%, respectively, during the growth of the crops. After germination, the number of plants in each pot was reduced to 3 for maize and 10 for wheat. Daylight was maintained for 12 h after September 15 using artificial light. The complete plants were harvested after ripening on October 2 for wheat (after 11 weeks of growth) and October 18 for maize (allowing 12 weeks of growth). Wheat stems and spikes as well as maize stalks and cobs were separated. Samples were cut using a knife and subsequently minced under cryogenic conditions to obtain homogeneous samples and to improve extraction efficiency. Also soil samples, cleared of root material, were taken.

Experimental Design of the Chloramphenicol Additions. Chloramphenicol was added to the plants after germination and an initial 2-week growth phase to avoid any chloramphenicol-induced effects in the early growing stage. After reaching a plant height of approximately 20 cm for both crops, chloramphenicol was added weekly via 100 mL solution additions containing the appropriate amount of chloramphenicol. In total, three treatment levels were performed, including a zero-treatment receiving the same volume of deionized water, a low dose (7.5 mg chloramphenicol total addition per pot), and a high dose (75 mg chloramphenicol total addition per pot). These levels were selected to ensure detection of chloramphenicol in plant tissues, even if the transfer rate was found to be low. A chloramphenicol stock solution was prepared by dissolving 112.5 mg of chloramphenicol in 500 mL of deionized water. After mechanical stirring for 30 min all chloramphenicol was dissolved and the solution transferred to brown, 2 L glass flasks. The total volume was brought to 1500 mL. One hundred milliliters of this stock solution equals an addition of 7.5 mg, which was added weekly for 10 consecutive weeks to the 12 pots of the high-dose treatment. From this stock solution 150 mL was diluted 10 times to a total volume of 1500 mL, which served as the low treatment dose. Again, 10 additions of this solution were added to the low-dose treatment pots during the growing phase of the plants. To ensure that all chloramphenicol accumulated by the

plants occurred through root action and to avoid direct contact between chloramphenicol in the water and the above-ground plant material, the chloramphenicol-containing solutions as well as the deionized water (zero-treatment) were added to the pots via a vertical plastic cylinder with a diameter of 4 cm and a total height of 10 cm that was installed in each pot. This cylinder was buried in the soil to a depth of 3 cm, and the solutions could seep into the soil via small holes below the soil surface. To avoid retention or degradation of chloramphenicol, the cylinder was filled with inert quartz sand with a high infiltration rate. After addition of 100 mL of the treatment solutions, 100 mL of deionized water was subsequently added to the cylinder to force the chloramphenicol into the soil and to minimize potential loss of chloramphenicol retained in the sand layer.

Stability Study. Two-gram aliquots of the dried soil material from the topsoil and subsoil were transferred into individual polypropylene test tubes (n = 24), and 0.5 mL of milli-Q water was added. Of both soil types, half of the soil-containing test tubes were sterilized at 121 °C for 15 min during 2 consecutive days, the other tubes were stored at room temperature. At t = 0, an aqueous solution (125 μL) of chloramphenicol was added to two aliquots of each soil type, resulting in a nominal concentration of 50 μg/kg. The spiked samples were shaken for 10 s using a vortex mixer and placed at room temperature exposed to daylight. At t = 1, 2, 3, and 4 days this spiking procedure was repeated, and at day 4, 5 μg/kg of internal standard (*RR-p*-chloramphenicol-d_5) was added to all samples as an analytical reference, including the last set of blank samples, which were then analyzed at random by chiral liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Chloramphenicol Production in Soil. To determine the natural production rate of chloramphenicol in soil by bacteria, eight 100 g batches of dried topsoil were transferred into 250 mL glass containers, which were covered with aluminum foil. Half of the containers were sterilized at 121 °C for 15 min during 2 consecutive days. *S. venezuelae* (DSM40230, Leibniz Institute DSMZ, Braunschweig, Germany) was cultured in GYM (4 g/L glucose, 4 g/L yeast extract, 10 g/L malt extract) for 2 days at 28 °C (200 rpm) until an optical density of 600 (OD_{600}) of ~2.0 was reached. The culture (650 mL) was harvested by centrifugation (10 min at 10000g) and washed three times with 100 mL of PBS (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, pH 7.4) to remove any chloramphenicol that might have been produced during culturing. After the last washing step, the pellet was taken up in 65 mL of PBS and stored at 4 °C until further use. Enumeration of this inoculant yielded 2.0×10^9 colony forming units (cfu) per mL. The inoculant was added to the sterile and nonsterile soil (in duplicate) at two concentrations: 2×10^6 and 2×10^8 cfu/g soil. The moisture concentration was adjusted to 20% for all containers. The beakers were covered with Parafilm and placed into a humidity chamber at 28 °C. After 1, 8, 15, and 22 days the soil samples were homogenized by stirring with a wooden rod and 2.5 g aliquots were taken in duplicate. The aliquots were stored at <−70 °C until analysis.

Sample Preparation for Chloramphenicol Analysis. The samples were prepared according to a previously reported and validated method[23] with minor adjustments, making it suitable for plant material and soil. An additional validation was carried out for plant materials to ensure good method performance. Of the cryogenically minced crop samples, 2.5 g was extracted using 20 mL of acetonitrile (ultrasonic assisted). After centrifugation (3500g, 15 min) the organic phase was isolated, evaporated until dry (45 °C, N_2), and reconstituted in 5 mL of water. A Strata-X 200 mg/6 mL solid phase extraction (SPE) cartridge (Phenomenex, Torrance, CA, USA) was conditioned with 5 mL of methanol and 5 mL of water. The sample extract was applied onto the cartridge, and subsequently the cartridge was washed with 6 mL of 40% methanol in water containing 1% acetic acid followed by 6 mL of 40% methanol in water containing 0.25% ammonia. The cartridges were dried by applying vacuum for 3 min, and chloramphenicol was eluted from the cartridge using 3 mL of 80% methanol in water. The methanol in the eluent was evaporated (45 °C, N_2). Two milliliters of ethyl acetate was added to the remaining aqueous extract, which was shaken for 1 min using a rotary tumbler. After centrifugation (3500g, 5 min) the ethyl acetate layer was isolated and evaporated (40 °C, N_2) until dry. The residue was redissolved in 500 μL of water and transferred into an LC-MS/MS sample vial. Soil samples were analyzed using the same method, but then the samples were extracted with 10 mL of water, as was proven sufficient from previous experiments.

LC-MS/MS Analysis. Samples of wheat stems and spikes, maize stalks and cobs, and soil were analyzed separately. Quantitation was carried out by preparing a calibration line in blank material, previously found to be free of chloramphenicol, ranging from 0 to 200 μg/kg. Internal standard (*RR-p*-chloramphenicol-d_5) at 10 μg/kg was added to all individual samples before extraction. All final extracts were injected as such and after 50-fold dilution in water to obtain a response within the calibration range.

The LC system consisted of a vacuum degasser, autosampler, and a Waters (Milford, MA, USA) model Acquity binary pump equipped with a Chromtech (Apple Valley, MN, USA) Chiral AGP (α1-acid glycoprotein) analytical column of 2.0 × 150 mm, 5 μm, placed in a column oven at 30 °C.[23] Isocratic elution was performed using a mobile phase consisting of 2% acetonitrile in 10 mM ammonium formate buffer adjusted to pH 4.0 with formic acid at a flow rate of 0.4 mL/min. The injection volume was 10 μL. Detection was carried out using a Waters model Xevo TQS triple quadrupole mass spectrometer in the negative electrospray ionization (ESI) mode. The operating parameters were as follows: capillary voltage, −1.5 kV; cone voltage, 20 V; source offset, 50 V; source temperature, 150 °C; desolvation temperature, 550 °C; cone gas flow, 150 L/h; and desolvation gas, 750 L/h. Chloramphenicol and chloramphenicol-d_5 were fragmented using collision-induced dissociation (CID). The selected reaction monitoring (SRM) transitions are 321.0 > 152.0 (17 eV), 321.0 > 194.0 (10 eV), and 321.0 > 257.0 (eV) for chloramphenicol and 326.0 > 199.0 (10 eV) for chloramphenicol-d_5. Using this method, the detection limit was <0.05 μg/kg for all matrixes.

RESULTS AND DISCUSSION

Stability in Soil. The results of the stability study are presented in Figure 2. Chloramphenicol is rapidly degraded in nonsterile topsoil with a half-life of approximately 1 day. The rapid degradation is in line with results reported earlier[24,25] showing degradation kinetics depending on the soil composition. In sterilized topsoil, also a decrease of chloramphenicol concentrations was observed but at a significantly lower rate. At least part of the degradation of chloramphenicol can therefore be attributed to bacterial activity.[13] Subsoil is expected to contain a much lower bacterial load, and indeed the reduction of chloramphenicol concentrations in nonsterile subsoil is less.

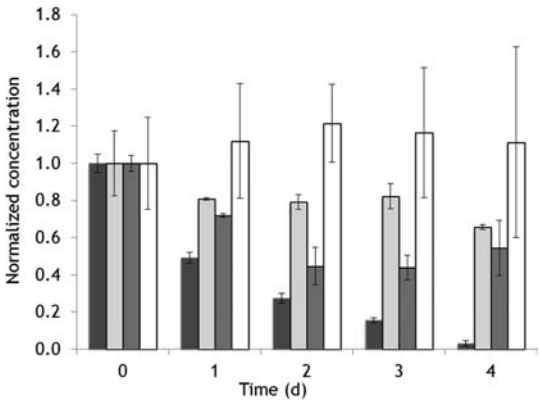

Figure 2. Normalized chloramphenicol concentration during incubation of chloramphenicol in (black) topsoil, (light gray) subsoil, (dark gray) sterilized topsoil, and (white) sterilized subsoil. Error bars represent the standard deviation (n = 2).

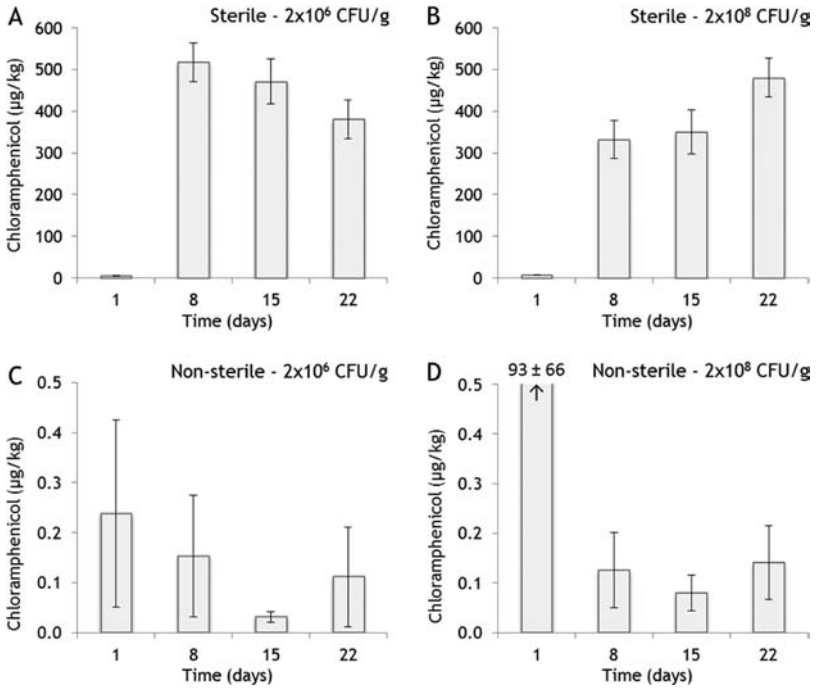

Figure 3. Average chloramphenicol concentration including standard deviation (error bars) in soil after incubation at 28 °C of (A) sterilized topsoil containing 2×10^6 cfu/g, (B) sterilized topsoil containing 2×10^8 cfu/g, (C) nonsterile topsoil containing 2×10^6 cfu/g, and (D) nonsterile topsoil containing 2×10^8 cfu/g, $n = 4$ (2 experiments analyzed in duplicate).

For sterilized subsoil no degradation of chloramphenicol seems to have occurred, but high standard deviations in the experiment with sterilized subsoil prevent accurate comparison with the nonsterilized samples.

The instability of chloramphenicol in nonsterile soil explains the low number of positive chloramphenicol findings in soil samples.[10] Note that when chloramphenicol is produced in soil, especially in close proximity to the plant roots, it may very well be available for uptake by crops. Note that the processes occurring at the root−soil interface are complex and that the underlying transfer process is unknown. This probably explains the relatively high number of positive plant samples compared to the number of positive soil samples as previously reported.[10]

Chloramphenicol Production in Soil. Both the inoculant used for this experiment and the topsoil were found not to contain any chloramphenicol. The results of the chloramphenicol production in sterile and nonsterile soil inoculated with *S. venezuelae* are presented in Figure 3. Substantial chloramphenicol production is observed in the sterile soil samples. The concentration level of approximately 500 µg/kg that is reached during the first week is sustained throughout the incubation period and remarkably appears to be independent of the size of the inoculant. In nonsterile soil, inoculation with *S. venezuelae* yielded significantly lower chloramphenicol levels. The samples spiked with 2.0×10^8 inoculant showed a remarkable peak concentration at day 1 (47 and 140 µg/kg for the individual containers), suggesting that the presence of (competing) microbial flora triggers a specific physiological response. The subsequent collapse of the chloramphenicol concentration is in agreement with results for nonsterilized soil in the stability experiment. Furthermore, if chloramphenicol was produced only in the beginning of the production experiment, no chloramphenicol would be detectable after several days. Because also at 22 days of incubation chloramphenicol is detected, it is concluded that chloramphenicol is produced continuously during the experiment.

From the stability and production experiments it is concluded that chloramphenicol can be produced in sterile as well as in natural soils and that biosynthesis and biodegradation occur simultaneously. Chloramphenicol production and *S. venezuelae* growth rate are strongly related[26] and depend on many environmental factors. For instance, *S. venezuelae* growth at pH 6.0 in MYM (maltose, yeast extract, malt extract) broth is optimal at 28−32 °C and is slightly lower at 22 °C. At pH 7.5, the bacterial growth rate is reported to be optimal at 22 °C (lowest temperature tested).[27] It is concluded that chloramphenicol can also be produced in the soil at lower temperatures, which are common in deeper soil layers. Furthermore, soil organic matter is of importance, showing increased chloramphenicol production after addition of carbon and nitrogen sources, e.g., in fertilized soils.[13,28,29] Because of the simultaneously occurring processes and many environmental variables, the production experiment is considered to be proof of principle that chloramphenicol can be produced in natural soils rather than a precise quantitation of the chloramphenicol levels that can be produced in soil. Nevertheless, we showed that over 100 µg/kg chloramphenicol can be produced in nonsterile topsoil within a single day at high *S. venezuelae* inoculation levels. Note that the experimental setup was limited to detect free chloramphenicol and that the amounts of total chloramphenicol, e.g., present as conjugate or other metabolite, might be higher.

Transfer Study. The transfer study was designed to study the influence of the soil, crop type (wheat versus maize), and administered chloramphenicol concentration. Chloramphenicol solution was administered on a weekly basis, so chloramphenicol was bioavailable during all stages of the growth. The determined average chloramphenicol concentrations ($n = 4$, 2 samples, both analyzed in duplicate) in the wheat stems and

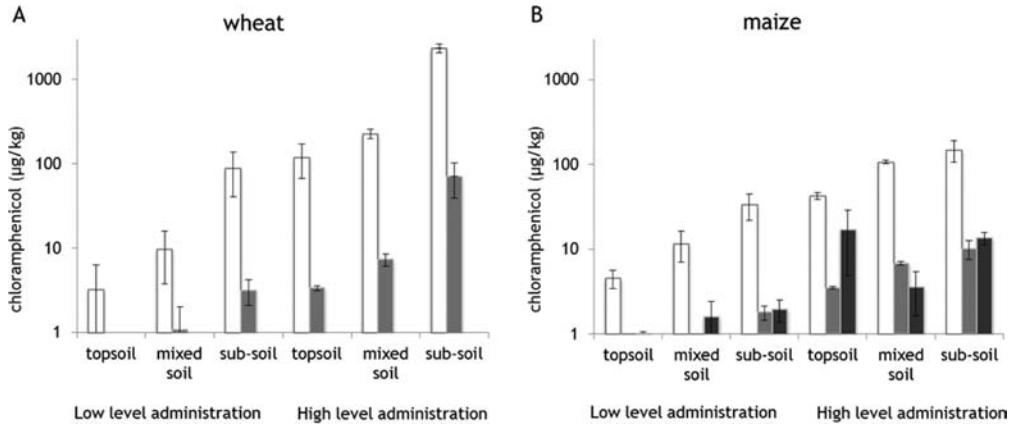

Figure 4. Average free chloramphenicol concentration including standard deviation (error bars) determined in the transfer study for (A) wheat: (white) stems, (gray) spikes, and (black) soil, and (B) maize: (white) stalks, (gray) cobs, and (black) soil at low and high level administration (2 samples, both analyzed in duplicate), $n = 4$ (2 samples, both analyzed in duplicate). No chloramphenicol was detected in the soils used for wheat cultivation.

spikes, maize stalks and cobs, and soil are presented in Figure 4. Surprisingly, only low levels of chloramphenicol were detected in the soil-grown wheat, whereas no chloramphenicol was detected in the soil-grown maize. This is explained only by a high degradation of chloramphenicol in soil. The difference between maize and wheat might have been caused by the different storage time at −20 °C (maize soil was stored four weeks longer than wheat soil), but also unknown effects caused by the root systems might have occurred.

The fate of chloramphenicol in the system was assessed by adding up the absolute amounts of free chloramphenicol detected per pot (sum of soil and plant). This then was compared to the total amount of chloramphenicol administered over 10 weeks (7.5 mg/pot at the low level and 75 mg/pot at the high level). Thus calculated, the total amount of free chloramphenicol recovered was <0.5%, indicating that over 99% of free chloramphenicol disappeared during the experiment. The major cause of chloramphenicol loss is most likely the microbial degradation in soil. Also chloramphenicol conjugation, metabolism, or degradation in the crops may occur, which may account for another part of the chloramphenicol loss.

The free chloramphenicol concentration in the wheat stems and the maize stalks is significantly higher than that in the wheat spikes or maize cobs. The average difference is approximately a factor 30 for wheat and 15 for maize, which suggests a relatively higher transfer into maize cobs compared to wheat spikes. The data were studied in more detail using analysis of variance (ANOVA), in which the total amount of administered chloramphenicol and the soil type were considered as the factors under investigation. Although severe biovariability is observed (variation between pots was significantly higher than variation between duplicate analyses of the same pot), for all plant materials, the effect of the administered chloramphenicol level, the soil type, and the interaction of both were statistically significant ($\alpha < 0.001$). The administered chloramphenicol concentration and the soil type are both related to the theoretical bioavailability of the antibiotic, and thus, it is most likely that the observed effects are related to a single parameter of importance, being the bioavailability of the antibiotic. The effects were more significant for wheat spikes and stems compared to maize stalks and cobs. As an example, the difference in chloramphenicol concentration in wheat grown on topsoil and subsoil is a factor 20, whereas this is a factor 4 for maize.

For all experiments, the absolute amount of free chloramphenicol in the plants (total of stems and spikes for wheat, and stalks and cobs for maize) was divided by the total administered amount of chloramphenicol. The results for each experiment are presented in Table 1. Note that the calculated transfer rates strongly depend on the experimental setup; for example, if chloramphenicol is administered continuously instead of weekly, the bioavailability of chloramphenicol would increase and a higher uptake is expected. Furthermore, only free chloramphenicol is taken into account, so if chloramphenicol is metabolized or conjugated in the crops, the actual transfer of chloramphenicol is higher than calculated here.

Table 1. Detectable Amount of Free Chloramphenicol in Wheat and Maize Grown on Topsoil, Mixed Soil, and Subsoil versus Administered Chloramphenicol

		detected chloramphenicol vs administered chloramphenicol (%)			
		wheat ($n = 2$)		maize ($n = 2$)	
soil type	chloramphenicol administration (mg/pot)	pot 1	pot 2	pot 1	pot 2
topsoil	7.5	0.007	0.001	0.02	0.02
	75	0.02	0.009	0.02	0.02
mixed soil	7.5	0.02	0.006	0.04	0.07
	75	0.006	0.07	0.04	0.05
subsoil	7.5	0.17	0.06	0.19	0.10
	75	0.19	0.10	0.05	0.07

A final observation from the transfer study is that chloramphenicol was detected in two of the wheat samples (up to 7 µg/kg) and two of the maize samples (up to 2 µg/kg) belonging to the untreated population. External contamination in the greenhouse was excluded by the setup of the experiment, and contamination in the laboratory was excluded by repeated analyses on another occasion; thus, it was concluded that this most probably must have been the result of natural production of chloramphenicol in the pots.

Can Chloramphenicol Production in Soil Yield Residues in Crops? The production and degradation of chloramphenicol in soil occur simultaneously, and both processes depend on many environmental parameters. Therefore, it is not possible to determine the total amount of free chloramphenicol produced in nonsterile soil that is available for uptake by the crops. Consequently it is also not possible to calculate what level of free chloramphenicol in crops can still be explained through natural chloramphenicol production by soil bacteria and subsequent uptake by crops. On the basis of the experimentally determined uptake rates (Table 1) however, calculations were made to obtain an estimate of the required magnitude of chloramphenicol production in soil to explain the observed noncompliant findings in crops. Here we calculate what level of chloramphenicol should be produced by soil bacteria to result in detection of 0.1 μg/kg free chloramphenicol in crops.

The average mass of a single full-grown wheat plant as determined in the transfer study is 16 g (fresh weight). Therefore, 0.1 μg/kg equals 1.6 ng of chloramphenicol per wheat plant. Considering an average transfer rate for a wheat plant growing on topsoil of 0.009% (average transfer rate using topsoil) (Table 1), a single plant should be exposed to 17 μg of chloramphenicol during its lifetime. A regular field contains 200 wheat plants per square meter, and thus in this square meter 3.4 mg of chloramphenicol should be produced to yield a level of 0.1 μg/kg chloramphenicol in all of these wheat plants. Considering the availability of nutrients in a 30 cm layer of soil, having a density of 1.4 kg/L, these crops have 420 kg of soil available, and therefore chloramphenicol production should be 8 μg/kg soil in total during the growing period of the crops to yield 0.1 μg/kg chloramphenicol in wheat. For maize a level of 2 μg/kg in the topsoil is required assuming a plant density of 10 maize plants per square meter and an average maize plant mass of 185 g (fresh weight). Chloramphenicol levels as high as 10 μg/kg in wheat, being the highest concentration detected, can be explained if in total 800 μg of chloramphenicol is produced per kg of soil when considering topsoil. Note that these levels do not indicate the level that should be present in soil at a certain time, but rather the total amount of chloramphenicol that should be produced per kg of soil during the whole crop production time of approximately 10 weeks.

The results from the chloramphenicol production experiment described here showed that over 100 μg/kg chloramphenicol can be produced by *S. venezuelae* in nonsterile topsoil within a single day. This is a level that significantly exceeds the chloramphenicol concentration calculated that could result in the detection of residues in crops. This suggests that the low-ppb concentrations of chloramphenicol and possibly also the high concentrations observed in crops in monitoring studies can be explained by the natural production of chloramphenicol by soil bacteria and the subsequent uptake of the drug by crops.

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Notes

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