Detection of veterinary antibiotics in water and soil using spectral approaches

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Introduction

There is currently a serious global environmental threat posed by the uncontrolled spread of antibiotics in wastewater and natural settings. The main concerns are the accumulation of drug resistance genes in bacterial populations and the disruption of stable ecosystem functioning across all trophic levels. Given the widespread use of pharmaceuticals in agriculture, veterinary medicine, and aquaculture - not just for treatment and prevention but also as growth stimulants - the development of rapid detection methods for antibiotics in the environment is highly relevant

Materials and methods

Ciprofloxacin (ready-to-use 2 mg/mL solution) was obtained from Mosfarm (Russia). Tylosin (200 mg/mL solution) was acquired from Nita-farm (Russia). Tetracycline hydrochloride was purchased from Sigma-Aldrich.

UV-Vis absorption spectra were measured in quartz cuvettes with a 1 cm path length using a Solar PB2201 spectrophotometer over the wavelength range of 200–500 nm, and fluorescence spectra were registered using a Solar CM 2203 luminescence spectrometer. Emission spectra were corrected for the inner-filter effect using absorbance values at excitation and emission wavelengths.

Result

Absorption spectra

All three antibiotics demonstrated absorption bands in the UV region, which were separated by mathematical processing from the broad background of absorption of natural organic matter of water, soil or organic fertilizers, monotonically decreasing with rising wavelength. The concentration of the antibiotic was determined by the area of the absorption band or optical density at a fixed wavelength using calibration dependencies. In this way, it is possible to determine concentrations of tylosin up to 100 mg/l, and for tetracycline and ciprofloxacin up to 60 mg/l. At higher concentrations it is necessary to dilute the samples or use the absorption at longer wavelengths.

The minimum concentrations of antibiotic that can be detected using spectrophotometry are determined by the instrumental error of the spectrophotometer. For a cuvette with an optical path length of 1 cm, we estimate the lower limit of concentration measurement as 1 mg/L for tylosin and 2 mg/L for tetracycline and ciprofloxacin in water.

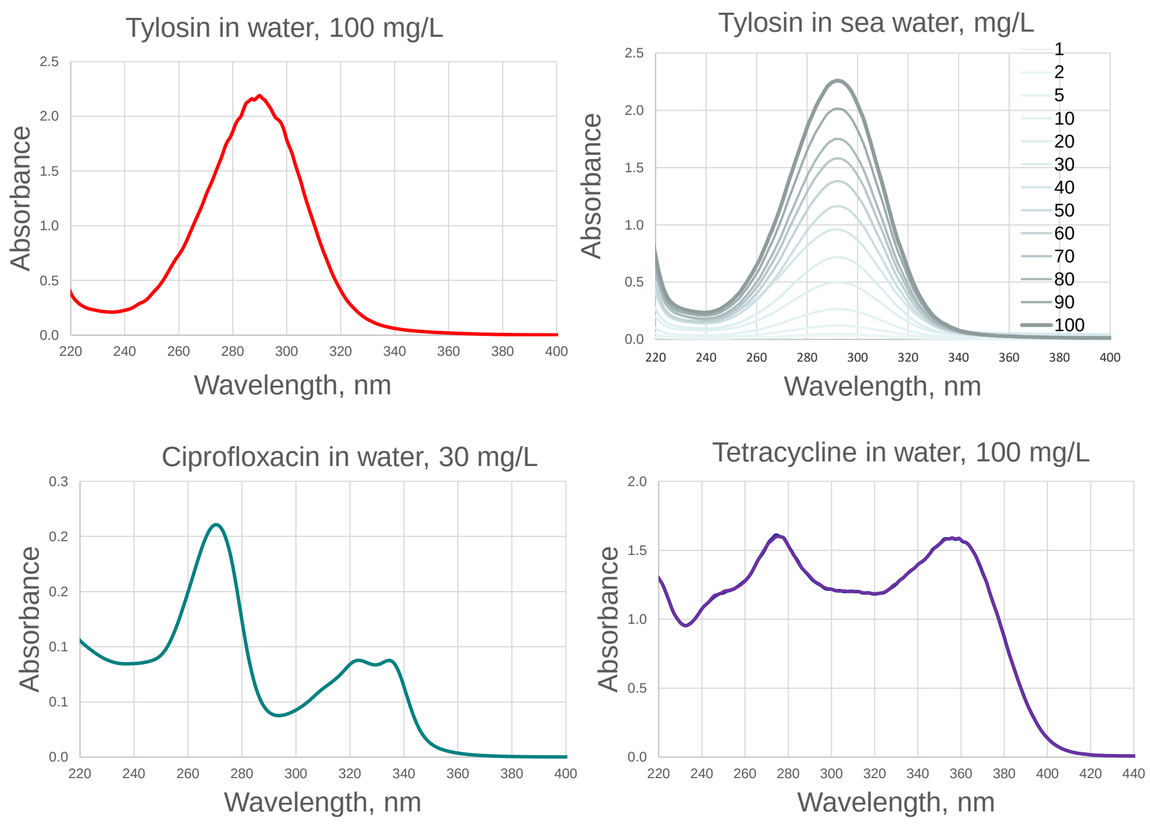


Figure 1. Absorption spectra of solutions of tylosin in distilled water seawater, ciprofloxacin and tetracycline in distilled water.

Objective and tasks

The work studied the spectral characteristics of three veterinary antibiotics (tylosin, tetracycline, ciprofloxacin) of various concentrations in the presence of organic substances of natural origin or by-products of animal husbandry (dissolved organic matter of natural water, aqueous extracts of soils and organic fertilizers).

Antibiotics and organic matter of the environment

However, when determining antibiotic concentration via spectrophotometry in natural waters, soils, or in the presence of organic fertilizer, the light absorption by dissolved organic matter (DOM) must be accounted for. It is known that the absorption spectrum of natural humic substances (the chromophoric fraction of DOM) decreases monotonically with increasing wavelength. Occasionally, a slight local maximum or inflection around 260-270 nm may be visible in the UV absorption spectra of natural DOM, which is attributed to phenolic or protein compounds. Nevertheless, natural humic substances lack distinct, well-defined absorption bands in the UV range.

A similar pattern was observed in the aqueous extract of an organic fertilizer based on manure. The absorption bands of antibiotics, when introduced into the manure filtrate, are less intense than the absorption from the organic matter of the fertilizer itself.

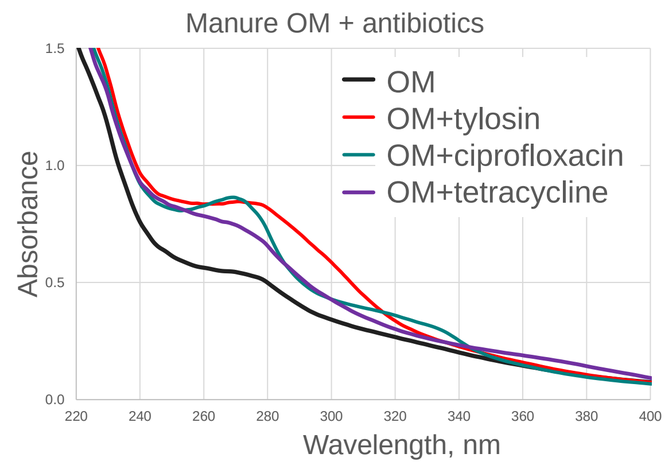


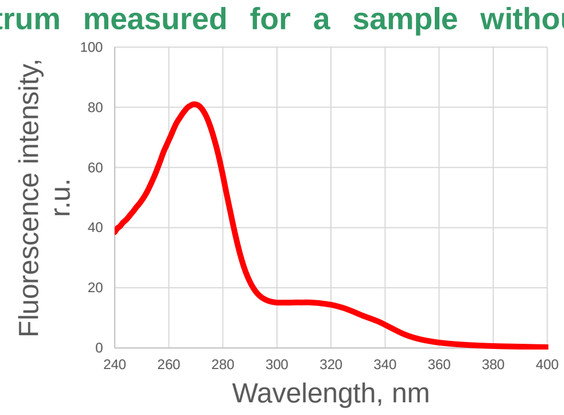
Figure 2. Absorption spectra of OM from manure-based organic fertilizer in water with added antibiotics.

To measure the concentration of antibiotics, the areas under the UV absorption bands of antibiotics were taken. The dependences of the absorption band area on the concentration for the corresponding preparation in water were used as a calibration dependence. This method allows measuring the concentrations of antibiotics in natural water, soil extracts or organic fertilizer solutions up to approximately antibiotic concentrations of 5-10 mg/L. For lower concentrations of antibiotics, more sensitive fluorescent methods should be used.

Fluorescence spectra

Fluorescence spectroscopy was used to monitor concentrations of ciprofloxacin below 5 mg/L. The emission band of ciprofloxacin partially overlaps with the fluorescence of organic matter in water and soil, so to isolate the contribution of this antibiotic to the spectra, synchronous fluorescence spectra were measured with a constant difference in excitation and recording wavelength. In this case, the antibiotic concentration was determined by the area of the synchronous fluorescence spectrum band in the UV range 270-370 nm after subtracting a similar spectrum measured for a sample without antibiotic.

Figure 4. Synchronous fluorescence spectrum of ciprofloxacin solution of 5 mg/L concentration, the offset between the excitation and recording wavelength is 160 nm.



Tylosin in aqueous solutions did not exhibit fluorescence emission, and the fluorescence band of tetracycline overlaps with the fluorescence bands of the components of soil organic matter or organic fertilizer. That makes difficult their fluorescent diagnostics.

Conclusions

Research demonstrates that the veterinary antibiotics tylosin, tetracycline, and ciprofloxacin can be quantified using absorption spectroscopy in various aqueous environments, even those containing dissolved organic matter from soil or fertilizers. However, while fluorescence spectroscopy offers greater sensitivity, its application is limited: tylosin is non-fluorescent in aqueous solution, and tetracycline's luminescence band overlaps with the fluorescence of organic matter components.

For ciprofloxacin, a method was successfully developed to determine low concentrations (below 5 mg/L) in organically-rich aqueous media. This was achieved by measuring synchronous fluorescence spectra with a constant wavelength offset between excitation and emission. The ability to measure antibiotic concentrations directly in complex matrices like fertilizer extracts or soil solutions represents a promising methodological advancement for improving the standardization and regulation of these non-traditional environmental toxicants.

Acknowledgments

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