Residual and migration characteristics of norfloxacin in two mangrove ecosystems

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Abstract. Direct discharge of aquaculture wastewater in coastal areas could increase concentrations of antibiotics in coastal mangrove forests. This study focused on the Gaoqiao Mangrove Nature Reserve in Zhanjiang City, Guangdong Province. Norfloxacin (NOR) residues in rhizosphere sediments, plant roots, branches, and leaves of two dominant mangrove communities, Rhizophora stylosa and Avicennia marina, the correlation between physical properties of rhizosphere deposition and residual NOR in sediments, and NOR accumulation in the root system were analyzed. Significant differences were noted in NOR residues in R. stylosa and A. marina, with higher NOR concentrations than those in other wetland sediments locally and abroad. NOR accumulation in R. stylosa was higher in the branches than in the roots and was also significantly higher than that in A. marina. Thus, both species could accumulate NOR from the environment with R. stylosa showing a stronger potential to purify the environment. Cation exchange capacity and total organic carbon could affect NOR distribution in the rhizosphere sediment, and total organic carbon content could reduce NOR uptake by mangrove roots. This study contributes to research on the migration and adsorption characteristics of antibiotics in mangrove wetlands.

1. Introduction
Fluoroquinolones (FQs) are a class of synthetic broad-spectrum antimicrobial agents with good inhibitory and killing effects against prawn pathogens and are one of the most commonly used antibiotics in shrimp farms¹. Because of the low metabolic rate (< 25%) of prawns, more than 70% of FQs are not metabolized and are directly introduced into the environment through animal excreta or aquatic feed. In typical coastal aquaculture zones in Thailand, Vietnam, and China, FQs were found in both water and sediment²–⁴, including norfloxacin (NOR), enrofloxacin, and the residual levels were in g·kg⁻¹–mg·kg⁻¹. Environmental antibiotics often remain in the system for several months⁵. They may not only affect human health through the food chain but also induce resistance in microorganisms in the environment, which may pose a potential threat to the ecosystem⁶.

There is evidence that mangroves near important breeding areas have been contaminated by FQs⁷. They are considered “emerging contaminants” of the environment, and the induced resistance genes may ultimately affect human food safety through the food chain. However, there are relatively few studies regarding FQs, and studies are more focused on laboratory simulations⁸. Therefore, the study of FQs in mangrove ecosystems is necessary.

Sampling was conducted in Gaoqiao mangrove forest, a core area of the Zhanjiang Mangrove
National Nature Reserve in Guangdong Province, and the residues of NOR in rhizosphere sediments and plant organs such as roots, twigs, and leaves of two dominant mangrove communities (Rhizophora stylosa, Rs and Avicennia marina, Am) were analyzed. The correlation analysis of NOR content in mangrove rhizosphere sediments and roots and physico-chemical properties of rhizosphere sediments was conducted. This study has theoretical significance for understanding the migration, adsorption, and purification effect of organic pollutants (including antibiotics) by mangrove plants and can provide a scientific reference for the restoration of coastal mangroves and reduction in the ecological risk of environmental pollution.

2. Materials and methods

2.1. Study areas and samples

The Gaoqiao mangrove forest is the key core area of the Zhanjiang Mangrove National Nature Reserve. Samples of rhizosphere sediment, roots, twigs, and leaves were collected from two dominant mangrove communities (Rs and Am) in December 2017. There were seven sampling points in each community, and their distribution is shown in Figure 1 (Rs1–Rs7, Am1–Am7).

![Figure 1. Sampling sites in Gaoqiao mangrove area (Rs1–Rs7 represent Rhizophora stylosa, Am1–Am7 represent Avicennia marina)](image)

2.2. Chemical analysis of sediments

Cation exchange capacity (CEC) was measured using the ammonium acetate exchange method. Total organic carbon (TOC) was determined by the potassium dichromate volumetric method.

2.3. Analysis of NOR in sediment and plant samples

2.3.1. Sample precondition. 1 g of sediment was ground and sieved through an 80-mesh sieve. Five milliliters of acid-acetonitrile and 5 mL Na2EDTA-McIlvaine buffer (pH 4) was added to each sediment sample. The samples were vortex-mixed for 30 s, placed in an ultrasonic bath for 10 min and then centrifuged for 15 min (4500 rpm, 4 °C). The extraction procedure was repeated three times. The combined supernatant was filtered through a nylon syringe filter (0.45 μm) and diluted to 400 mL with Milli-Q water. The extraction was performed by solid phase extraction (SPE) using a SAX-HLB cartridge and concentrated under a gentle nitrogen stream. The final extract was redissolved to 1.0 mL with the mobile phase and filtered through a nylon syringe filter (0.22 μm).
The extraction procedure of the plant samples were the same as that followed for the sediment. The sediment and plant extracts were analyzed by high-performance liquid chromatography (HPLC).

2.3.2. Quality control. The external standard method was used with the addition of 50 μg·kg\(^{-1}\) and 100 μg·kg\(^{-1}\) of standard. The recoveries of the two levels were 70.1% and 75.4%, and relative standard deviations (RSD) were 6.2% and 5.3%, respectively. The result was satisfactory.

2.3.3. Quantity analysis. The wavelength of excitation for fluorescence detection was 280 nm and that of emission was 452 nm. Using the NOR concentrations of the standard as the abscissa and the peak area as the ordinate, the calibration curve was calculated as \( y = 0.107x - 5.142 \) (\( r = 0.996 \)). The limit of detection was 7.0 μg·kg\(^{-1}\).

2.4. Transfer factor and bioconcentration factor formulae

Bioconcentration factors (BCFs) can measure the ability of pollutant transfer in plants\(^9\). In this study, RCF, TCF, and LCF were used to represent the ability of NOR in rhizosphere sediments to transfer into the roots, branches, and leaves of mangrove plants, respectively. The calculation formulae are as follows:

\[
\begin{align*}
\text{RCF} &= \frac{C_r}{C_s} \quad (1), \\
\text{TCF} &= \frac{C_t}{C_s} \quad (2), \\
\text{LCF} &= \frac{C_l}{C_s} \quad (3),
\end{align*}
\]

where \(C_r\), \(C_t\), and \(C_l\) are the contents of NOR (g·kg\(^{-1}\)) in mangrove roots, branches, and leaves, respectively, and \(C_s\) is the concentration of NOR (g·kg\(^{-1}\)) in mangrove rhizosphere sediments.

The transfer factor (TF) can measure the ability of NOR to move aboveground (branch and leaf) from the root. The transfer factor was calculated as follows\(^{10}\):

\[
\text{TF} = \frac{C_u}{C_r} \quad (4),
\]

where \(C_u\) is the concentration of NOR (g·kg\(^{-1}\)) in aboveground organs (branches and leaves), that is, \(C_u = C_t + C_l\), and \(C_r\) is the concentration of NOR (g·kg\(^{-1}\)) in the root system.

2.5. Reagents and instruments

The standard substance of NOR was chromatographically pure, which was produced by Dr. Ehrenstorfer in Germany. The main instruments used were HPLC (1260 fluorescence detector. Agilent, USA), termovap sample concentrator (N-EVAPTM-112, Organamation, USA), solid-phase extraction of plant (12-bit, ANPEL Scientific Instrument Co. Ltd, Shanghai, China), high speed freezing centrifuge (3K30, Sigma, Germany), and vortex mixing apparatus (XW-80A, Huxi Analysis Instrument Factory Co. Ltd, Shanghai, China).

2.6. Data treatment and statistical analysis

Statistical analysis and chart drawing were performed using Excel 2007, SPSS 19.0, and OriginPro 8.0. The t-test and one-way analysis of variance were used to analyze kinds of the differences. Significance level was set as \(p = 0.05\).

3. Results and discussions

3.1. Physico-chemical properties of mangrove rhizosphere sediments

The pH value and ksoil of rhizosphere sediments showed no significant differences between the two species. The CEC value showed that the buffering capacity of rhizosphere sediments of Rs was larger than that of Am sediments. In this study, the TOC content of the two mangrove rhizosphere sediments ranged from 16.72 to 74.94 g·kg\(^{-1}\).
3.2. **NOR residue characteristics in mangrove rhizosphere sediments**

Quantitative analysis of the NOR chromatogram of the sediment samples showed that the detection rate of NOR in the rhizosphere sediments of the two mangroves was 100%. The concentration of NOR in the rhizosphere sediments of Rs was 55.3 ± 2.6 μg·kg⁻¹, while the concentration of NOR in the rhizosphere sediments of Am was 52.2 ± 1.4 μg·kg⁻¹. The values observed in this study are much higher than the NOR residues reported in most rivers, estuaries, and wetland sediments in China[^1^][^2^]. The water–soil partition coefficient of NOR (Kd, sediment L·kg⁻¹) has a maximum index of 277,874[^3^], indicating that it is easily adsorbed in sediments.

Pearson correlation analysis of NOR residues and physico-chemical properties in rhizosphere sediments showed that there is a significant positive correlation between NOR content in sediments and the CEC of sediments as well as the TOC content (p < 0.01). This indicates that the CEC and the TOC content in the sediments have an important influence on the distribution of NOR in the sediments.

![Figure 2. Norfloxacin (NOR) concentration in mangrove rhizosphere sediment](image)

3.3. **Distribution of NOR in mangrove organs**

The NOR chromatogram of plant samples was analyzed quantitatively, as shown in Figure 3. The detection rates of NOR were 100% in both mangrove species. The distribution of NOR in the branches (600.8 ± 66.6 μg·kg⁻¹) of Rs was higher than that in the roots (119.6 ± 48.4 μg·kg⁻¹). Simultaneously, there were significant statistical differences (t-test, t = 15.496, p < 0.01). The distribution of NOR in Am was detected only in the roots and the concentration of NOR was 147.5 ± 26.2 μg·kg⁻¹, which varied from 121.1 to 189.4 μg·kg⁻¹. The concentration of NOR residues in Rs (720.35 ± 110.05 μg·kg⁻¹) was significantly higher than that in Am (147.47±26.21 μg·kg⁻¹), with a statistically significant difference (t = 13.398, p < 0.05).

There was a large amount of NOR distributed in the roots of both mangrove species and the content showed no significant differences (t = -1.342, p = 0.204 > 0.05). The NOR content of the upper branches in Rs was about 5 times that of the root, while there was no transfer from the root to the upper parts in Am. In the study area, the average age of the Rs community (48-year-old) was about twice that of the Am community (23-year-old) with a longer exposure to pollutants in the environment. Moreover, the NOR content of the rhizosphere sediments in Rs was higher than that in Am. At present, studies on the structure of both mangrove species are limited, and the factors affecting the distribution of pollutants in the mangrove species need to be studied further.
Figure 3. Concentration of fluoroquinolones (FQs) in mangrove plants

3.4. Accumulation and transport of NOR in mangrove organs

The accumulation factors (RCF, TCF, and LCF) and transfer factor (TF) of NOR in the organs of both mangrove species were calculated according to the formulae (1) to (4). The results are shown in Table 1. In Rs, TCR > RCF > 1 > LCF (= 0) and TF > 1, indicates a large NOR transfer from roots to branches. The NOR in Am is only accumulated in the roots, RCF > 1, which indicates that there is no transfer to the upper parts.

Thuy et al. [9] found that the accumulation of NOR in *Acrostichum aureum* and mangroves significantly reduced the NOR concentration in the culture solution in a hydroponic experiment. In the present study, BCF of Rs > BCF of Am > 1 showed that both mangrove species could accumulate the NOR in the environment, indicating that Rs and Am have a good purification effect for NOR in the environment, and Rs has a stronger purification effect for NOR. In addition, as the TF of Rs > 1, a large amount of NOR in the roots of Rs can be transferred to the upper part of the plant (in the branches).

Table 1. Bioconcentration factors (BCF) and transfer factors (TF) of norfloxacin (NOR) in mangrove plants

| NOR  | *R. stylosa* | *A. marina* |
|------|--------------|-------------|
|      | Range | Mean±SD  | Range | Mean±SD  |
| RCF  | 1.04-3.55 | 2.20±0.97a | 2.26-3.55 | 2.82±0.47 |
| TCF  | 8.36-12.58 | 10.91±1.56 | na    | 0.00    |
| LCF  | na    | 0.00    | na    | 0.00    |
| TF   | na    | 5.72±2.08 | na    | 0.00    |

na: not available

4. Conclusions

NOR was detected in rhizosphere sediments and mangrove organs in two mangrove communities in the Zhanjiang mangrove reserve. The content of NOR in the rhizosphere sediments of both mangrove species was higher than that in the sediments of most wetland systems, indicating that coastal aquaculture wastewater is an important source of FQs in the mangrove ecosystem. Correlation analysis of NOR residues in rhizosphere sediments with the physico-chemical properties of the sediments showed that TOC content and CEC were the key factors affecting the distribution of NOR in rhizosphere sediments.

NOR showed different distribution characteristics in the organs of both mangrove species. NOR was detected both in the roots and branches of Rs, and the content of NOR in the branches was 5 times more than that in the roots. NOR was detected only in the roots of Am with the concentrations ranging
from 121.1 μg·kg$^{-1}$ to 189.4 μg·kg$^{-1}$.

SCR > RCF > 1 > LCF (= 0) and TF > 1 in Rs indicated that a large amount of NOR in the roots of Rs is transferred to the branches. In Am, RCF > 1, indicated that NOR accumulated only in the roots and was not transferred to the upper parts. This indicates that both mangrove plants could accumulate substantial amounts of NOR. The higher NOR concentration in the upper parts of Rs than that in the roots indicates that Rs plays a better role than Am in the removal of NOR and purification of the environment.

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