Study on the removal efficiency of antibiotics in coastal wetlands by *Suaeda* and *Nelumbo nucifera*

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Abstract: This study mainly focused on the current situation of antibiotic pollution in coastal wetlands by screening for four common antibiotics (norfloxacin – NOR, ofloxacin – OFL, azithromycin – AZM, and roxithromycin – RXM) and two coastal wetland plants (*Suaeda* and *Nelumbo nucifera*), to determine the removal of antibiotic pollution by phytoremediation technology and its mechanism. We aimed to provide ideas for the remediation of antibiotics in coastal wetlands and their mechanisms of action in the context of intensive farming. The results showed that both plants had remediation effects on all four antibiotics, the phytoremediation of NOR and OFL was particularly significant, and the remediation effect of *N. nucifera* was better than that of *Suaeda* . The removal rates of the four antibiotics by *Suaeda* and *N. nucifera* at low antibiotic concentrations (10–25 μg/L) reached 48.9–100% and 77.3–100%, respectively. The removal rates of the four antibiotics at high antibiotic concentrations (50–200 μg/L) reached 7.5–73.2% and 22–84.6%, respectively. Moreover, AZM was only detected in trace amounts in the roots of *N. nucifera*, and RXM was not detected in either plant body.

Introduction

Coastal wetlands are at the junction of terrestrial and marine ecosystems and are widely distributed in the area of salt and freshwater convergence. They are highly dynamic, special, and complex ecosystems with unique hydrology, soil vegetation, and biological species, which also have abundant biological and mineral resources, meanwhile, provide critical habitat for a huge diversity of wildlife (Mauricio and Francis, 2017; Andria et al. 2021). In recent years, coastal wetlands have been seriously polluted due to land-based pollution, seawater intrusion, port industries, coastal development, and marine--based resource farming factors. With frequent heavy metal spills in marine resources, coastal greening, red tide serious, and bad odor, it is difficult to stop a series of ecological crises, and coastal wetland pollution and restoration problems should not be underestimated. Factory aquaculture is usually located in the coastal areas of developed and developing countries, resulting in high concentrations of antibiotics as one of the major contaminants of coastal wetland resources (Blasco., 1994). In China, the annual use of antibiotics is approximately a quarter of the amount used in all countries worldwide, with an average annual production of approximately 210 tons, of which approximately 52% is used as antibiotics in livestock cultivation and aquaculture (Yao et al. 2017). Such high levels of antibiotic use has made coastal wetlands an area grappling with the serious threat of antibiotic pollution. Although several antibiotics do not have a long half-life, their heavy use has led to their persistent and widespread presence in the environment. Several studies (Jiang et al. 2011; Yan et al. 2013) have found that antibiotic residues can be detected in rivers, sediments, and soils (a case of strong bioaccumulation), and the induction and spread of drug-resistant pathogenic bacteria due to antibiotic residues is seriously endangering environmental ecology and human health.

Extensive studies (Calheiros et al. 2007; Ellis., 2006) have been conducted worldwide on the concentration levels of antibiotics in different water bodies, and the frequency of antibiotic detection has reached 40–50%. Kolpin et al. (2002) conducted a universal survey of 128 rivers in 30 states in the United States, which showed that sulfonamide antibiotics and ofloxacin were commonly found in these rivers. Moreover, lincomycin, erythromycin, and sulfamethoxazole were frequently detected in two rivers in South Wales (KasprZyk-Hordern et al. 2008). Previous studies also found that sulfamethoxazole,

sulfamethoxazole, trimethoprim, and clarithromycin antibiotics were detected in the Juangong River Delta of Vietnam (Managaki et al. 2007), while sulfamethoxazole and norfloxacin antibiotics have been detected in the water of the Pearl River Delta in Guangzhou, China (Peng et al. 2008). Such studies have reiterated that antibiotic contamination in the aquatic environment should not be underestimated. Currently, the following treatment methods mainly adopted globally for antibiotic pollution in aquatic environments are: chemical oxidation, adsorption, membrane technology, and bioremediation. However, the first three methods entail high operation and management costs, which make them unsuitable for large-scale use. Bioremediation refers to the use of microbes and plants for the absorption, migration, transformation, and decomposition of antibiotic pollutants in water and soil to reduce the concentration of antibiotics to a safe range. It overcomes drawbacks such as high operation costs, incomplete purification, secondary pollution, endangering breeding function and destroying the ecological balance, thereby enabling rapid restoration of damaged ecosystems (Burken et al. 1998). Coastal wetlands are an important part of the aquatic environment; however, research on the phytoremediation of coastal wetlands is at a preliminary stage, and there are few relevant studies.

We selected two dominant populations of coastal wetland plants, *Suaeda* and *Nelumbo nucifera*, and screened four common antibiotics in coastal wetlands to compare their removal efficiency in an aqueous environment. Moreover, we investigated the effects of the two plants on remediation and enrichment degree of different single antibiotics to identify the plant with more effective antibiotic bioremediation. The results can be applied to the bioremediation of coastal wetlands contaminated with antibiotics in China and provide a reference for the protection and restoration of coastal wetlands in China.

Materials and methods

Growing Suaeda and N. nucifera

Suaeda seeds were purchased from Panjin City, Liaoning Province, China. The seeds were soaked in YM bacterial solution (1:500) for 24 h before planting to promote seed germination. The seeds were filtered out with gauze, washed 1–2 times with tap water, and planted into the soil. Before germination, the soil was sprayed 1–2 times a day with a spray bottle, watered shallowly and diligently to keep the soil surface moist. After germination, the plants were watered every other day. The planting temperature should be above 10°C and the plants should receive at least 4 h of sunlight per day. The plant should be used for experiments only when it is well established (approximately 15 cm tall, above soil level). The growth period of *Suaeda* in an outdoor culture was approximately 30 days, from April to September, and 25 days in an artificial climate culture room from October to March.

Nelumbo nucifera was purchased from Suqian, Jiangsu, China. After removing the yellow leaves, the plants were placed directly into tap water, and plant nutrient solution was added at a ratio of 1:2000. The water was changed once every 5 days to keep the water clean and nutritious. During the cultivation period, if the water hibiscus split, the split plant should be removed and placed in a pot, which will enable it to continue cultivation; in addition, dead leaves should be removed.

Sampling

For each sampling, the culture solution with the plants was slowly stirred in hydroponic pots to mix the solution well. A pipette was used to draw 300 mL of the culture solution from each culture pot. The collected water samples were placed in high-temperature, sterilized polypropylene plastic sampling bottles. All plants in the experiment were harvested, and the whole plants were gently washed with distilled water to remove root attachments, dried, and stored at −80°C.

First, the roots, stems, and leaves of each *Suaeda* and *N. nucifera* were separated, cut into segments, and put into crucibles. They were labeled and marked, dried in a vacuum freeze dryer for 72 h, and crushed in a pulverizer. This powder was then passed through a 60 mesh sieve, after which 1.0 g was weighed and placed in a centrifuge tube. To this tube, 10 mL of formic acid-methanol (1:99) was added and extracted by a vortex mixer for 30 s. The extract was then shaken in an ultrasonic cleaner for 10 min and centrifuged in a high-speed refrigerated centrifuge (4°C, 6000 r/min) for 5 min. Following this, the supernatant was removed, and the centrifugal extraction step was repeated three times. After three rounds of centrifugation, the supernatant was filtered through a 0.45 μm membrane, diluted to 300 mL with ultrapure water, and transferred to an HLB column for solid-phase extraction. Lastly, the eluate was blown to near dryness with a nitrogen blowing apparatus; 0.8 mL methanol and 1.2 mL ultrapure water were added to dissolve the mobile phase, filtered through a 0.22 μm needle filter into a clean injection bottle, and stored at −4°C in the refrigerator for measurement. The water samples were pretreated by solid-phase extraction, and the eluate was nitrogen blown to dryness at 60°C. Methanol 0.8 mL and ultrapure water 1.2 mL were added, turbine spun for 1 min, filtered through a 0.22 μm needle filter to a clean injection bottle, and stored in a refrigerator at −4°C for measurement.

Antibiotic detection

Solid-phase extraction (SPE) was used to enrich the antibiotics in the samples. The target antibiotics were determined through LC-MS/MS (USA). The column temperature was 30°C, the injection volume was 5 μL, and the flow rate was 0.3. The mobile phase consisted of 0.1% formic acid solution (A) and acetonitrile (B). The injection port temperature was maintained at 40°C. The ion source temperature was 120°C, the dissolvent gas temperature was 350°C, and the drying and atomizing gas flow rates were 15 L/min and 3 L/min, respectively. The detection conditions for antibiotics by mass spectrometry are listed in Table S1.

Statistical analysis

To analyze the significance between data, a one-way ANOVA analysis was performed using SPASS, and graphs were plotted using EXCLE and Origin Pro 9.

Results

Removal efficiency of antibiotics in different concentration gradients using Suaeda and N. nucifera

Figure 1 (a-d) shows the changes in the removal rates of NOR, OFL, AZM, and RXM from water samples after 15 days of

incubation at five concentration gradients (10 µg/L, 25 µg/L, 50 µg/L, 100 µg/L, and 200 µg/L) in the presence of *Suaeda* and *N. nucifera*. It was found that as the culture progressed, varying concentrations of the four antibiotic solutions were removed by treatment with *Suaeda* and *N. nucifera*. At a concentration of 10 µg/L of the four antibiotics, *N. nucifera* removed 100% of all four antibiotics, and *Suaeda* removed up to 100% of NOR and OFL. At a concentration of 25 µg/L of the four antibiotics, *N. nucifera* could still remove all of the NOR and OFL, but could not completely remove RXM and AZM. However, at this concentration, *Suaeda* could not completely remove all four antibiotics. Both plants showed a gradual decrease in removal rate as the concentration of the four antibiotics increased, and the removal rate did not change significantly when *N. nucifera* was treated with 100 µg/L and 200 µg/L OFL and NOR. The removal rate significantly decreased when treatment was carried out with 200 µg/L AZM and RXM, compared to treatment with 100 µg/L of both antibiotics, with a 50% reduction in removal rate. The removal rate of *S. aureus* did not change significantly at 25 µg/L and 50 µg/L AZM treatment and decreased significantly at 100 µg/L and 200 µg/L. The removal rate of *Suaeda* was significantly lower in the concentration range of 25–200 µg/L for the treatment of RXM. It can be seen that *Suaeda* was the least effective in the removal of RXM, especially at high concentrations. It can be seen that both plants possess the ability to remove the four antibiotics from water, especially at low concentrations, and the removal rate of *N. nucifera* was higher than that of *Suaeda*. For the two quinolone antibiotics, NOX and OFL, the removal rates of *Suaeda* and *N. nucifera* were significantly better than those for the two macrolide antibiotics, AZM and RXM.

The degree of enrichment of different concentrations of antibiotics in various organs of Suaeda

The variation of the content in each organ of *Suaeda* at different concentration gradients of the antibiotics is shown in Figure 2. AZM and RXM were not detected in *Suaeda* harvested at any concentration of AZM and ROX water samples in their plants. It can be seen that RXM was not enriched in *Suaeda*, probably because AZM and RXM were not easily absorbed by it, and the removal rate of both antibiotics was mostly derived from adsorption and root microbial degradation. In addition, the enrichment of NOX and OFL in *Suaeda* was mainly in the roots at low concentrations (10–25 µg/L), and was not detected in the stems and leaves. At high concentrations (50–200 µg/L), the enrichment was highest in the stem, followed by the root and the least enrichment was observed in the leaf, and the enrichment of NOX was higher than that of OFL. In the concentration range of the experiment, the *Suaeda* showed continuous uptake of these two antibiotics and did not show their own avoidance of NOX and OFL.

The degree of enrichment of different concentrations of antibiotics in various organs of N. nucifera

The changes in the root, stem, and leaf contents of *N. nucifera* treated with different concentrations of antibiotics are shown in Figure 3. We found that the enrichment of NOR and OFL was mainly in the roots at low concentrations (10–25 µg/L) and was not detected in the stems and leaves. At high concentrations (50–200 µg/L), the highest enrichment was observed in the leaves, followed by roots, and the lowest was observed in the
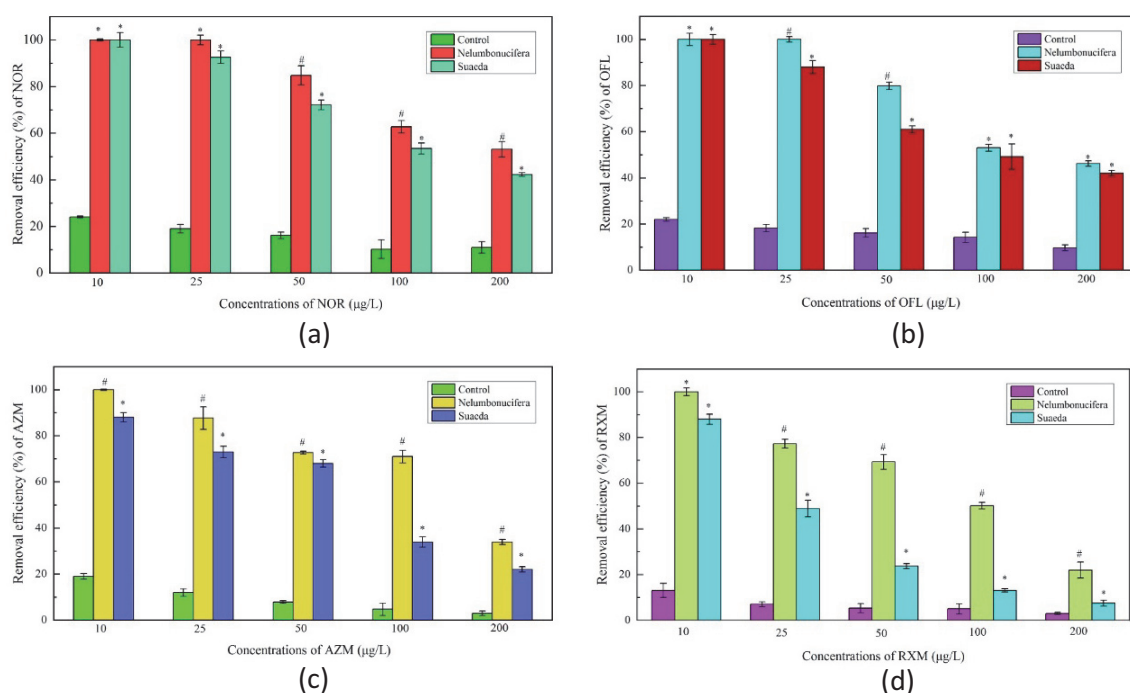
Fig. 1. The removal rates of different antibiotics concentration gradients treated by *Suaeda* and *Nelumbo nucifera*
(a) Fluoroquinolone antibiotics (NOR) (b) Fluoroquinolone antibiotics (OFL) (c) Macrolides antibiotics (AZM)
(d) Macrolides antibiotics (RXM).
Note: Different symbols (* and #) for the same antibiotics concentrations represent significant differences in the control, *Suaeda* treated experimental group and *Nelumbo nucifera* treated experimental group (P <0.05)

stems. The enrichment of NOR was higher than that of OFL. Similar to the results of *Suaeda*, the accumulation of NOR and OFL in the plants increased alongside their concentrations. Within the experimentally set concentration range, *N. nucifera* showed a continuous uptake of these two antibiotics and did not show its own avoidance of NOR and OFL.

AZM was not found in *N. nucifera* at concentrations of 10–50 µg/L. At concentrations of 100 µg/L and 200 µg/L, *N. nucifera* showed minimal uptake of AZM and was only enriched in the roots, although it did not change significantly with increasing concentrations, and no residues were detected in the leaves and stems of the plant. The reason for this observation may be that AZM is not easily absorbed by *N. nucifera*, and the enrichment of AZM by *N. nucifera* is weak.

RXM was not detected in any of the harvested water samples at any concentration of RXM in the plants. Therefore, it can be concluded that RXM was not enriched in *N. nucifera*, probably because it is not easily absorbed by *N. nucifera* and most of the removal of AZM and RXM by the plant is due to root adsorption and microbial degradation in the root system.

Discussion

Removal of different concentrations of antibiotics by Suaeda and N. nucifera

The results of this experiment showed that *Suaeda* and *N. nucifera* had some ability to remove NOX, OFL, AZM,

and RXM from water and achieved significant removal in a short period of time. The removal rates of NOR were 100%, 92%, 73.2%, 58.9%, 42.2%, and 100%, 100%, 84.6%, 62.1%, and 53.4% when comparing *Suaeda* and *N. nucifera* under the laboratory configuration of series concentrations (10–200 µg/L) of antibiotics after half a month of incubation, respectively. Comparing the removal rates of OFL by *Suaeda* and *N. nucifera*, which were 100%, 88%, 60.1%, 50.4%, 62%, 100%, 100%, 79.8%, 53%, and 46.2%, respectively, it can be seen that the removal effect of *N. nucifera* was superior to that of *Suaeda*. Comparing the removal of AZM by *Suaeda* and *N. nucifera*, which were 88.2%, 73.7%, 78.1%, 40.9%, 22%, and 100%, 87.7%, 72.3%, 71.6%, and 33.9%, respectively, it can be seen that the removal effect of AZM by *N. nucifera* is superior to that of *Suaeda*. Comparing the removal of RXM by *Suaeda* and *N. nucifera*, which were 87%, 48.9%, 23.7%, 13.1%, 8.1%, and 100%, 77.3%, 69.3%, 50.2%, and 22%, respectively, it can be seen that the removal effect of *N. nucifera* was superior to that of *Suaeda*. Comparing the effectiveness of *Suaeda* on the removal of the antibiotics, *Suaeda* was more favorable for the removal of NOX and OFL. Comparing the removal effect of *N. nucifera* on the four antibiotics, *N. nucifera* was far more favorable to remove NOX, OFL, and AZM.

As the concentration of each antibiotic increased, the treatment efficiency of *Suaeda* and *N. nucifera* decreased gradually, which is consistent with the experimental findings of Chen (Chen et al. 2012), who found that the removal rates of
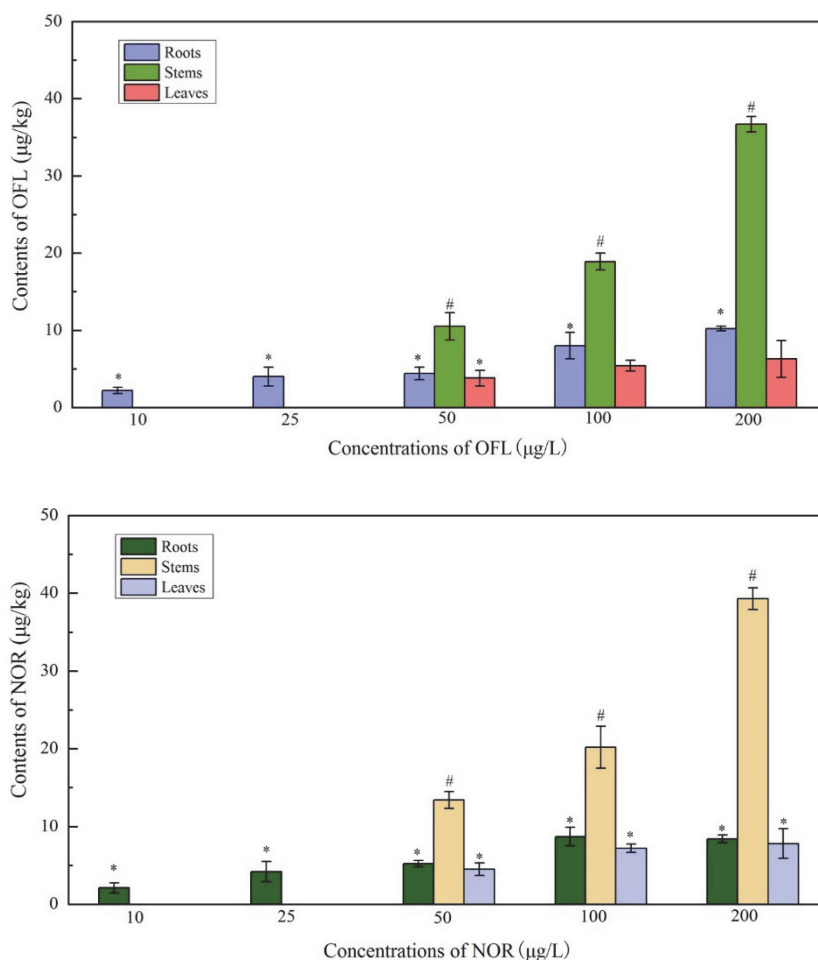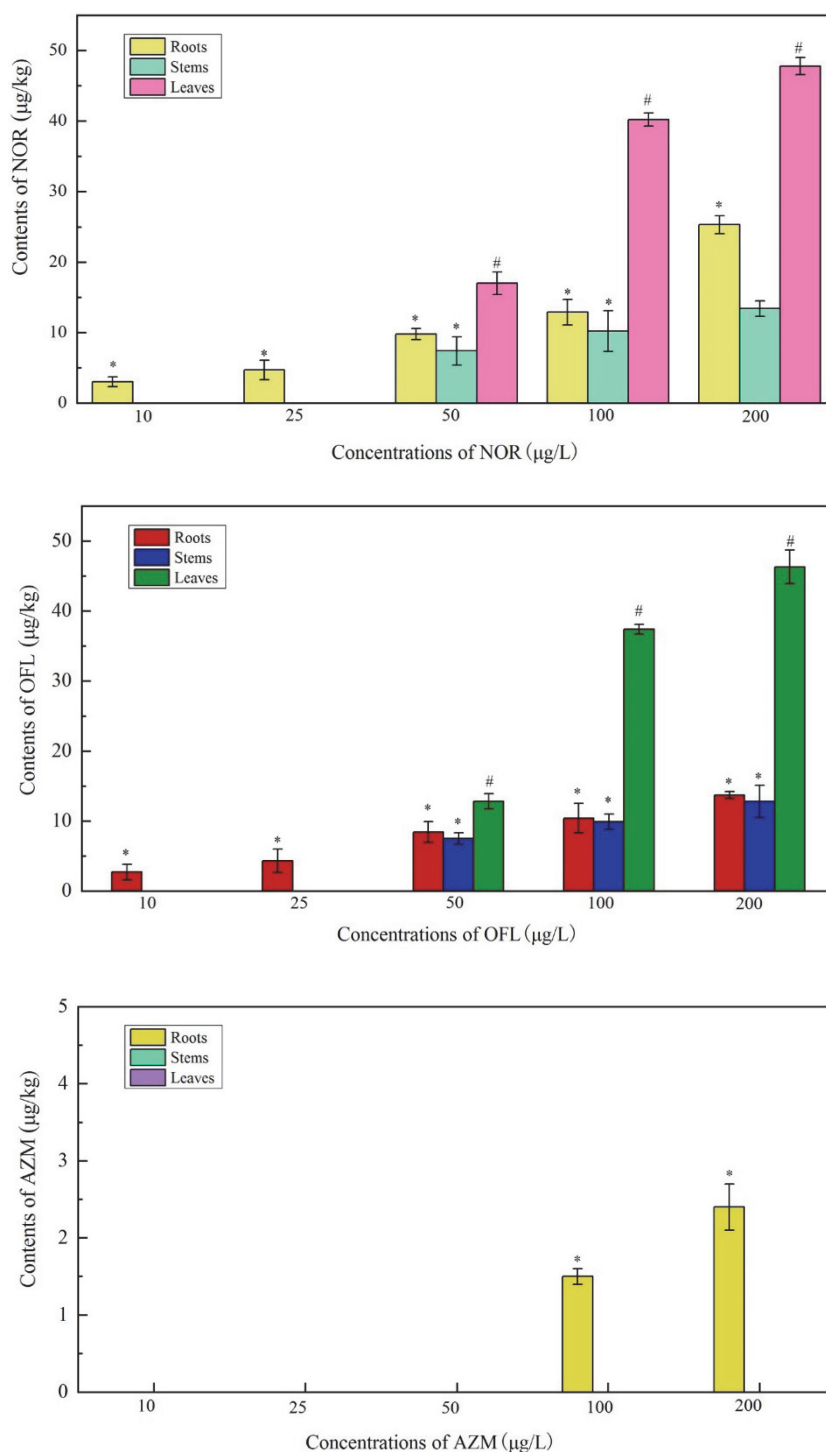

Fig. 2. The contents of antibiotics in roots, stems, and leaves in *Suaeda* at different concentrations of antibiotics

Fig. 3. The contents of antibiotics in roots, stems, and leaves in *Nelumbo nucifera* at different concentrations of antibiotics

the 20 μg/mL and 50 μg/mL treatment groups were 73.4% and 13.2%, respectively, after 1 day of incubation. No ampicillin was detected in the 20 μg/mL treatment group after 4 days of incubation, while the removal rate was 33.85% in the 50 μg/mL treatment group. This suggests that the initial concentration of antibiotics is also an influential factor in the removal efficiency of antibiotics by plants. A previous study (Hoang et al. 2013) found residual concentrations of macrolides, sulfonamides, tetracyclines, and quinolones ranging from 0.08 to 0.79 μg/L, and the concentration range set in this experiment was much higher than the actual level of contamination by these

four antibiotics. Thus, both plants can be considered good phytoremediants for these four antibiotics.

Enrichment of Suaeda and N. nucifera organs under different concentrations of antibiotics

In this experiment, *Suaeda* and *N. nucifera* showed strong absorption of NOX and OFL, and extremely weak or even no absorption of AZM and RXM. A comparison of the levels of the four antibiotics in the roots, stems, and leaves of *Suaeda* and *N. nucifera* revealed that the maximum value of the four antibiotics in the organs of the two plants was 47.8 μg/kg,

a result slightly higher than that of Hoang (Hoang et al. 2013). Hoang et al. detected varying concentrations in the roots of bamboo rootwort (22–45 µg/kg), haplophyllum fern (12–26 µg/kg), and ortho-mangrove (20–43 µg/ kg). The reason may be that the antibiotic concentrations under the experimental conditions of hydroponics were slightly higher than those in the wetlands, as studied by Hoang et al. The growth conditions of *Suaeda* and *N. nucifera* under hydroponic conditions were highly different from those of the other wetland plants in their natural state, as was the uptake of antibiotics.

NOR and OFL belong to the quinolone class of antibiotics, which are easily photolyzed (Hu et al. 2007). In the present study, the relatively faster degradation of NOR and OFL under the incubation conditions at low concentrations (10–25 µg/L) and the degradation in water resulted in reduced uptake by the plants. This is consistent with the observations of Thuy (Thuy et al. 2014), who studied the degradation of NOR and CIP by goldfish algae and vetiver grass. At high concentrations (50–200 µg/L), the migration from roots to stems and leaves, in vivo, began gradually, and the enrichment gradually increased with increasing concentrations. The enrichment distribution of NOR and OFL in N. nucifera was: leaf > root > stem, while that in Suaeda was: stem > root > leaf. However, previous study found that antibiotics tended to accumulate in plant root (Geng et al. 2022), which indicated that antibiotics accumulated to different degrees in different parts in plants. The enrichment amount of N. nucifera was greater than that of Suaeda. A previous study (Chiou et al. 2001) found that roots are the basic connection point between plants and soil, and roots are enriched or decomposed for antibiotic removal through physical, chemical, and biological pathways of antibiotic uptake and inter-root microbial action. The stems of plants are the conduit for antibiotics, and NOR and OFL in leaves mainly originate from upward transport after uptake by plant roots. The uptake of most antibiotics is a passive process in which antibiotics are transported upward along the vascular tube under the action of transpiration flow in plants (Dettenmaier et al. 2009; Grote et al. 2007; Kumar et al. 2005), and the ability of plants to absorb and transport pollutants depends on their transpiration intensity. Different plants differ in their shape and their transpiration capacities (Chiou et al. 2001). Due to the small and thin needle-like shape of Suaeda's leaves, transpiration is greatly reduced. Moreover, the plant's thick and long stems, which have many branches, transport very little water to the leaves from the roots (Chiou et al. 2001). Thus, the transport of antibiotics to the leaves was slightly lower for NOR and OFL, with most remaining in its stems. The stems of *N. nucifera* are extremely short, the leaves are large and full, and transpiration is strong; therefore, NOR and OFL absorbed by *N. nucifera* were most enriched in the leaves, followed by the roots, and the lowest in the stems. A previous study (Thuy et al. 2014) indicated that the plant uptake of NOR depends on the plant species. The translocation of ciprofloxacin in two mangrove wetlands in a study by Sun (Sun et al. 2017) showed that plant lipid content has a direct effect on plant uptake of organic matter and plants with high lipid content have a higher capacity for organic matter enrichment via their roots.

AZM was detected only at very high concentrations in the roots of *N. nucifera*, while ROX was neither detected in *Suaeda* nor in *N. nucifera*. The uptake of AZM and ROX by *Suaeda* and *N. nucifera* was not significant. This may be because the physicochemical properties of AZM and ROX indicate that they are not easily absorbed by plants. The molecular weight of substances that plants can absorb through active transport generally does not exceed 500, and compounds with larger molecular weights are instead strongly adsorbed by plants. The molecular weight of AZM is 748.98, and that of ROX is 837.04, which makes it difficult for these antibiotics to be absorbed by plants. Second, when the LogKow value of organic matter is >3.5, it is strongly adsorbed on the root surface of plants, and the LogKow values of AZM and ROX were 3.7 and 4.3, respectively (Kay et al. 2005; Maier et al. 2018), thus making it difficult for them to migrate upwards. Therefore, based on the mass balance of AZM and ROX in water samples, it can be concluded that most of the degradation rates of AZM and ROX by both plants originated from root adsorption and rhizosphere microbial degradation.

Conclusions

The removal of the four antibiotics by *N. nucifera* was significantly higher than that by *Suaeda*, and *N. nucifera* could achieve 100% removal of the four antibiotics at low concentrations (10–25 µg/L). In contrast, *Suaeda* could achieve 100% removal rate of only 10 µg/L NOR and OFL. The removal rates of the four antibiotics were 7.5–73.2% and 22–84.6% at high concentrations (50–200 µg/L) for *Suaeda* and *N. nucifera*, respectively. Meanwhile, it has also been observed that the more developed the root system, the larger the leaves, and the faster the growth and metabolism of the plant, the better the removal of antibiotics. The smaller the molecular weight of antibiotics, the easier they are to be absorbed by plants, and those with molecular weights greater than 500 are not easily absorbed by plants. In addition, AZM was extremely weakly enriched in *N. nucifera*, and at high concentrations, it was enriched in the roots. However, *Suaeda* and *N. nucifera* did not take up RXM at all.

Acknowledgement

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