Environment Protection Engineering

Vol. 49 2023 No. 1

DOI: 10.37190/epe230101

LIDAN PANG (ORCID: 0000-0001-7813-8753)[1], HAIDONG ZHOU (ORCID: 0000-0003-3821-7427)[1]
MENGWEI LI (ORCID: 0000-0001-5687-1401)[1], ZIMING ZHAO (ORCID: 0000-0002-9936-6437)[1]
XINXUAN XU (ORCID: 0000-0001-9547-8549)[1], MIXUAN YE (ORCID: 0000-0001-8996-1358)[1]
XIAOJING LIU (ORCID: 0000-0002-6633-9280)[1]

REMOVAL OF TYPICAL ANTIBIOTICS FROM THE SYMBIOTIC SYSTEM OF *MICROCYSTIS AERUGINOSA* AND EMERGENT PLANTS

In a simulated urban river system, the conversion and distribution of six typical antibiotics were investigated under the following conditions: no plant, only *Microcystis aeruginosa* (algae) and algae combined with *Juncus effusus*, *Cyperus alternifolius*, and *Acorus calamus.* Through the calculation of the mass balance, the quantitative distribution of antibiotics in the water phase, sediment, *Microcystis aeruginosa,* and plant tissues, and the total elimination efficiency of the antibiotics were determined. The results showed that higher concentrations of sulfathiazole (STZ) and sulfamethoxazole (SMZ) were detected in the water phase of the non-plant group, which were 52.81% and 56.88%, respectively, and ciprofloxacin (CIP) and tetracycline (TCY) were detected higher in the sediment, up to 1562 ng/g and 1829 ng/g, respectively. The antibiotic removal rates have been greatly improved, and those in the system containing *Microcystis aeruginosa* were higher than that in the system without aquatic plants or algae. The calculation of the mass balance showed that the removal effect of algae combined with *Juncus effusus* was the best, and the removal rates (azithromycin (AZM) and clarithromycin (CLM)) were the highest, reaching 68.88% and 61.96%. It seems that algae and plants play an important role in the removal of antibiotics.

1. INTRODUCTION

Antibiotics are a class of chemicals that interfere with or block the normal growth and metabolism of other cells widely used to treat various diseases. According to statis-

[1]School of Environment and Architecture, University of Shanghai for Science and Technology, Shanghai 200093, China, corresponding author H. Zhou, email address: zhouhaidong@usst.edu.cn

tics, China's average annual consumption of antibiotics per capita is 138 g, approximately twice that of Europe and 10 times that of the United States [1]. However, due to intestinal malabsorption or incomplete metabolism, antibiotics are excreted as maternal compounds or metabolites. They can enter groundwater through the leaching of contaminated surface water and landfill leachate, where researchers detected antibiotics [2]. Sulfa antibiotics (such as sulfathiazole (STZ), and sulfamethoxazole (SMZ)) were found to have high migration capacity, and sulfa metabolites left in the faeces still maintained significant activity for a long time after being discharged into the environment [3]. Senta et al. [4] explored the high obstinacy of macrolide antibiotics (such as azithromycin (AZM), clarithromycin (CLM)) in sediment. The results showed that such antibiotics could stay in sediment for long and enter groundwater after several years. Tetracycline (TCY) and quinolones (such as ciprofloxacin (CIP)) are often used in medical care and animal husbandry. Because of their low absorption rate, their residues are often detected in water, sediment, soil, and biota samples. Because of the wide use of antibiotics and their water solubility, stability and low volatility characteristics, antibiotics exist in a "lasting" state in water, and their impact on the environment has attracted more and more attention.

Therefore, some people studied antibiotic degradation methods, including the activated sludge process, adsorption and biological methods, membrane separation technology, chemical oxidation, etc. As a biological method, phytoremediation technology can directly use plants to remove, decompose, or absorb pollutants (heavy metals, organic matter, etc.) in contaminated land or groundwater. It characterises long-term effects, low cost, no production and no secondary pollution, and is widely used in the remediation of various pollutants, including heavy metals, polycyclic aromatic hydrocarbons, persistent organic pollutants, dyes, etc. [5]. When organic pollutants exist in the water environment, they are adsorbed by plant roots first, part of which is degraded by the root exudates, enzymes, and rhizosphere microorganisms, and the other part is transferred from the root surface to the plant, which is degraded or destroyed under the action of enzymes, and finally forming CO_2 and H_2O. In recent years, more and more research were reported on plant regulation of antibiotic contamination.

Algae are the bottom organisms in the food chain, they can photosynthesise like plants. In recent years, there has been increasing research on the use of algae-based technologies in wastewater treatment. Su et al. [6] explored the metabolic process of carbon, nitrogen, and phosphorus in organic wastewater by microalgae, providing a potential method for the large-scale application of microalgae wastewater treatment technology. Li et al. [7] summarised the research status of using algae to remove antibiotics from wastewater and explained its removal mechanism, namely biosorption, bioaccumulation, biodegradation, photodegradation, volatilisation and hydrolysis. In addition, treatment has been put forward, which could effectively treat organic wastewater using the interaction between algae and bacteria [8]. Research shows that nutrients from wastewater are assimilated into biomass for algae growth and can be used as a biological fertiliser. Algae can produce valuable products while treating wastewater, such as biofuels, proteins, carbohydrates, pigments, and vitamins [9]. As a new pollutant that has been a concern of the scientific community in recent years, there are increasing studies on using algae technology to control antibiotics. Algae-based technology can remove antibiotics from wastewater; the process mainly includes adsorption, biodegradation, and photodegradation. Therefore, compared to traditional wastewater treatment technology, algae-based technology is more sustainable.

In this paper, six representative antibiotics frequently detected in urban rivers were selected as the target, and three types of aquatic plants such as *Juncus effusus*, *Cyperus alternifolius,* and *Acorus calamus* were planted separately in a simulated urban river and inoculated with *Microcystis aeruginosa*. The purpose of this study was to explore the effects of algae and aquatic plants in urban river systems on the removal of antibiotics and the restoration of water quality. The changes of target antibiotics under different ecosystem conditions in water bodies and sediments, as well as the removal effects of aquatic plants and algae on target antibiotics in simulated urban rivers were investigated.

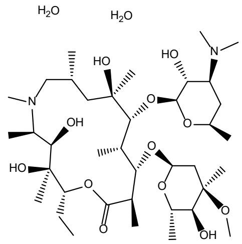
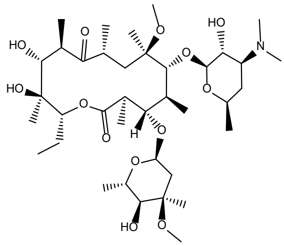
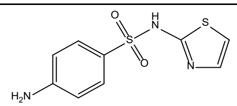
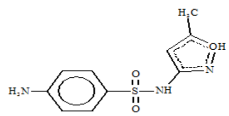
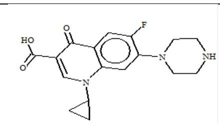
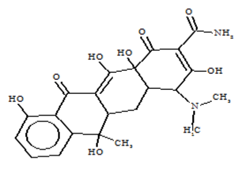
2. MATERIALS AND METHODS

Chemicals and materials. Six types of antibiotics such as AZM, CLM, STZ, SMZ, CIP, and TCY were selected for the study. Their reference standard (purity >98.5%) was purchased from Dr. Ehrenstorfer, Augsburg, Germany. The physicochemical properties of all targets are presented in Table 1. Internal standard methyl chlortetracycline, roxithromycin, $^{13}C_6$-sulfamethoxazole, and moxifloxacin were purchased from Witega, Germany. The standard and internal standard of the antibiotics were dissolved in methanol and prepared in 1 g/dm^3 stock solution which was then diluted with methanol to the desired concentration of the standard solution. All reserve liquid and standard liquid were stored at –20 °C protected from light. The concentrations of antibiotics in the stock solutions were checked every 3 months with newly prepared solutions of their reference standards. High performance liquid chromatography (HPLC) grades of methanol and dichloromethane were supplied by Merck & Co., Darmstadt, Germany. HPLC grade formic acid and ammonium acetate were purchased from Aladdin, Shanghai, China. Na_2-EDTA, NaOH, and other reagents (analytical grade or better) were provided by Sigma-Aldrich, USA.

Solid phase extraction (SPE) was carried out using Poly-Sery HLB cartridges (6 cm^3/200 mg, ANPEL, USA). Polyether sulfone syringe filters (∅0.22 μm) were also bought from ANPEL. And ultrapure water was used for the whole procedure of sample analysis, produced by an EPED ultrapure water machine, China. The nitrogen purging and enrichment device was purchased from Tianjin Autoscience Instrument Co., Ltd., China.

Table 1

Physicochemical properties of the target antibiotics

Drug	Type	Molecular weight [g/mol]	Formula	Molecular structure	$\log K_{OW}$	$\log K_{OC}$[2]
AZM	macrolide antibiotic	749.5	$C_{38}H_{72}N_2O_{12}$		4.02[1]	1.676
CLM	macrolide antibiotic	748.4	$C_{38}H_{69}NO_{13}$		3.16[1]	1.371
STZ	sulphonamide	256	$C_9H_9N_3O_2S_2$		0.7[1]	1.071
SMZ	sulphonamide	253.28	$C_{10}H_{11}N_3O_3S$		0.48[2]	1.536
CIP	quinolone antibiotic	331.35	$C_{17}H_{18}F_1N_3O_3$		0.28[2]	–0.004
TCY	tetracycline antibiotic	444.44	$C_{22}H_{24}N_2O_8$		–1.30[2]	–0.128

[1]Data from Kosma et al. [26].
[2]Data estimated by EPI SUITE ™ 4.11.
$\log K_{OW}$ – the octanol–water partition coefficient, $\log K_{OC}$ – soil organic carbon–water partition coefficient.

Sediment and water collection. The river water and sediment used in this experiment were collected from the Qiujiang River (QJ), an urban river in Yangpu district, Shanghai.

The river water was collected and stored in a plastic bucket. After standing for one hour, the large particles in the water were removed. The grab sampler was used to collect sediment, which was placed at the bottom of the experimental device after solid impurities such as branches and stones were removed. Then river water was slowly injected into the device and the follow-up experiment after standing for 1 to 2 days. The main chemical characteristics of the collected river water are shown in Table 2. It could be seen from Table 2 that the water quality was poor and the urban river might be contaminated by wastewater.

Table 2

Chemical Characteristics of water collected from the Qiujiang River

No. of sampling campaign	DO [mg/dm^3]	COD [mg/dm^3]	TN [mg/dm^3]	NH_4^+-N [mg/dm^3]	TP [mg/dm^3]	Turbidity [NTU]	pH
1	2.78±0.5	89±3.2	7.92±1.76	3.05±0.7	0.575±0.13	39.3±6.8	7.69–7.82
2	5.21±1.1	94±4.1	7.58±0.8	3.26±0.2	0.763±0.21	46.7±6.4	7.77–8.21
3	4.67±1.3	77±5.8	7.89±1.2	3.11±1.2	0.692±0.07	48.3±7.8	6.71–7.89
4	3.68±0.8	89±3.0	7.35±1.6	3.21±0.8	0.632±0.04	41.7±8.1	7.11–7.94
5	3.65±0.2	73±1.9	7.12±0.9	3.56±0.5	0.721±0.14	38.5±6.6	7.56–8.15

Experimental setup. The experiment was carried out in a circulating flume simulating urban water system [10]. The main body of the river simulation device was made up of two parts: straight part and an arc part. The total effective volume of the water flume was 500 dm^3. The water inlet pipe and the water outlet pipe were set on the side wall of the straight channel of the water flume, and the upper and lower sampling ports were set on the straight channel and bend, respectively, to collect water samples and sediment samples during the test operation. A blow-down pipe was installed under the water flume to discharge river water and sediment from the unit after the test cycle. The heating pipe was built into the annular water flume and the water temperature in the device was regulated by the intelligent temperature control system. The straight channel of the water flume was equipped with three waterproof lamps to simulate the lighting conditions in the environment, so that the environmental conditions in the entire simulation device tended to the actual urban river environment.

Three types of aquatic plants, *Juncus effusus*, *Cyperus alternifolius*, and *Acorus calamus*, were selected and purchased from the Shanghai Orchid Rain flower shop to explore their performance in removing antibiotics and restoring water quality in urban rivers. The whole survey was carried out under the following ecological conditions, that is, blank (no *Microcystis aeruginosa* or any aquatic plants), only *Microcystis aeruginosa*, *Microcystis aeruginosa* combined with *Juncus effusus*, *Cyperus alternifolius*, or *Acorus calamus*. The aquatic plants were initially free of any antibiotic contamination and were thoroughly rinsed with ultrapure water prior to incubation in the system. They were planted on the floating bed made of acrylic board. During the experiment, *Microcystis aeruginosa* was added to the water phase to explore the effect of water purification and antibiotic removal by the symbiotic system of ecological floating island and algae. The plant coverage rate for each test in the system was 30%, and the *Microcystis aeruginosa* was 10^5 cells/cm^3. At the beginning of each test, plants and algae were trained in the system for 5 days to accommodate the sink environment. Each antibiotic was dissolved in a 10 μg/dm^3 solution and added to the circulating flume to initiate the 15-day experiment. After each test, the plants were manually removed to complete the discharge of river water and sediment. After cleaning, the flume was laid with the collected sediment and added with the collected river water. The flume was balanced for 7 days before the next test. Water and sediment samples were collected at 0, 0.5, 1, 2, 5, 10, and 15 days of the experiment, while plants at 5, 10, and 15 days of the experiment. At each sampling, 300 cm^3 of river water (3–5 cm below the water surface) and 10 cm^3 of sediment were collected for the measurements of the target antibiotics, and another 100 cm^3 of river water was taken for the analyses of conventional indicators of water quality. The same volumes of collected river water and sediment were replenished into the flume after each sampling and 200 cm^3 of collected river water were added daily into the flume to compensate for evapotranspiration during the tests.

Sample pretreatment. Detailed pretreatment of water, sediment, and plant samples can be found in the literature [11]. For algae samples, 50 cm^3 water samples were taken and centrifuged at 3000 rpm (nondestructive) for 20 minutes. The supernatant was removed immediately. 5 cm^3 of methanol was added to the remaining algal cell samples, which were completely shook, centrifuged at 3000 rpm, and the supernatant was collected. The process was repeated 3 times, and the supernatant taken 3 times was combined, and ultrapure water was added to make up the volume to 100 cm^3. Subsequently, the sample was pretreated with the pretreatment of the same procedure in the water sample.

The solid phase extraction (SPE) procedure was carried out on a vacuum manifold (Supelco, USA) that had 12 extraction positions. First, the Poly-Sery HLB SPE cartridges were placed in the vacuum manifold and pretreated with 5 cm^3 of methanol and 5 cm^3 of ultrapure water for 3 times for activation and equilibrium. Before extraction, each sample was added with Na_2-EDTA at 0.2 g to chelate divalent cations, such as Ca^{2+} and Mg^{2+}, and adjusted to pH 7.0 with 1 mol/dm^3 NaOH. Then, the flow rate of the samples was adjusted to 3 cm^3/min, the HLB filter cartridges were washed with 5 cm^3 ultrapure water for 6 times and dried under vacuum for 30 min to remove excess water. Next, 3 elutions were performed using a mixture of 2 cm^3 of methanol, dichloromethane, and acetone (40:40:20). The eluent was collected in a 10 cm^3 glass tube and then evaporated to dryness with a nitrogen stream at 40 °C. Then methanol was added to the glass tube to 1 cm^3 and 1 ng of internal standard sulfamethoxazole-D4 was added. Finally, polyether sulfone syringe filters (∅0.22 μm) were used to filter the solution, which was then analyzed on UHPLC-MS / MS (ThermoFisher, USA).

Analysis methods. Antibiotics were detected by an ultra-high performance liquid chromatography system (UHPLC, UltiMate 3000, Dionex, USA) combined with a tandem quadrupole mass spectrometer equipped with a hot electrospray ionisation source (HESI-MS/MS, Thermo Scientific TSQ Vantage TM, USA). A detailed description of UHPLC-MS/MS analysis can be found in the literature [2]. Details of antibiotic testing are available in the literature [11]. Recovery of target compounds was calculated by adding standard antibiotics to river water, sediment, and plant samples, repeated three times for each group, and considering blank subtraction. The average recoveries ranged from 66.1 to 104.1%.

Analyses of conventional indicators such as COD, dissolved oxygen (DO), total ammonia nitrogen (NH_4^+-N), total phosphorus (TP), pH, total solids (TS), turbidity and volatile solids (VS) were performed using Chinese National Standard methods for detection of water quality. Total mitrogen (TN) was analysed using the Multi N/C 3100 TOC/TN analyser (Analytikjena, Germany).

Data processing. A mass balance calculation of antibiotics was performed to estimate their total removal efficiencies (TRE) in the urban river system. The TREs of antibiotics were calculated as follows:

$$\mathrm{TRE} = \frac{M_0 - C_w V_w - 10^{-3}\left(C_s M_s + C_p M_p\right)}{M_0} \times 100\%$$

where M_0 is the initial total mass, μg, of antibiotics measured in the water phase and sediment at the beginning of the experiment; C_w, C_s and C_p are the concentrations in the water phase, μg/dm^3, sediment, ng/g, and plant tissue, ng/g, at the end of the experiment, V_w is the effective volume of the water phase, dm^3, M_s is the dry mass of sediment, g; M_p is the dry mass of plant tissue, g.

3. RESULTS

3.1. CONVENTIONAL INDICATORS UNDER DIFFERENT SYMBIOTIC SYSTEMS

The DO ranged from 2.78 to 5.21 mg/dm^3 at the beginning. After 24 h, the DO content fluctuated from 5.33 to 8.56 mg/dm^3, mainly because the ecosystem was started after the belt operation, the holding equipment ensures sufficient oxygen content of the system. The pH was kept in the range of 6.81~8.32.

The removal rates of COD, NH_4^+-N, TP and TN in the symbiotic systems are shown in Fig. 1. In the blank group, the COD remained constant for 15 days in the test period, and the removal rate was only 24.7% at 15 days. Compared to the blank group, the CODin other systems gradually decreased over time, and the removal rate reached 62.8, 72.7, 67.4, and 69.9% at 15 days, respectively. The degradation of COD in the water environment depends on many processes such as photodegradation, hydrolysis, biodegradation, bioconcentration, and adsorption. Organic matter is consumed as a source of nutrients for dominant organisms composed of aerobic and anaerobic bacteria. Most organic matter is degraded by anaerobic bacterial activity.

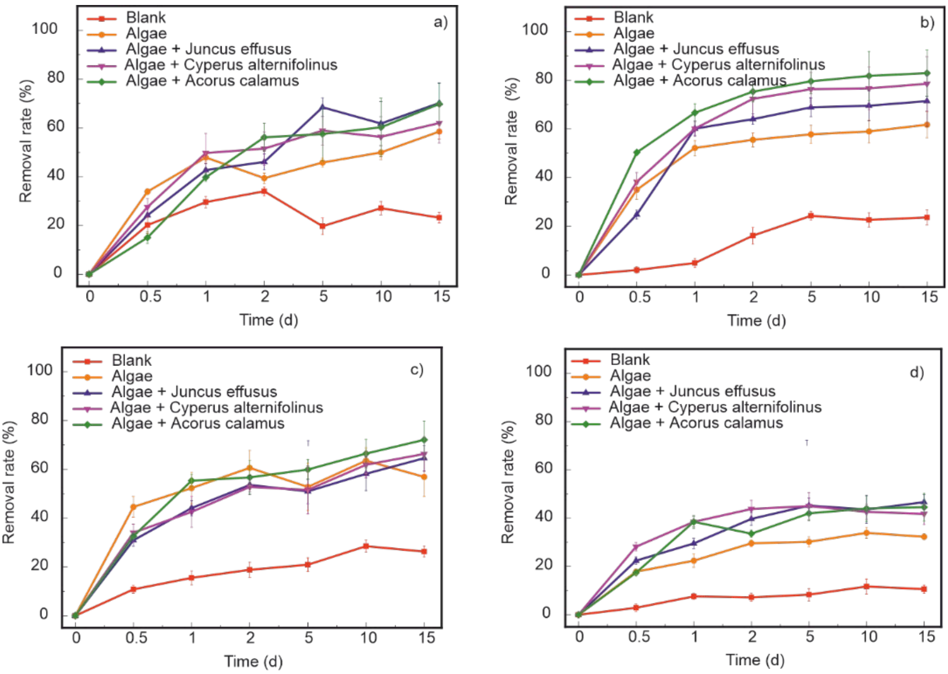


Fig. 1. Removal rates of water quality routines: a) COD, b) NH_4^+ N, c) TP, and d) TN under various aquatic plant ecological conditions of aquatic plants within 15 days

However, there are some aerobic and facultative bacteria in the simulated urban river system, including shallow water and an aerobic environment (under planting conditions, oxidation-reduction potential from –50 to 300 mV, DO from 2.9 to 4.2 mg/dm^3). Therefore, relatively limited COD degradation efficiencies were calculated throughout the test period. In addition, the presence of plants has a certain enhancement in organic matter [12], there are three main ways for plants to remove organic pollutants: 1) direct absorption of plants, 2) release of exudates and enzymes from the plant root, and 3) the combined action of plants and microorganisms of the rhizosphere. The efficiency of the removal of organic pollutants depends on the type and mix of plants.

In planting systems, both NH_4^+-N and TN have higher removal rates, indicating that aquatic plants had a strong removal effect on NH_4^+-N and TN. In the three systems containing algae and aquatic plants, the removal rates of NH_4^+-N at treatment for 15 days were 71.4, 78.5, and 82.9%, respectively. At 15 days of treatment, the TN removal rates were 46.6, 41.8, and 44.5%, respectively. The removal rate of NH_4^+-N and TN in the system with only *Microcystis aeruginosa* was 61.7 and 32.5%, respectively, which was much higher than 23.6% and 10.6% of the blank experimental group. However, this is lower than in ecosystems where algae and aquatic plants coexist. The efficiency of removal of the simulated urban river system is affected by various factors such as mineralisation, ammonia volatilization, nitrification, denitrification, plant and microbial absorption and adsorption. Under growing conditions, the growth and metabolism of aquatic plants will absorb and utilise nutrients (such as nitrogen and phosphorus) and organic matter. These plants will also provide suitable environments and large breeding spaces for nitrifying and denitrifying bacteria, which will accelerate nitrification and denitrification and better remove nitrogen.

The removal rates of TP at 15 days in the five ecosystems were 26.3, 56.9, 64.6, 66.3, and 72.1%, respectively. In the presence of aquatic plants, the removal of phosphorus may result from two processes. One is that phosphate is attached to suspended particles and the other is that soluble phosphorus is absorbed by aquatic plants and microorganisms [13]. The best removal effect was the simultaneous symbiosis of algae and *Acorus calamus*, with a removal rate of 72.1%.

3.2. CONCENTRATION OF ANTIBIOTICS IN WATER

The structure may be affected by conjugation processes, which change the properties of the compound. Compounds produced by binding processes were not examined in this study; therefore, as shown in Fig. 2, the relative concentrations of antibiotics such as STZ and SMZ may be higher than 100%. Most antibiotics showed lower concentrations at the end of the test. Therefore, aquatic plants play an important role in the removal of antibiotics in the aqueous phase.

The macrolide antibiotics AZM and CLM are widely used in human and animal medicines, and their degradation pathways are similar. Under the experimental conditions, their concentrations continued to decrease. After 15 days, in the group without plants or algae, the concentration levels were 23.20 and 26.89% of the initial concentration. This may be partly due to biodegradation [1]. Compared with the blank group, the concentration of AZM and CLM was slightly lower in the systems inoculated with *Microcystis aeruginosa* and remained at 18.21% and 19.04% of the initial concentration at a 15th day, respectively. That was slightly higher than in the group that had both algae and aquatic plants. Compared to the blank experimental group, the groups containing algae or aquatic plants had better ability to remove AZM and CLM, algae + *Juncus effusus* and algae + *Acorus calamus* were superior to the other two groups, and after 15 days, the concentration levels of the two antibiotics were low – 10.98, 12.3 and 13.65, and 13.9% of the initial concentration, respectively. Wang et al. [14] reported that pollutants with high logK_{OW} values will strongly adhere to nearby plants (CLM logK_{OW} 3.16, Table 1). For CLM, the relatively high removal rate may be mainly due to the adsorption and accumulation of aquatic plants.

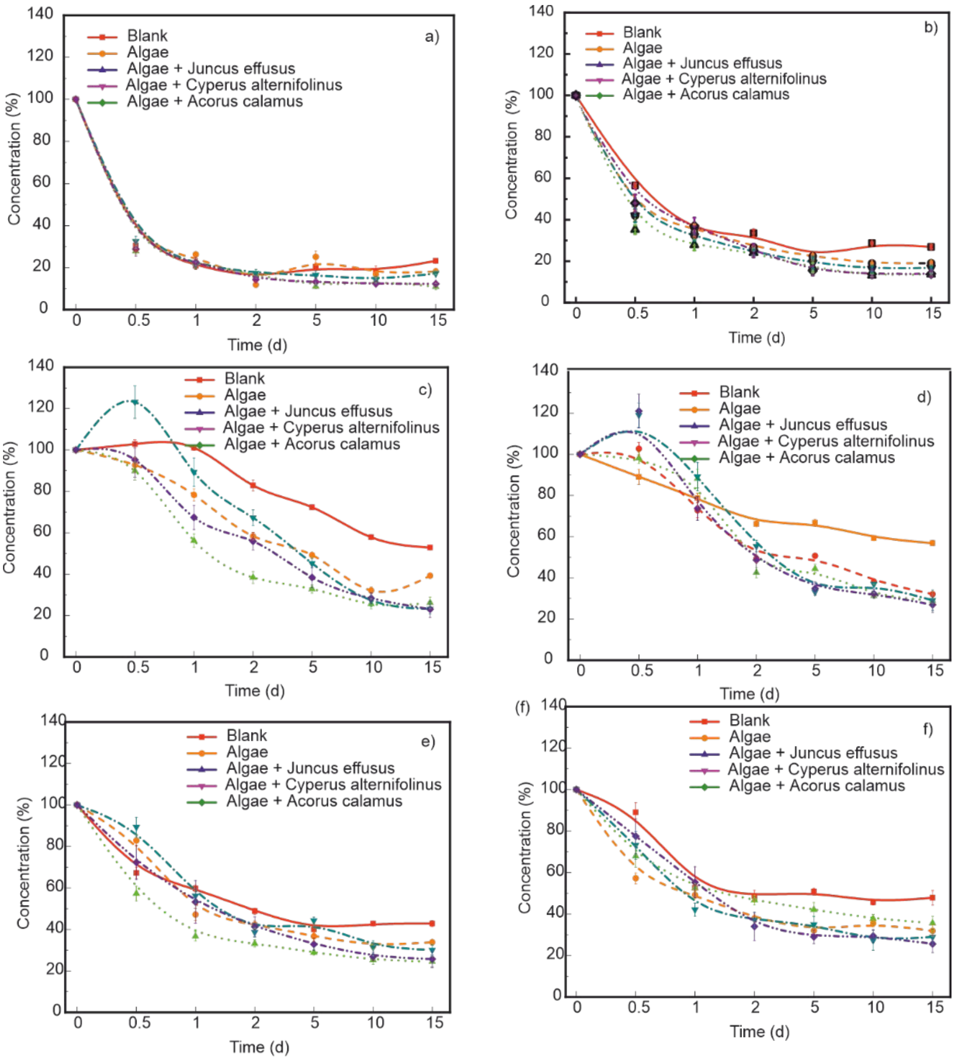


Fig. 2. Concentrations of various antibiotics: a) AZM, b) CLM, c) STZ, d) SMZ, e) CIP, f) TCY in 15 days under aquatic plant ecological conditions

Relatively low levels of sulfa antibiotics STZ and SMZ were observed under growing conditions, which means that phytoremediation may be a particularly important route for their removal. At the end of the test, the concentrations of sulfa antibiotics STZ and SMZ were 52.81% and 56.88% of the initial concentration, respectively. However, the concentrations of the experimental group inoculated with *Microcystis aeruginosa* at 3 days were 39.25% and 49.09% of the initial concentration, respectively, which were better than that of the experimental group without algae or aquatic plants. Therefore, *Microcystis aeruginosa* at a certain concentration has the ability to remove sulfa antibiotics. In the experimental groups containing both algae and plants, the two antibiotics remained at 26.21, 23.1, 22.98%, and 28.89, 29.12, 26.98% (in relation to the initial concentration), respectively, at 15 days, slightly lower than the experimental group containing only algae. Therefore, aquatic plants are the main way to remove sulphonamides.

Under aquatic plant conditions, most of the target antibiotics are detected at lower concentrations, which means that aquatic plants play an important role in their removal. Phytoremediation shows great potential for removing compounds in water that are difficult to degrade or adsorb, such as CIP and TCY. Due to the direct adsorption by plants, some antibiotics achieve relatively high removal rates by floating aquatic plants. The removal rate of photosensitive drugs such as CIP and TCY is relatively low under the conditions of planktonic aquatic plants, indicating that the coverage of floating plants on the water surface reduces the illumination area. For highly hydrophobic drugs such as AZM and CLM, no significant differences were detected in aquatic plant conditions. Therefore, all selected antibiotics exhibit different trends and removals in the aqueous phase due to the different physicochemical properties and lifestyles of the selected aquatic plants.

3.3. ANTIBIOTIC CONCENTRATION IN SEDIMENT

All antibiotics under examination showed a tendency to increase first and then decrease during the experimental period (Fig. 3). In the initial stage, the antibiotics were adsorbed by the sediment. Then various microorganisms present in the sediment biodegraded the target, accompanied by photodegradation, desorption or adsorption into deep sediments. At the end of the test, lower concentrations of antibiotics were measured in the systems containing algae and aquatic plants.

Hydrophobic contaminants AZM, CLM ($\log K_{OW} > 1$, Table 1) show strong adsorption in the sediment. During the first 48 hours, the rapid adsorption of AZM and CLM in plant-free sediments dominated and then continued to decrease, suggesting that biodegradation became later the major removal pathway [1]. At the end of the experiment, the detection concentrations of AZM and CLM were relatively low in the experimental group containing algae (compared to the no-plant group), indicating that *Microcystis aeruginosa* contributed to the removal of antibiotics. The lowest concentration of antibiotics was detected in the experimental group with both aquatic plants and algae.

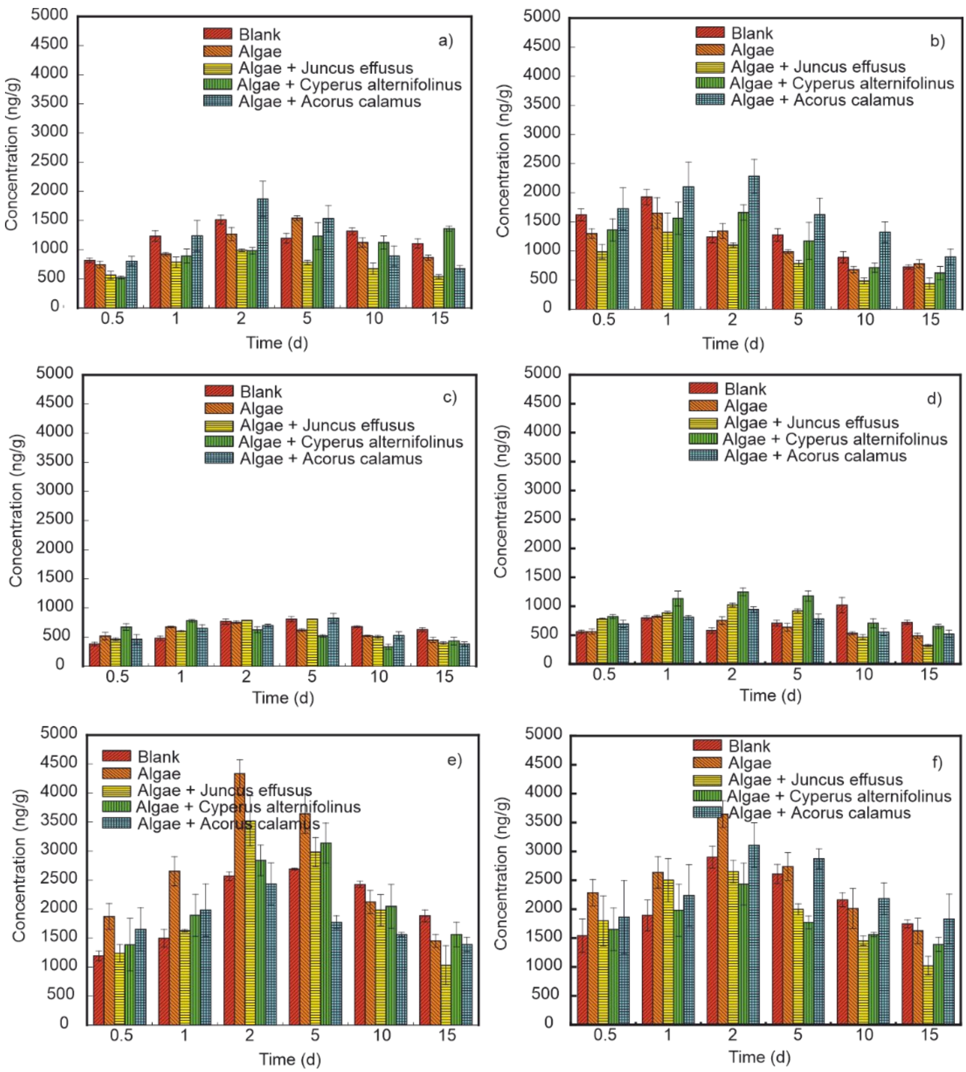


Fig. 3. Concentrations of various antibiotics: a) AZM, b) CLM, c) STZ, d) SMZ, e) CIP, f) TCY in sediment within 15 days under ecological conditions of aquatic plants

STZ and SMZ showed lower concentrations in the sediment and weaker adsorptivity than AZM and CLM. STZ and SMZ were absorbed by the sediment and removed mainly through biodegradation and photodegradation. Therefore, in the middle and late stage of the experiment, the two sulphonamides showed a downward trend, but the trend was not very strong. Baran et al. [15] studied the toxicity and biodegradability of sulfamethoxazole and sulfadiazine and their photodegradation products in water. The results showed that the biodegradability of sulfamethoxazole drugs was poor. Richardson et al. [16]

confirmed the biodegradability of 12 sulphonamide's in an activated sludge. The biodegradation characteristics of some sulphonamides showed consistency, and the migration and conversion characteristics of the two sulphonamides in this study also showed consistency.

The tetracycline antibiotic TCY showed strong adsorption in the sediment. At the end of the experiment, the detection concentrations in the sediment of the five experimental groups were in the range of 1021–1829 ng/g. Figueroa et al. [17] showed that under a wide range of environmental conditions, tetracycline antibiotics have strong adsorption in sediments, and the adsorption mechanism is mainly ion exchange, which may be related to the nature of tetracycline antibiotics. Their concentration peaks at 48 h, amounted up to 3645 ng/g. After that, there is a downward trend, but tetracycline antibiotics are not prone to biodegradation, mainly caused by photodegradation.

The fluoroquinolone antibiotics (FQs) CIP detected a higher concentration in the sediment, and the concentration at the end of the experiment was 482–1562 ng/g, indicating that the adsorption in the sediment was stronger. Studies [18] have shown that, the migration of FQs to the lower layer is weak, and it is easy adsorbed on the surface layer of the sediment, which is related to the contribution of –COOH to the adsorption of FQs. The quinolone antibiotics have a strong adsorption ability, and they easy accumulate in the soil. The degradation of CIP in the environment mainly includes biodegradation and photodegradation; the concentration of CIP detected in the sediment begins to decrease after 48 hours of experimentation.

3.4. CONCENTRATION OF ANTIBIOTICS IN ALGAE AND PLANT TISSUES

Figure 4 shows the level of antibiotics in plant tissues. Water purification of plants used in this experiment is a common ecosystem of floating islands and planktonic algae. Since no antibiotics were detected in algae cells, the contribution of algae to the absorption of antibiotics was not considered. However, this does not mean that *Microcystis aeruginosa* did not contribute to the removal of the antibiotics from the system, which may be due to the rapid metabolism or degradation of antibiotics into other substances after they are absorbed by the algal cells. Aquatic plants are planted in a floating form on floating islands, and the roots are far away from the sediment. Therefore, the antibiotics adsorbed in the sediment cannot be directly transferred to the plants. The antibiotics desorbed in the sediment enter the water phase and are captured, adsorbed and absorbed by plant roots together with the original free antibiotics in water. The roots, stems and leaves of the plants were tested separately, and it was found that the antibiotics retained high in the roots of the plants, while less relatively in the stems and leaves.

For hydrophobic AZM and CLM, there may be a relatively low amount of plant uptake. Relatively low concentrations of AZM (root 28.8–39.6 ng/g, stem and leaf 12.3–17.41 ng/g), and CLM (root 31.4–39.6 ng/g, stem and leaf 18.3–22.4 ng/g) were found in aquatic plants, which is in line with expectations. In addition, they are more susceptible to biodegradation [1], therefore, the lower cumulative concentrations of AZM and CLM in plant tissues may reflect their high biodegradation potential in the short period of plant uptake. Their concentration in the roots is higher than in the stems and leaves, probably due to their weaker ability to migrate in plant tissues.

The hydrophilic drugs STZ and SMZ showed strong enrichment ability in various aquatic plants. The enrichment concentrations of STZ and SMZ in the roots of three aquatic plants, *Juncus effusus*, *Cyperus alternifolius* and *Acorus calamus*, were 198.9, 226.7, 332.5 ng/g and 178.4, 196.7, 221.3 ng/g, respectively. However, their enrichment concentrations in stem and leaf tissues were 29.3, 42.8 112.7, and 43.1, 33.8, 69.2 ng/g, respectively, which were much lower than in the roots. Boxall et al. [19] found that plants have a good enrichment effect on sulphonamides.

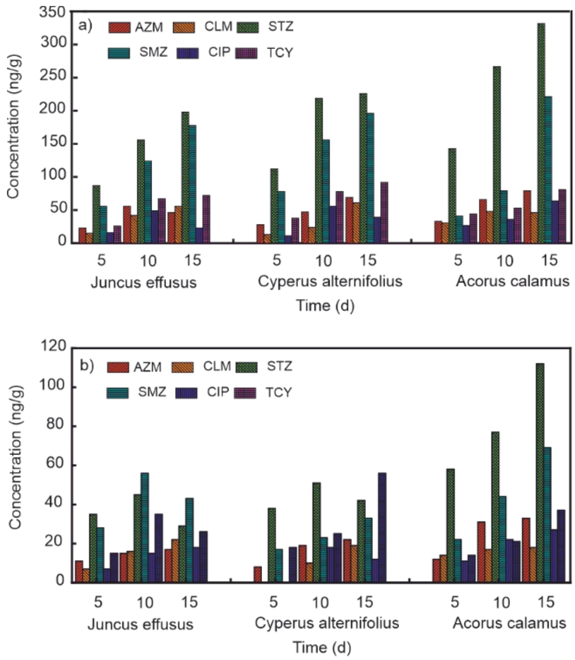


Fig. 4. Contents of antibiotics in the roots (a) and stems and leaves (b) of the three aquatic plants in various symbiotic system conditions at 5, 10 and 15 d (in triplicate)

CIP was quickly adsorbed by the sediments in the aquatic ecosystem. Lower concentrations of CIP were detected in the floating island plants, and the results were as expected (root 23.2–64.7 ng/g, stem and leaf 12.7–27.3 ng/g). Yu et al. [20] conducted examined the transfer of four FQs in the roots, branches and leaves of two dominant mangrove species, *Aegiceras corniculatum* and *Kandelia candel*, in Guangdong Bay, and found that FQs in their roots could be transferred to the aboveground part in large quantities and entered into the stems and leaves, which was consistent with the results presented in this study. Medium concentrations of TCY (root 72.7–92.7 ng/g, stem and leaf 26.8–56.7 ng/g) were detected in plant tissues.

3.5. ACTUAL REMOVAL RATE OF ANTIBIOTICS

The TREs of each antibiotic can be obtained using mass balance calculation. Figure 5 shows the efficiency of removal (TREs) of the examined antibiotics in various ecosystem conditions. For the macrolide antibiotics, the removal rate was the highest and amounted to 38.97–68.88% (AZM) and 31.28–61.96% (CLM), the experimental group of algae + *Juncus effusus* had the highest removal rate. Secondly, the removal rates of sulfa antibiotics STZ (35.71–54.55%) and SMZ (38.42–54.12%) were relatively good, while the removal rates of CIP (7.33–39.35%) and TCY (24.8–36.96%) were the lowest.

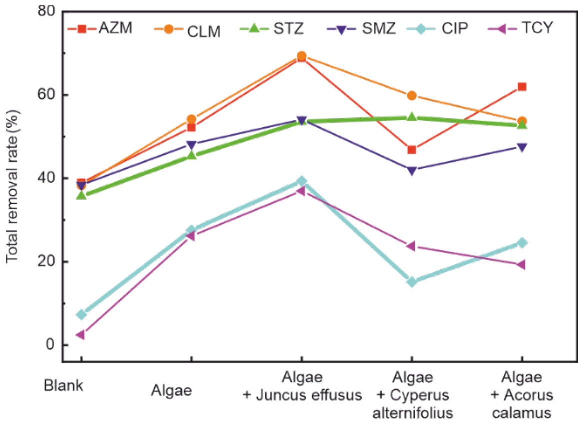


Fig. 5. Total removal efficiencies of antibiotics with different aquatic plants through mass balance calculations in the simulated urban river system (conducted in triplicate)

Compared with the system without algae and plants, the total removal rate increased by 25.5–39.6%. In the previous experiment [11], researchers investigated the removal rates of antibiotics in the presence of different aquatic plants, in which the removal rates of STZ, AZM and TC were 31.7–53.7%, 25.1–31.3%, and 17.4–29.0%, respectively. By comparison, the removal rate of antibiotics was greatly improved when *Microcystis aeruginosa* was added in this study.

4. DISCUSSION

Antibiotics can achieve higher TREs in growing conditions. In aqueous, sediment and plant tissues, the changes and differences in the examined antibiotics may be mainly attributed to hydrolysis, biodegradation, photolysis, adsorption, desorption and plant uptake enrichment. Cui et al. [21] reported that drugs may be adsorbed into aquatic plants through roots and then transferred to shoots and leaf tissues.

In this study, aquatic plants were planted on floating islands, and the roots of the plants were not in direct contact with the sediments, resulting in some antibiotics that were easily adsorbed by the sediment. At the beginning, the antibiotics were adsorbed in the sediments and rarely transferred to water or adsorbed by the plants, such as quinolone antibiotic CIP (deposition phase adsorption: 32.60–53.43%; plant absorption: 0.84–2.43%) and tetracycline antibiotic TCY (deposition phase adsorption: 34.93–59.69%; plant absorption 2.49–3.28%). In addition, roots and branches of aquatic plants can adsorb suspended particles and reduce the turbidity of water. Refraction and reflection occur when light strikes the suspended particles, while lower turbidity water accelerates the rate of photodegradation of the drug. Furthermore, aquatic plants provide the necessary reproductive environment and space for microorganisms, which indirectly accelerates the biodegradation of target antibiotics such as AZM and CLM.

In addition, various physicochemical properties of the antibiotics (Table 1) may also contribute to their differences in water, sediment and plant tissues. Dettenmaier et al. [22] found that high polarity ($\log K_{OW} < 1$) and water-soluble organic compounds showed a higher tendency to be absorbed by plant roots. In the experiment, the water-soluble organic substances STZ ($\log K_{OW} = 0.7$) and SMZ ($\log K_{OW} = 0.48$) showed poor adsorption in the sediment, but strong enrichment ability in various aquatic plants [14]. The highly hydrophobic drug ($\log K_{OW} > 4.0$) did not show significant differences throughout the experiment in the sediment.

The adsorption properties of CLM in sediments are slightly different in different experimental groups. In the experimental group, the adsorption of antibiotics in the deposition phase was higher, and the adsorption in the group of *Acorus calamus* was the highest (899 ng/g). This may be due to different adsorption procedures in different aquatic plant conditions. Compared with other aquatic plants, the roots of *Acorus calamus* are less developed. Studies have shown that the roots of aquatic plants can intercept some organic matter in sewage [23]. In addition, small aquatic organisms and microorganisms attached to the roots of plants maintain their normal physiological activities by decomposing or taking in organic substances such as antibiotics. Because the root system of *Acorus calamus* is relatively simple and has fewer types or microbes attached, the antibiotics adsorbed or enriched in it are more difficult to disappear, so more antibiotics are detected in *Acorus calamus* compared to the other two aquatic plants. Therefore, *Juncus effusus*, which has more developed roots of three aquatic plants, has the best effect on CLM removal, followed by *Cyperus alternifolius*, and the worst is *Acorus calamus*.

Adsorption capacity f STZ ($\log K_{OW} = 0.7$) and SMZ ($\log K_{OW} = 0.48$) in sediments is low, and the minimum TREs amount (35.71 and 38.42%) is lower than that of macrolide antibiotics AZM and CLM. Hydrophilic drugs seem to be adsorbed more difficult by sediment and are easily concentrated by plants.

CIP and TCY have lower TREs, which are 7.33–39.35% and 2.48–36.96%, respectively. The FQs have a larger adsorption coefficient (K_d), and stronger adsorption capacity, and are easy to accumulate in soil and sediments. Figueroa et al. [17] showed that in a wide range of environmental conditions, tetracycline antibiotics have strong adsorption in sediments, and the adsorption mechanism is mainly ion exchange, which may be related to their nature. Therefore, both CIP and TCY are easily adsorbed by the deposition phase. Since these antibiotics are quickly absorbed by the sediment after entering the aquatic environment, the shorter roots of floating island plants cannot capture CIP and TCY in time, so they are located in much lower amounts in plant tissues than STZ and SMZ, which have less adsorption. The presence of antibiotics was still not detected in the cells of *Microcystis aeruginosa*, probably due to the faster metabolism of cells.

Besides, photosynthesis and metabolism of aquatic plants and potential volatilization may also be an additional means of removing antibiotics. For example, Dordio et al. [24] detected a metabolite of carbamazepine from cattail tissue, indicating that some drugs were indeed converted to other products by catabolism within aquatic plants.

The synergistic effect of algae and aquatic plants may also be one of the reasons for the better removal effect of antibiotics. Xinjie et al. [25] effectively reduced the high concentration of ammonia nitrogen and phosphorus in swine wastewater by co-culture of plants and algae, and obtained the following synergistic mechanism of algae and plants: common utilization of nutrients; plant root respiration leads to acidification of wastewater, which reduces ammonia toxicity; the consumption of bicarbonate by algae reduces the toxicity of bicarbonate to plants; the oxygen released by algae photosynthesis reduces hypoxia stress and promotes plant growth.

It can be inferred that under the condition of plant-algae co-culture, the roots of plants will release carbon dioxide due to respiration, which will acidify the water body, while the decrease of pH will convert the original ammonia in water into ammonium ions and exist stably, reduce the toxicity, which is conducive to the growth of algae and other organisms in the water body. On the other hand, the normal growth of algae in the water is conducive to photosynthesis. In this process, it will release oxygen, help to increase the content of dissolved oxygen in water, and then promote the oxidative decomposition of organic matter and the respiration of plant roots. During the growth of algae, a large amount of carbon will be consumed. The consumption of bicarbonate reduces the toxicity of bicarbonate to plants and is also conducive to plant growth. Therefore, the effect of sewage treatment by co-culture of algae and plants has been greatly improved.

5. CONCLUSIONS

The removal effect of symbiotic systems composed of *Microcystis aeruginosa* and aquatic plants on antibiotics in water, sediment and plant tissue in urban rivers was examined. In water and sediment, antibiotics in the ecological conditions containing *Microcystis aeruginosa* and aquatic plants were present in lower concentrations than those without algae or plants. The residues of antibiotics were more in the roots of plants than in the stems and leaves. Overall, the removal rates of AZM and CLM in all ecological conditions were very high, reaching 68.88%. The system of *Microcystis aeruginosa* combined with *Cyperus alternifolius* had the best removal effect on the two sulfa antibiotics, and the highest removal rate reached 54.6%. For CIP and TCY that are difficult to be removed, the system of *Microcystis aeruginosa* combined with *Juncus effusus* had the best removal effect, with the highest removal rate of 39.3%.

The combined restoration of plants and algae is an effective process for removing frequently detected pharmaceuticals in urban water environments, and the symbiotic systems constructed by plants and algae can become an effective means to purify water quality and improve the urban river water environment.

ACKNOWLEDGEMENTS

This work was financially supported by the Key Projects of the Joint Fund of the National Natural Science Foundation of China (U2240205), and the USST Program of Science and Technology Development (2018KJFZ117).

REFERENCES

[1] TOPP E., RENAUD J., SUMARAH M., SABOURIN L., *Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin and azithromycin in agricultural soil following several years of exposure in the field*, Sci. Total Environ., 2016, 562, 136–144. DOI: 10.1016/j.scitotenv.2016.03.210.

[2] ZHOU H., YING T., WANG X., LIU J., *Occurrence and preliminarily environmental risk assessment of selected pharmaceuticals in the urban rivers*, China, sci. Rep. UK, 2016, 6, 34928. DOI: 10.1038 /srep34928.

[3] ZUO R., LIU X., ZHANG Q., WANG J., YANG J., TENG Y., CHEN X., ZHAI Y., *Sulphonamide antibiotics in groundwater and their migration in the vadose zone: A case in a drinking water resource*, Ecol. Eng., 2021, 162, 106175. DOI: 10.1016/j.ecoleng.2021.106175.

[4] SENTA I., TERZIC S., AHEL M., *Analysis and occurrence of macrolide residues in stream sediments and underlying alluvial aquifer downstream from a pharmaceutical plant*, Environ. Pollut., 2021, 273, 116433. DOI: 10.1016/j.envpol.2021.116433.

[5] NATARAJAN S., STAMPS R.H., MA L.Q., *Phytoremediation of arsenic-contaminated groundwater using arsenic hyperaccumulator Pteris vittata L. effects of frond harvesting regimes and arsenic levels in refill water*, J. Hazard. Mater., 2011, 185, 983–989. DOI: 10.1016/j.jhazmat.2010.10.002.

[6] SU Y., *Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment*, Sci. Total Environ., 2021, 762, 144590. DOI: 10.1016/j.scitotenv.2020.144590.

[7] LI S., SHOW P.L., NGO H.H., HO S.-H., *Algae-mediated antibiotic wastewater treatment: A critical review*, Environ. Sci. Ecotechnol., 2022, 9, 100145. DOI: 10.1016/j.ese.2022.100145.

[8] CHAN S.S., KHOO K.S., CHEW K.W., LING T.C., SHOW P.L., *Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium. A review*, Bioresour. Technol., 2022, 344, 126159. DOI: 10.1016/j.biortech.2021.126159.

[9] CUELLAR-BERMUDEZ S.P., ALEMAN-NAVA G.S., CHANDRA R., GARCIA-PEREZ J.S., CONTRERAS--ANGULO J.R., MARKOU G., MUYLAERT K., RITTMANN B.E., PARRA-SALDIVAR R., *Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater*, Algal Res., 2017, 24, 438–449. DOI: 10.1016/j.algal.2016.08.018.

[10] ZHOU H., CHEN X., YING T., XUAN Y., WANGJIN Y., LIU X., *Variations and behavior of wastewater-marking pharmaceuticals influenced under hydrodynamic conditions in urban river systems*, Int. J. Environ. Sci. Technol., 2019, 16, 5669–5684. DOI: 10.1007/s13762-018-2038-7.

[11] ZHOU H., LIU X., CHEN X., YING T., YING Z., *Characteristics of removal of waste-water marking pharmaceuticals with typical hydrophytes in the urban rivers*, Sci. Total Environ., 2018, 636, 1291–1302. DOI: 10.1016/j.scitotenv.2018.04.384.

[12] NIU X., FAN M., CHANG J., *Organ matter accumulation in a constructed wetland during operation*, Acta Ecol. Sin., 2002, 22, 1240–1246.

[13] SUDIARTO S.I.A., RENGGAMAN A., CHOI H.L., *Floating aquatic plants for total nitrogen and phosphorus removal from treated swine wastewater and their biomass characteristics*, J. Environ. Manage., 2019, 231, 763–769. DOI: 10.1016/j.jenvman.2018.10.070.

[14] WANG W., QU X., LIN D., YANG K., *Octanol–water partition coefficient (logK_{OW}) dependent movement and time lagging of polycyclic aromatic hydrocarbons (PAHs) from emission sources to lake sediments: A case study of Taihu Lake, China*, Environ. Pollut., 2021, 288, 117709. DOI: 10.1016/j.envpol.2021.117709.

[15] BARAN W., SOCHACKA J., WARDAS W., *Toxicity and biodegradability of sulphonamides and products of their photocatalytic degradation in aqueous solutions*, Chemosphere, 2006, 62, 1295–1299. DOI: 10.1016/j.chemosphere.2006.04.040.

[16] RICHARDSON B.J., LAM P.K.S., MARTIN M., *Emerging chemicals of concern: pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China*, Mar. Pollut. Bull., 2005, 50, 913–920. DOI: 10.1016/j.marpolbul.2005.06.034.

[17] FIGUEROA R.A., LEONARD A., MACKAY A.A., *Modeling tetracycline antibiotic sorption to clays*, Environ. Sci. Technol., 2004, 38, 476–483. DOI: 10.1021/es0342087.

[18] ZHANG J., DONG Y., *Effect of low-molecular-weight organic acids on the adsorption of norfloxacin in typical variable charge soils of China*, J. Hazard. Mater., 2008, 151, 833–839. DOI: 10.1016/j.jhazmat.2007.11.046.

[19] BOXALL A.B.A., JOHNSON P., SMITH E.J., *Uptake of veterinary medicines from soils into plants*, J. Agr. Food Chem., 2006, 54, 2288–2297. DOI: 10.1021/jf053041t.

[20] YU F., SUN Q., LIU Y., PENG Y., REN K., *Study on the absorption and transport characteristics of fluoroquinolones (FQs) antibiotics in mangrove organs of Zhenhaiwan, Guangdong province*, J. Environ. Sci., 2017, 37, 4427–4435.

[21] CUI H., HENSE B.A., MULLER J., SCHRODER P., *Short-term uptake and transport process for metformin in roots of Phragmites australis and Typha latifolia*, Chemosphere, 2015, 134, 307–312.

[22] DETTENMAIER E.M., DOUCETTE W.J., BUGBEE B., *Chemical hydrophobicity and uptake by plant roots*, Environ. Sci. Technol., 2009, 43, 324–329.

[23] KIM Y., KIM W., R*oles of water hyacinths and their roots for reducing algal concentration in the effluent from waste stabilization ponds*, Water Res., 2000, 34, 3285–3294.

[24] DORDIO A.V., BELO M., MARTINS TEIXEIRA D., PALACE CARVALHO A.J., DIAS C.M., PICO Y., PINTO A.P., *Evaluation of carbamazepine uptake and metabolization by Typha spp., a plant with potential use in phytotreatment*, Biores. Technol., 2011, 102, 7827–7834.

[25] XINJIE W., XIN N., QILU C., LIGEN X., YUHUA Z., QIFA Z., *Vetiver* and *Dictyosphaerium sp. co-culture for the removal of nutrients and ecological inactivation of pathogens in swine wastewater*, J. Adv. Res., 2019, 20, 71–78. DOI: 10.1016/j.jare.2019.05.004.

[26] KOSMA C., LAMBROPOULOU D.A., ALBANIS T.A., *Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece*, J. Hazard. Mater., 2010, 179, 804–817. DOI: 10.1016/j.jhazmat.2010.03.075.
[27] KIM Y., CHOI K., JUNG J., PARK S., KIM P., PARK J., *Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulphonamides, and their potential ecological risks in Korea*, Environ. Int., 2007, 33, 370–375. DOI: 10.1016/j.envint.2006.11.017.