

Article

Phytotoxicity and Accumulation of Antibiotics in Water Lettuce (*Pistia stratiotes*) and Parrot Feather (*Myriophyllum aquaticum*) Plants under Hydroponic Culture Conditions

Young-Jae Park¹ and Jae-Gwon Son^{2,*}¹ Horse Industry Complex Center, Jeonju Kijeon College, Jeonju 54989, Korea; song352@naver.com² Department of Rural Construction Engineering, Jeonbuk National University, Jeonju 54906, Korea

* Correspondence: sjg@jbnu.ac.kr

Abstract: The aim of this research was to investigate the accumulation of antibiotics in two kinds of plants. The hydroponic culture solution included a mixture of antibiotics, including three commonly used antibiotics in South Korea: norfloxacin (NOR), sulfamethazine (SMZ), and tetracyclines (TC). None of these antibiotics were detected in the shoots of water lettuce plants, only in the roots. However, in parrot feather plants, antibiotics were detected in both the shoots and the roots, with higher amounts detected in the shoots than in the roots. SMZ and TC were most likely to be detected in the roots and shoots of water lettuce and parrot feather plants, and about one-third of the NOR administered was later detected in the plants. The BCF (bioconcentration factor) of antibiotics ranged from 0.24 to 0.78, while that of NOR was much lower, ranging from 0.24 to 0.38. The SMZ (0.59–0.64) and TET (0.72–0.78) exhibited higher uptake accumulation in the water lettuce tissues compared with the parrot feather plants.

Keywords: antibiotics; parrot feather; phytoremediation; water lettuce; water plants



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1. Introduction

Phytoremediation is a technique that utilizes plants to remove or transform toxic chemicals incorporated in soils, ground water, and surface water. Various plants are used for removal or degradation of inorganic and organic contaminants, such as pesticides, antibiotics, heavy metals, and nutrients.

Phytoremediation techniques are used extensively because they have a number of advantages. Phytoremediation is low cost compared with conventional techniques and is suitable for treating very large land areas [1]. Furthermore, these techniques may be more effective than conventional techniques based on chemical extraction because the use of plants for environmental cleanup does not cause secondary pollution. However, phytoremediation also has limitations. A significant disadvantage of phytoremediation by aquatic plants is their shallow root systems, which limit the depth of soil treatment by the rhizosphere. Another disadvantage is the slow rate of environmental cleanup, which may take more than 10 years [2]. Phytoremediation effectiveness decreases during winter, and plant diseases or pests may occur in the condition of plant damage [3].

For decades, a wide variety of antibiotics has been extensively applied worldwide for preventing or treating human, animal, and plant infections and as feed additives for animals to prevent or treat diseases and promote growth [4]. Consumption of veterinary antibiotics has been increasing in both industrialized and developing countries. However, in Korea, the total production of antibiotics decreased significantly from 1500 tons in 2007 to 820 tons in 2013. This dramatic reduction in use of veterinary antibiotics was due to restrictions on their use for feed supplementation. Tetracyclines, sulfonamides, and penicillin are the most commonly used antibiotic types, with usage of 165, 60, and 160 tons, respectively, in 2014. These antibiotics are commonly found in both aquatic and agricultural

environments, associated with the discharge of wastewater to the environment. Despite the low concentrations usually found in water ecosystems (from nanograms to micrograms per liter), continuous discharge of antibiotics and their degradation products through different pathways makes them 'pseudo-persistent' in the environment [5].

Macrophytes accumulate pollutants by incorporating them into the structures of their cells. In addition, these plants have a natural ability to absorb and metabolize xenobiotics while adapting to harsh conditions in a contaminated environment [6]. Some studies have used aqueous solution amended with different antibiotics to address plant uptake. The water plants *Myriophyllum aquaticum* (parrot feather) and *Pistia stratiotes* (water lettuce) were previously used for the study of phytoremediation of tetracyclines and oxytetracyclines in aqueous media [7]. Comparable levels of antibiotic removal by filtered root exudates from these two species suggested involvement of root-secreted enzymes/metabolites in degrading/transforming the antibiotics [7].

The aim of the present study was to investigate the accumulation of antibiotics in two kinds of plants and to survey the phytotoxicity of antibiotics. The study was performed under hydroponic culture conditions to avoid potentially confounding effects of adsorption of antibiotics on soil particles [8]. The hydroponic culture solution contained a mixture of three commonly used antibiotics in South Korea: norfloxacin, sulfamethazine, and tetracyclines.

2. Materials and Methods

2.1. Antibiotics and Chemicals

Target antibiotic compounds were selected based on frequency of detection in the environment and physicochemical differences. In this study, tetracycline of the tetracycline type and sulfamethazine of the sulfonamide type, which are widely used in South Korea, were first selected as target antibiotics. In addition, norfloxacin, a quinolone type, was frequently detected in sewage wastewater and was additionally selected. We examined tetracyclines (TC), sulfamethazine (SMZ), and norfloxacin (NOR), which belong to three different antibiotic classes. All antibiotic standards and some internal standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). All organic solvents used were HPLC grade and purchased from Merck Corporation (Darmstadt, Germany). Stock solutions and internal standards were prepared at 100 mg/L in methanol and stored in amber glass vials at 4 °C.

2.2. Experimental Design

Selection of plants is important for achieving significant phytoremediation in aquatic ecosystems. The plants must be able to tolerate pollution and be easy to grow and fast growing. Prior to the experiments, 2 different types of water plants that have been used in wetland systems were cultured for 1 month in modified Hoagland's nutrient solution for acclimatization at a nearby plant nursery. We then obtained the plants and washed them to remove sediment particles. Identical plants were assigned to 9 glass containers, each containing 10 individuals arranged in parallel rows. We covered the surface of each container with silver foil to prevent the photodegradation of the antibiotics and to mitigate the effects of sunlight on the root systems. Throughout the study, hydroponic culture systems were supplied with 5 L Hoagland's nutrient solution fortified with the test antibiotics, the pH was adjusted to 6.5. The experiments took place in a greenhouse environment (12 h photoperiod, 30/25 ± 2 °C day/night), and all plants were treated for 30 days with the following concentrations of TC, SMZ, or NOR in nutrient solution: 0, 1, 5, and 10 mg/L. The Hoagland's nutrient solution was prepared as described by Dordio et al. [9]. The antibiotics in wastewater were frequently detected and ranged from ng/L up to lower µg/L (norfloxacin: 2.775 µg/L; sulfamethazine: 1.280 µg/L; and tetracycline: 0.412 µg/L; respectively) [10,11]. However, in this study, antibiotics were treated at relatively high concentrations to search for antibiotic hyperaccumulator water plants. We measured the following phytotoxicity parameters: leaf chlorophyll and fresh

and dry weights of the water plants. At the end of the treatment period, samples of roots and shoots were obtained from the same pot. Roots were carefully washed in tap water. Plant materials were dried for 2 days at 60 °C and then frozen until analyzed. Control and treated plant materials were dried in separate drying ovens to prevent cross-contamination. The distributions of antibiotics in the hydroponic culture solution and water plants were analyzed.

2.3. Assay of Phytotoxicity Parameters of Water Plants

Chlorophyll contents (Chl a, Chl b and total Chl) of water plants were measured according to the method described by Huang et al. (2004) [12]. Plant leaves (0.2 g/3 parallel individuals in each container) were cut into 0.2 cm segments and treated in 80% (v/v) acetone for 24 h in the dark. Absorbance of the solutions was measured with a spectrophotometer (DR 5000, HACH, Loveland, CO, USA) at 663 nm and 645 nm. After 30 days, the plants were harvested and washed with tap water and distilled water. Fresh weights of the plants were determined for each treatment, and dry weights of shoots and roots were determined after drying at 65 °C for 48 h.

2.4. Antibiotic Analysis in Water and Plant Samples

Liquid chromatography in combination with tandem mass spectrometry (LC–MS/MS) was used to analyze the antibiotics in the plant and water samples. Prior to extraction, the water was filtered through Whatman no. 1 filter paper to eliminate the suspended matter and then filtered through a 0.45 µm mixed cellulose membrane. The pH of water samples was adjusted to 4.0 with 1 mol/L H₂SO₄. The antibiotics were extracted in 250 mL of filtered water. The plant samples were freeze-dried and ground to 80 mesh. A mixed solution (5 mL) containing phosphate buffer, acetonitrile, and the plant samples was added to each centrifuge tube. The tubes were shaken in a shaker at 250 rpm for 30 min, sonicated for 10 min, and then centrifuged at 12,000 × g for 10 min.

The extraction process was performed 3 times for each sample. The samples were extracted using Oasis HLB extraction cartridges (500 mg, 6 mL, Waters, Milford, MA, USA) in the following manner. Each cartridge was sequentially pre-conditioned with 6.0 mL acetone, 6.0 mL methanol, and 6.0 mL 0.1% formic acid solution. The samples were passed through an extraction cartridge at a rate of 3 mL/min. Subsequently, the cartridge was rinsed with 5 mL 0.1% formic acid solution containing 5 mmol/L ammonium acetate and then eluted with 5.0 mL methanol. Finally, the target fraction was re-dissolved in 10% methanol solution to a final volume of 1.0 mL for LC–MS/MS (ABI 3200 Q TRAP) analysis. The antibiotics were analyzed using a LC–MS/MS system with an Inertsil® ODS-SP column for separation. The chromatographic mobile phase consisted of methanol (A) and 0.1% formic acid solution dissolved with 5 mmol/L ammonium acetate (B). The column temperature was set to 40 °C. The target antibiotics were detected by electrospray tandem mass spectrometry in positive-ion mode. Data acquisition was performed by multiple reaction monitoring (MRM), recording two MRM sessions per compound. The optimal conditions are summarized in Table 1. The correlation coefficients (R^2) of the calibration curves exceeded 0.99 for all analytes. Recoveries of the antibiotics in the plant and water samples were determined at different concentrations in triplicate and calculated as percentages of the measured concentration, relative to the spiked concentration. Limits of quantification (LOQ) of the antibiotics were calculated with signal–noise ratios of 10. Retention time, correlation coefficient, LOQ, and recovery of the samples are summarized in Table 2.

Table 1. Optimum LC–MS/MS parameters of precursor and product ions and collision energy (CE).

Antibiotics	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
NOR	152	110.0	16
TC	445	410.0	25
SMZ	279	186.1	24

NOR—norfloxacin; SMZ—sulfamethazine; TC—tetracycline.

Table 2. Retention time, correlation coefficient, recovery, and limit of quantification (LOQ) of samples.

Antibiotics	Retention Time	R ²	LOQ (ng/g)	Recovery (%)	
				Plant	Water
NOR	3.52	0.99	2.59	80.1 ± 2.8	91.2 ± 3.2
TC	7.49	0.99	5.17	74.1 ± 3.6	84.6 ± 2.0
SMZ	7.68	0.99	1.54	79.9 ± 2.2	88.7 ± 3.2

2.5. Bioconcentration Factor

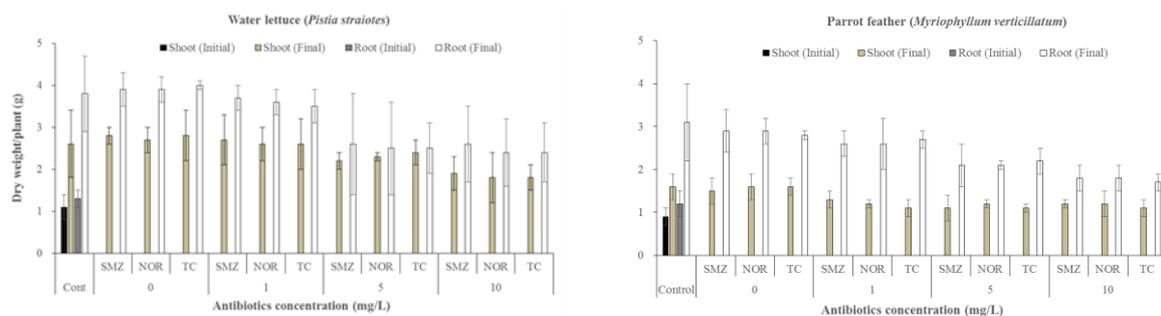
To evaluate the abilities of the two water plant species to extract and accumulate antibiotics into plant tissues, the bioconcentration factor (BCF) was determined for roots, according to the method described by Zayed et al. [13], as follows:

$$\text{BCF} = \frac{\text{Pollutant concentration in plant tissue at harvest (mg/kg)}}{\text{Initial concentration in the external growth medium (}\frac{\text{mg}}{\text{L}}\text{)}}$$

3. Results and Discussion

3.1. Effects of Antibiotics on Growth of Water Plants

The objective of this study was to identify water plant species capable of efficient removal of antibiotics from aquatic environments without adverse effects on their growth and morphology. During the 30-day experimental period using 1–5 ppm treatment concentrations, we noticed no visible signs of detrimental effects or yellow leaves in either species. The growth of plants in the hydroponic culture solution was good, as demonstrated by significantly greater biomass yield at the end than at the beginning of the experiment (Figure 1). By the 10th day of the experiment, the containers were completely covered by plants. However, at 10 ppm treatment concentrations, plant growth was inhibited by 40–50%. In particular, we observed chlorosis and early abscission of leaves. Neither water lettuce nor parrot feather plants showed difference in fresh plant weight according to type of antibiotic used. The fresh weight of plants decreased in a concentration-dependent manner as antibiotic concentration increased (Figure 2).

**Figure 1.** Dry weights of water lettuce and parrot feather under various treatments at the end of the experimental period.

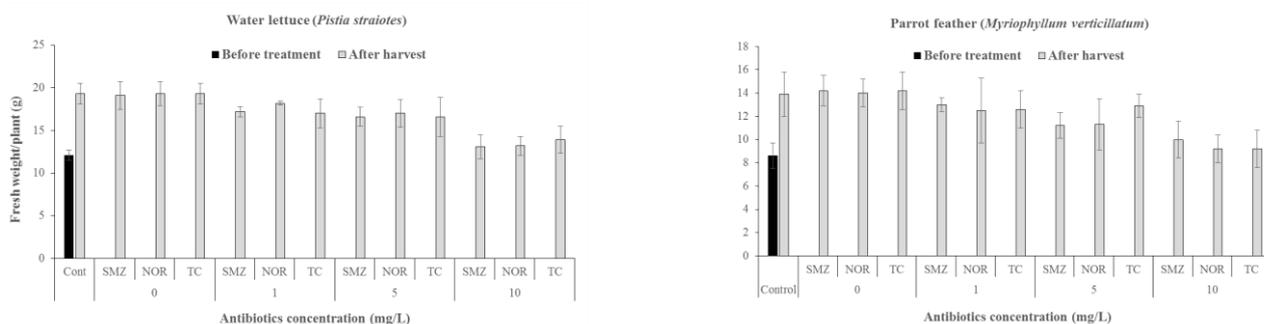


Figure 2. Fresh weights of water lettuce and parrot feather plants under various treatments at the end of the experimental period.

3.2. Effects of Antibiotics on Chlorophyll Content

Plant chlorophyll content is an important parameter for evaluating photosynthetic activity, and such measurements can be used as indicators of pollutant-induced plant stress [12]. Plant total chlorophyll, chlorophyll a, and chlorophyll b were all affected by antibiotic treatment. At the end of the treatment period, inhibition rates of total chlorophyll content of the 2 water plants for control, 1 ppm, 5 ppm, and 10 ppm treatments were 3.1–5.9%, 13.3–16.8%, 33.9–44.2%, and 41.5–45.2%, respectively (Tables 3 and 4). Antibiotics administered at concentrations greater than 5 mg/L seriously disrupted the normal chlorophyll content of water lettuce and parrot feather plants. The inhibition rate of chlorophyll b was higher than that of chlorophyll a after antibiotic treatment. Plant total chlorophyll, chlorophyll a, and chlorophyll b did not significantly differ between the water lettuce and parrot feather plants. The inhibition rates of chlorophyll in water plants increased as antibiotic concentrations increased, possibly because organic pollutants block electron transport flow from photosystem II to photosystem I, thereby inhibiting plant biosynthesis of chlorophyll b [11].

3.3. Antibiotic Residues in Aqueous Solution

Antibiotic concentrations in hydroponic culture solution under various treatment concentrations during the experiment period are shown in Figures 3 and 4. In water lettuce plants, SMZ and TC were lower than the limit of detection in aqueous solution from 21 days after treatment. However, 60% of the treated amount of NOR remained in aqueous solution. The water solubilities of treated antibiotics were SMZ 1500 mg/L H₂O (25 °C), TC 231 mg/L H₂O (25 °C), and NOR 0.28 mg/mL H₂O (25 °C). The high residual amount of NOR in aqueous solution is presumably due to water solubility. It was previously reported that absorption, abiotic transformation, and biotic transformation are major processes that antibiotics undergo in aquatic environments [14]. Hydrolysis and biodegradation also comprise an important degradation pathway for organic pollutants in aquatic ecosystem. In the present study, treated water plants showed greater removal efficiency of antibiotics compared with the control group (data not shown). We conclude that water plants play an important role in reduction in antibiotic contamination in aquatic environments, especially SMZ and TC. Parrot feather plants showed similar trends to water lettuce plants.

Table 3. Effects of antibiotics on concentrations of Chl a, Chl b, and total Chl in leaf tissues of water lettuce (*Pistia stratiotes*). Values are the mean ± S.D. of three parallel individuals in each container, with three measurements from each sample.

Item	DAT	Inhibition Rates of Chlorophyll (%)											
		0			1 mg/L			5 mg/L			10 mg/L		
		SMZ	NOR	TC	SMZ	NOR	TC	SMZ	NOR	TC	SMZ	NOR	TC
Chl a	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	−3.1 ± 0.1	−1.8 ± 0.2	0	−10	−9
	7	0	0	0	−6.9 ± 0.6	−3.0 ± 0.1	−1.2 ± 0.1	−6.5 ± 0.3	−10.5 ± 0.6	−12.9 ± 0.3	−21.3 ± 3.1	−24.4 ± 3.1	−32.9 ± 1.4
	14	−3.8 ± 0.2	−2.2 ± 0.1	−1.6 ± 0.2	−8.5 ± 0.4	−3.3 ± 0.2	−6.5 ± 0.2	−15.5 ± 1.1	−15.3 ± 1.5	−10.3 ± 1.1	−30.0 ± 2.0	−32.5 ± 2.9	−39.8 ± 2.0
	21	−3.9 ± 0.5	−3.8 ± 0.4	−3.7 ± 0.3	−13.1 ± 0.9	−8.2 ± 0.6	−6.0 ± 0.6	−25.6 ± 5.6	−16.3 ± 1.1	−16.6 ± 2.0	−35.3 ± 3.1	−30.1 ± 6.9	−42.2 ± 1.3
	30	−4.6 ± 0.3	−2.6 ± 0.3	−3.2 ± 0.3	−15.3 ± 1.2	−10.1 ± 1.9	−14.9 ± 1.9	−31.3 ± 6.2	−26.1 ± 5.6	−31.6 ± 2.3	−42.9 ± 3.9	−43.6 ± 3.4	−50.4 ± 3.5
Chl b	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	−1.9 ± 0.3	−3.0 ± 0.3	−3.4 ± 0.3	−2.3 ± 0.1	−3.8 ± 0.9	−3.2 ± 0.2	−4.2 ± 0.2	−5.2 ± 0.3	−5.3 ± 0.4
	7	0	0	0	−9.6 ± 0.6	−8.9 ± 1.2	−9.3 ± 0.3	−14.9 ± 2.5	−19.8 ± 1.1	−16.5 ± 1.5	−20.3 ± 1.4	−16.4 ± 0.9	−14.4 ± 1.0
	14	−1.2 ± 0.1	0	0	−11.4 ± 0.2	−15.2 ± 1.9	−16.3 ± 1.0	−32.4 ± 3.5	−25.3 ± 2.5	−29.9 ± 3.2	−33.4 ± 1.2	−31.6 ± 1.2	−22.1 ± 0.6
	21	−2.3 ± 0.1	−2.2 ± 0.1	−3.8 ± 0.1	−25.6 ± 0.9	−21.3 ± 3.6	−18.8 ± 1.9	−36.0 ± 5.2	−29.1 ± 3.3	−33.3 ± 3.0	−36.8 ± 2.2	−32.0 ± 2.9	−30.9 ± 2.9
	30	−3.7 ± 0.2	−3.7 ± 0.4	−4.3 ± 0.3	−26.2 ± 0.5	−22.5 ± 4.1	−21.6 ± 3.5	−41.3 ± 5.1	−32.5 ± 3.6	−39.8 ± 4.2	−36.9 ± 2.5	−34.1 ± 3.3	−33.2 ± 3.6
Tot Chl	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	−2.2 ± 0.1	−3.9 ± 0.6	−3.3 ± 1.0	−3.3 ± 0.6	−4.3 ± 0.1	−4.1 ± 0.2
	7	0	0	0	−5.4 ± 0.4	−6.1 ± 0.3	−5.1 ± 0.1	−5.6 ± 0.1	−12.1 ± 0.1	−9.8 ± 0.3	−18.2 ± 0.9	−26.3 ± 0.9	−29.6 ± 0.9
	14	0	−1.3 ± 0.2	−1.1 ± 0.1	−9.3 ± 0.2	−12.9 ± 0.3	−11.1 ± 0.6	−24.6 ± 0.6	−21.3 ± 3.3	−20.2 ± 1.3	−26.1 ± 1.5	−30.9 ± 0.6	−28.2 ± 0.3
	21	−3.4 ± 0.2	−3.2 ± 0.2	−4.3 ± 0.2	−13.9 ± 1.3	−13.3 ± 0.9	−12.9 ± 0.2	−29.1 ± 0.9	−25.5 ± 3.6	−26.6 ± 1.3	−41.6 ± 1.9	−38.2 ± 1.5	−33.6 ± 1.9
	30	−3.6 ± 0.5	−3.1 ± 0.1	−5.2 ± 0.5	−16.8 ± 1.0	−15.5 ± 1.9	−16.3 ± 0.5	−36.1 ± 0.2	−35.6 ± 5.2	−39.5 ± 3.0	−42.2 ± 1.8	−42.6 ± 2.9	−45.2 ± 2.1

DAT—days after treatment.

Table 4. Effects of antibiotics on concentrations of Chl a, Chl b, and total Chl in leaf tissues of parrot feather (*Myriophyllum verticillatum*). Values are the mean \pm S.D. of three parallel individuals in each container, with three measurements from each sample.

Item	DAT	Inhibition Rates of Chlorophyll (%)											
		0			1 mg/L			5 mg/L			10 mg/L		
		SMZ	NOR	TC	SMZ	NOR	TC	SMZ	NOR	TC	SMZ	NOR	TC
Chl a	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	-1.0 \pm 0.2	-1.1 \pm 0.1	-1.9 \pm 0.3	-3.7 \pm 0.1	-3.1 \pm 0.1
	7	0	0	0	-2.1 \pm 0.1	-2.3 \pm 0.4	-1.6 \pm 0.2	-2.9 \pm 0.1	-9.3 \pm 0.4	-3.2 \pm 0.1	-5.5 \pm 1.1	-20.5 \pm 1.1	-26.1 \pm 2.9
	14	-1.2 \pm 0.1	-1.6 \pm 0.1	-1.1 \pm 0.1	-3.9 \pm 0.2	-5.1 \pm 0.1	-5.3 \pm 0.3	-9.3 \pm 0.6	-11.4 \pm 0.9	-9.8 \pm 0.2	-11.4 \pm 1.3	-24.4 \pm 1.9	-26.9 \pm 3.9
	21	-3.0 \pm 0.3	-3.1 \pm 0.1	-3.2 \pm 0.1	-7.4 \pm 0.3	-11.6 \pm 0.9	-7.2 \pm 0.1	-13.2 \pm 2.3	-19.1 \pm 1.3	-19.3 \pm 0.6	-18.9 \pm 2.6	-29.6 \pm 2.6	-31.2 \pm 2.5
	30	-4.1 \pm 0.9	-3.2 \pm 0.2	-4.3 \pm 0.2	-9.5 \pm 0.9	-15.9 \pm 1.2	-13.3 \pm 0.6	-19.9 \pm 1.9	-26.0 \pm 3.4	-26.4 \pm 1.9	-29.6 \pm 2.9	-32.5 \pm 2.5	-34.9 \pm 3.4
Chl b	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	-2.2 \pm 0.1	-1.1 \pm 0.2	-2.1 \pm 0.2	-2.1 \pm 0.1	-6.5 \pm 0.1	-4.1 \pm 0.9	-6.6 \pm 0.2
	7	0	0	0	-2.1 \pm 0.6	-3.6 \pm 0.4	-6.0 \pm 0.5	-3.2 \pm 0.5	-12.9 \pm 0.5	-15.9 \pm 2.2	-16.2 \pm 0.3	-16.0 \pm 1.1	-10.1 \pm 1.8
	14	0	0	0	-6.3 \pm 0.2	-10.1 \pm 1.2	-10.1 \pm 0.2	-19.6 \pm 1.1	-21.4 \pm 0.9	-19.1 \pm 2.1	-29.9 \pm 0.9	-30.9 \pm 1.9	-20.9 \pm 1.1
	21	0	-2.5 \pm 0.2	-4.6 \pm 0.6	-9.1 \pm 0.9	-19.9 \pm 3.0	-13.6 \pm 1.2	-30.2 \pm 1.9	-41.2 \pm 2.9	-32.2 \pm 1.5	-29.1 \pm 1.1	-39.2 \pm 2.1	-41.2 \pm 2.0
	30	0	-1.3 \pm 0.1	-2.1 \pm 0.1	-19.9 \pm 0.5	-26.3 \pm 2.9	-20.1 \pm 2.1	-36.6 \pm 3.2	-45.9 \pm 4.1	-29.1 \pm 2.9	-42.0 \pm 1.9	-39.8 \pm 2.5	-41.0 \pm 2.9
Tot Chl	1	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	-2.9 \pm 0.1	-3.1 \pm 0.2	-3.0 \pm 0.1	-3.1 \pm 0.1	-3.6 \pm 0.6	-4.9 \pm 0.4
	7	0	0	0	-5.0 \pm 0.3	-6.0 \pm 0.1	-5.9 \pm 0.3	-5.2 \pm 0.3	-12.9 \pm 0.9	-8.1 \pm 0.6	-17.1 \pm 0.3	-25.4 \pm 1.8	-23.5 \pm 0.2
	14	0	-1.1 \pm 0.1	-1.5 \pm 0.2	-9.2 \pm 0.1	-12.1 \pm 0.2	-12.2 \pm 0.5	-23.1 \pm 0.2	-30.1 \pm 1.5	-21.4 \pm 0.6	-23.2 \pm 2.1	-31.2 \pm 2.9	-29.7 \pm 3.2
	21	-3.9 \pm 0.6	-3.3 \pm 0.5	-4.4 \pm 0.3	-13.1 \pm 0.6	-12.6 \pm 0.9	-13.0 \pm 0.5	-29.9 \pm 1.5	-33.1 \pm 2.6	-25.6 \pm 3.5	-40.9 \pm 3.3	-37.1 \pm 2.2	-30.3 \pm 2.1
	30	-3.3 \pm 0.2	-3.9 \pm 0.2	-5.9 \pm 0.2	-14.1 \pm 0.5	-13.3 \pm 2.1	-16.1 \pm 0.9	-44.2 \pm 1.3	-33.9 \pm 3.8	-41.1 \pm 2.9	-41.5 \pm 2.9	-42.5 \pm 4.2	-42.9 \pm 4.3

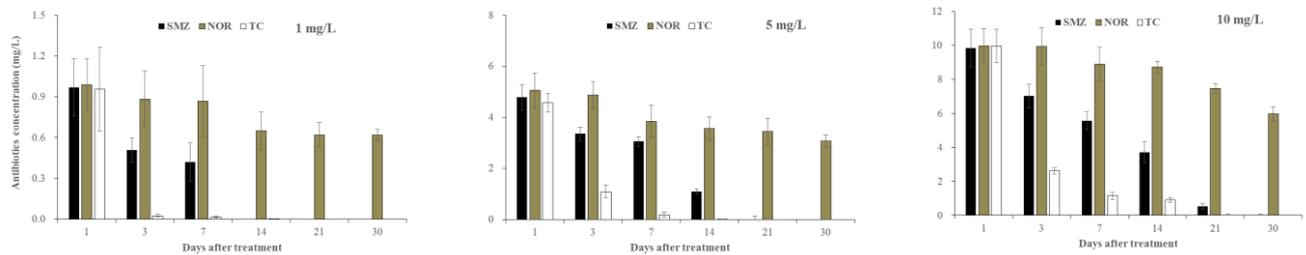


Figure 3. Selected antibiotic concentrations in hydroponic culture solution (water lettuce) under various treatments at the end of the experimental period.

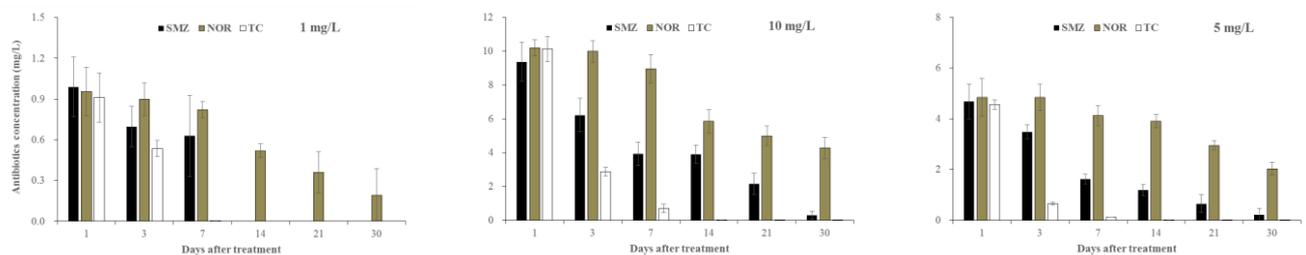


Figure 4. Selected antibiotic concentrations in hydroponic culture solution (parrot feather) under various treatments at the end of the experimental period.

3.4. Uptake Translocation of Antibiotics into Water Lettuce and Parrot Feather

In the harvest stage of the plant, we analyzed the SMZ, NOR, and TC contents of water lettuce and parrot feather. The results clearly indicated that the three antibiotics were taken up by both plants. Further, the uptake translocation of antibiotics was positively correlated with antibiotic concentration, a finding that is similar to those of previous studies [15,16]. None of the three antibiotics were detected in the shoots of the water lettuce plants, only in the root. However, in the parrot feather plants, antibiotics were detected in both the shoots and the roots, with higher amounts detected in the shoots than in the roots. Most SMZ and TC were detected in the roots and shoots of the water lettuce and parrot feather plants, and about one-third of the treated amount of NOR was detected (Figure 5). The low NOR uptake by plants is associated with water solubility. In this study, we found that the uptake translocation mechanisms of parrot feather differed from those of water lettuce. The antibiotics were taken up and transported via mass flow and active transport [15]. In general, there is a close correlation between antibiotic uptake translocation into plants and the antibiotic octanol/water partition coefficient ($\log K_{OW}$). There is an optimal hydrophobicity value for antibiotic uptake translocation, and that organic compounds that are either highly polar ($\log K_{OW} < 1$) or highly lipophilic ($\log K_{OW} > 4$) will not be significantly taken up by plants [15,17]. In this study, $\log K_{OW}$ values for SMZ, NOR, and TC were below 0.5. Further, we conducted experiments in a plastic film house environment at a temperature of $30/25 \pm 2$ °C day/night, and the volume of evapotranspiration water in all treatments was greater than 160–170 mL/day. Therefore, we hypothesized that antibiotics were taken up through mass flow and passive absorption. SMZ, NOR, and TC accumulated at different levels in water plants, likely because of their different solubilities, affinities, and absorption mechanisms. Specifically, the solubility value of SMZ was much higher than those of NOR and TC. Dettenmaier et al. [18] indicated that highly water soluble organic compounds are most likely to be taken up by plants. The three antibiotics were detected in shoots and roots, but there were different distributions for different antibiotics. The plant root was the primary point of contact with antibiotic solution; thus, most antibiotics accumulated in the root system via biological and physical–chemical uptake. Removal of antibiotics from hydroponic culture solution takes place through a variety of mechanisms, namely hydrolysis, biodegradation,

photodegradation, and plant uptake. In hydrosphere ecosystems, further research is needed to investigate the mechanism of phytoremediation of antibiotics by aquatic plants.

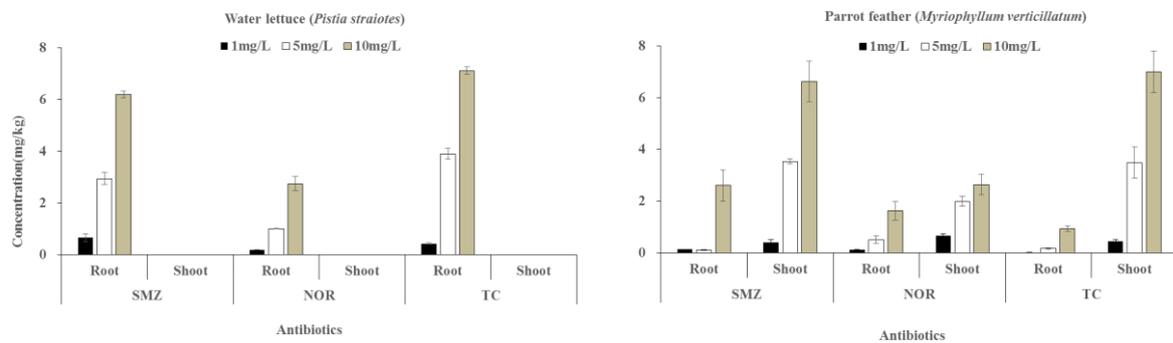


Figure 5. Selected antibiotic concentrations (on a dry weight basis) in water lettuce and parrot feather plants under various treatments at the end of the experimental period. Values are the mean \pm S.D. of three parallel individuals in each container.

3.5. Bioconcentration Factor

Plants having both phytostabilization and pollution-tolerant capacities are potentially useful for phytoremediation purposes. The mean BCF values for antibiotics in water lettuce and parrot feather tissues are shown in Figure 6. Uptake translocation of antibiotics varied among treatments. In water lettuce tissues, BCF of antibiotics ranged from 0.24 to 0.78, while that of NOR was much lower, ranging from 0.24 to 0.38. In addition, SMZ (0.59–0.64) and TET (0.72–0.78) showed higher uptake accumulation into water lettuce tissues. The BCF values of water lettuce and parrot feather showed similar trends but were higher for parrot feather (Figure 6). The differences in uptake accumulation of antibiotics in water plants are governed by antibiotic concentrations, crop species, and environmental conditions. SMZ and TC showed 60–80% removal, in the range of 1–10 mg/L of treatment concentration, but NOR removed only 20–30%. Parrot feather was slightly more effective in this respect than water lettuce. BCF values greater than 1 are generally believed to indicate the potential success of a plant species for phytoremediation. The BCF values of inorganic metal ions are often higher than 1 [19]; however, in this study, BCF values of antibiotics, which are toxic organic substances, were less than 1. The BCF values of antibiotics were similar to those measured in previous studies [18]. In this study, the metabolites of the three antibiotics could not be identified due to various research limitations. We present data for estimating metabolites of three antibiotics using previous research data. Biotransformation was shown to be the main removal process for the parent antibiotics from the aqueous phase and abiotic transformation, mineralization, and sorption to the suspended solids [20]. *Microbacterium* sp. strain 4N2-2 produces four metabolites (8-hydroxynorfloxacin, 6-defluoro-6-hydroxynorfloxacin, desethyl-norfloxacin, and N-acetyl-norfloxacin) from norfloxacin [21], *Streptomyces* sp. strain AL-16012 produces five metabolites (chlorotetracycline, doxytetracycline, meclotetracycline, minotetracycline, and rolitetracycline) from tetracycline [20], and ammonia-oxidizing archaeon strain produces two metabolites (acetyl + hydroxy (AcOH-sulfonamide) and N4-formyl-sulfonamide) from sulfamethazine [22] through glycosylation, decarboxylation, hydroxylation, oxidative defluorination, desethylation, and N-acetylation. These metabolites, of course, may be subjected to further degradation by other environmental microorganisms. Three antibiotic types—quinolones, sulfonamides, and tetracyclines—which are thought to be genetically toxic to fishes, have been reported to bioaccumulate in fish tissues, as well as in aquatic environments, such as rivers and surface water. The adverse effects of these antibiotics are known to cause damage to developmental, cardiovascular, and metabolic systems, as well as in altering antioxidant and immune responses, in fishes. Therefore, there are continuous concerns about the toxicity of antibiotics in fishes and management strategies are needed [20–22].

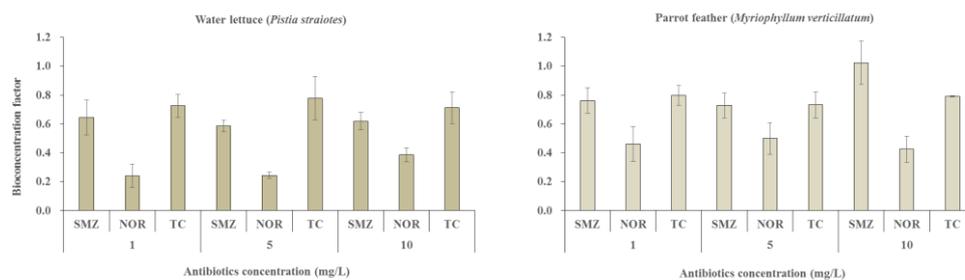


Figure 6. Bioconcentration factor (BCF) values of antibiotics in water lettuce and parrot feather plants (mean \pm SD, n = 3).

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