Toxicity of chlortetracycline and oxytetracycline on Vallisneria natans (Lour.) Hare

Jing Li
   Wuhan University College of Life Sciences

Lu Yang
   Wuhan University College of Life Sciences

Zhonghua Wu (✉️ wuzhonghua@whu.edu.cn)
   Wuhan University College of Life Sciences  https://orcid.org/0000-0003-0810-7470

Research Article

Keywords: Chlortetracycline, Oxytetracycline, Vallisneria natans, physiological responses

DOI: https://doi.org/10.21203/rs.3.rs-368657/v1

License: ☎️ This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Tetracyclines are frequently detected in water bodies due to their widespread use in aquaculture and animal husbandry. A hydroponic experiment was conducted to explore the phytotoxic effects of Vallisneria natans (Lour.) Hare exposed to various concentrations of chlortetracycline (CTC) and oxytetracycline (OTC) (0, 0.1, 1, 10, 30, 50 and 100 mg/L) for 7 days (7 D) and 14 days (14 D), respectively. The results showed that similar to OTC treatment for 7 D, the relative growth rates (RGR) and catalase (CAT) activity of V. natans, after 7 D of CTC exposure, decreased significantly at 10 mg/L and 30 mg/L, respectively. The content of soluble protein notably decreased when CTC ≥ 10 mg/L and OTC ≥ 30 mg/L. The hydrogen peroxide (H₂O₂) content was significantly stimulated when OTC ≥ 10 mg/L, while it hardly changed when exposed to CTC. After 14 D, the malondialdehyde (MDA) and H₂O₂ contents of V. natans were significantly higher than those of the control group under high concentration of OTC (≥ 30 mg/L), but they didn't change significantly under high concentration of CTC. The activity of polyphenol oxidase (PPO), under CTC treatment after 14 D, showed first a significant increase then decrease, the maximum value (125% of the control) was notice at 10 mg/L CTC, while it remained unchanged when exposed to OTC. The soluble protein content significantly decreased at 10 mg/L CTC and 0.1 mg/L OTC, respectively. The RGR, CAT and peroxidase (POD) activities, similar to OTC treatment after 14 D, decreased evidently when CTC was 10 mg/L, 30 mg/L and 0.1 mg/L, respectively. CTC and OTC harm the chlorophyll content of V. natans after 14 D, and the reduction of chlorophyll a and carotenoid were more pronounced than chlorophyll b. The results suggest that CTC and OTC both have negative effect on the growth of V. natans, and OTC can cause oxidative damage in V. natans but CTC harm the metabolism process without inducing oxidative damage. Overall, the toxicity of OTC to V. natans is stronger than that of CTC.

Introduction

Antibiotics are mainly used to treat microbial infections in human and animals and as feed additives to promote the growth of livestock (Anchordoquy et al., 2019). According to the survey, the annual consumption of antibiotics worldwide is estimated to be between 100,000-200,000 tons, and the global use of antibiotics is expected to be 200 percent higher by 2030 than in 2015 (Dutta and Mala, 2020). China is the world's largest producer and user of antibiotics, with 75-85% used in animal husbandry (Cheng et al., 2014). In China, the use of antibiotics for livestock and medicines was about 162,000 tons in 2013, compared with 150 times the consumption of antibiotics in the UK and 10 times that of the US (Wang et al., 2017). Tetracyclines, include tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC) and so on, are broad-spectrum bacteriostatic compounds and widely used due to their favorable properties such as low cost and high antimicrobial activity (Maged et al., 2020), and also as veterinary drugs to prevent and treat a variety of animal infectious diseases or as feed additives to promote animal growth (Dai et al., 2020). According to the survey, tetracyclines are the second most commonly used antibiotics in the world (Li et al., 2020), and CTC and OTC are two of the ten growth promoters approved in the United States (Jeong et al., 2010). In 2012, about 38,500 tons of antibiotics were used in veterinary
medicine in China, of which more than 60 percent were used for tetracyclines, sulfonamides and penicillin drugs (Lin et al., 2015). In Japan, tetracyclines for animal use account for about 43% of the total antibiotics (Fukushima et al., 2019).

Tetracyclines are frequently detected in water, with the highest reported residual concentrations at 2796.6 ng/L in the water of a high intensity aquaculture lake (Wang et al., 2017). The detection ranges of TC and OTC in surface water of the Yellow River Delta were 3.65-64.89 ng/L and 4.60-83.54 ng/L, respectively (Zhao et al., 2016). Chen et al. (2018) found that the average detected concentrations of OTC, CTC and TC in the surface water in China were 8.21, 7.71 and 3.57 ng/ mL, respectively. In addition, Wang (2017) found that the concentration of tetracyclines detected in water environment was affected by season. One reason for this is that the use of tetracyclines for preventing most respiratory infections increases during autumn and even winter in China, when animals are most susceptible to these diseases (Matsui et al., 2008). Another reason may be the longer half-life of tetracyclines in winter. The half-life of tetracyclines in low temperature seasons (e.g., 51-59 D at 13° in November) was longer than that in high temperature seasons (e.g., 24°21-40 D in May) (Wang et al., 2017). What is more, tetracyclines can be introduced into the water environment through different ways. Firstly, due to incomplete metabolism of organisms, most antibiotics are discharged from the body in the form of mother or its metabolites, which are directly released or indirectly discharged into the water environment through rainwater runoff (Christou et al., 2017). Secondly, the incomplete removal of sewage treatment plants. Studies have shown that concentrations of tetracyclines in downstream river are higher than upstream and midstream, which may due to the discharge of untreated livestock wastewater or incomplete treatment of sewage treatment plants to the downstream sampling points of the connected rivers (Krzeminski et al., 2019). Finally, antibiotics are added directly to the aquaculture water as feed additives in aquaculture (Pham et al., 2015). It is estimated that 80% of antibiotics used in aquaculture are released directly in the aquatic environment (Cabello et al., 2013). The high detection rate of tetracyclines (62.5~75%) in Poyang Lake, China is also closely related to the use of antibiotics in fish and poultry farming (Ding et al., 2017). And the results of tetracyclines in Taihu Lake showed that their contents (44.0-68.6 ng/L) were related to aquaculture of aquatic plants (Nkoom et al., 2018).

A large number of studies have shown unintended biological activity of residual tetracyclines in the water on non-targeted organisms. Moro (2020) found that 10mg/L of OTC altered the chloroplast shape of Isochrysis galbana, reduced pigment contents and inhibited its growth. Half of the growth inhibitions of Pseudokichneriella subcapitata and Selenastrum. capricornutum at different concentrations of CTC were 1.2-3.1 mg/L (Carusso et al., 2018). The toxic mechanisms of tetracyclines to aquatic animals (including Elliptio complanata, Danio rerio, Oncorhynchus mykiss, etc.) are mainly to damage the genetic material and nerve of organisms (Zargar et al., 2020). In addition, Ye et al. (2020) found that after 10 days of exposure, TC, CTC and OTC showed different toxicity to Microcystis aeruginosa. When M. aeruginosa was exposed to TC, it showed excitation effect; inhibition effect was observed when exposed to OTC, but no significant effect was observed when exposed to CTC. However, very limited information is available about the toxicity of CTC and OTC on aquatic plants.
Vallisneria natans (Lour.) Hare, one of the most common submerged plants, plays an important role in maintaining ecosystem stability and water health (Wang et al., 2008). In this study, the growth and the physiological responses of *V. natans* in different CTC/OTC concentrations were investigated. The resulting impact include biomass, chloroplast pigment, soluble protein content, membrane lipid peroxidation, hydrogen peroxide (H$_2$O$_2$) and antioxidant enzymes activity, including, catalase (CAT), guaiacol peroxidase (POD) and polyphenol oxidase (PPO). Our primary goals were to understand the toxicity of CTC and OTC on *V. natans* and to provide theoretical guidance for safety assessment of them.

**Materials And Methods**

2.1 Plant materials and experimental design

Whole plants of *V. natans* were collected from the East Lake in Wuhan (30°32' N 114°25' E). Before the experiment, each plant was thoroughly washed with running water to remove attachments and then cultivated for acclimatization in 5L 10% Hoagland’s solution (Hoagland and Arnon, 1950). After 14 days (14 D), each plant was again thoroughly cleaned in running tap water, and then rinsed in redistilled water, plants, with similar size and growth status, were chosen for experimental use. During acclimatization and experiment, the roots of the plants were fixed with sterilized pure white gravel and the plants were grown in nutrient solution in transparent plastic tanks at temperature 25±0.5 °C, 16/8 light/ dark cycle, relative air humidity 60%, and light intensity of 10000 lx. The antibiotics used in the experiment were obtained from chlortetracycline hydrochloride (USP grade, CAS NO.64-72-2) and oxytetracycline hydrochloride (USP grade, purity 95%, CAS NO.79-57-2), which were purchased from Wuhan Xinshen Chemical Technology Co., Ltd., China.

To encompass both environmental realism and concentrations that would elicit measurable toxic responses (Brain et al., 2005), the concentrations of CTC and OTC were set at 0.1, 1, 10, 30, 50 and 100 mg/L, respectively. A control treatment was setup using culture medium and plants without CTC/ OTC. Each treatment concentration was replicated three times, and the solutions were changed every 48 h. The plants were harvested after 7 days (7 D) and 14 D of exposure. They were first rinsed with double distilled water to remove the attachments on the surface of them and then packed individually for the estimation of various indexes.

2.2 Measurement of plant growth

The plant growth was presented as relative growth rates (RGR). After 7 D and 14 D of CTC/ OTC exposure, plants were harvested and washed with double distilled water. After being dried on filter paper, the fresh weight of each plant was weight by an electronic analytical balance (accuracy 0.1 mg). RGR was calculated according to Liu et al. (2019).

RGR was determined as:
RGR = [ ln (final weight) – ln (initial weight) ]/ \( \Delta t \) (g g\(^{-1}\) day\(^{-1}\)), where weight is the plant fresh weight over \( \Delta t \) days.

### 2.3 Photosynthetic pigment measurements

The photosynthetic pigments were measured according to the method of Jampeetong and Brix (2009). Plant leaves (0.05 g fresh weight, FW) were cut into pieces evenly. Then, 5 mL 95% (v/v) alcohol was added and flasks were placed in the dark for 48 h until the leaves turn white. The absorbance values of the extract at 470, 649 and 665 nm were determined by ultraviolet spectrophotometer with 95% ethanol as control. The contents of chlorophyll a, chlorophyll b and carotenoids (mg/g FW) were calculated by the method of Lichtenthaler and Wellburn (1987). All spectrophotometric analyses were conducted by a MAPADA UV-1200 spectrophotometer (Shanghai Meipuda Instrument Co. Ltd., Shanghai, China).

### 2.4 Lipid peroxidation measurements and soluble protein content

The level of lipid peroxidation in the plant leaves was determined by the quantification of malondialdehyde (MDA) (Cang and Zhao, 2013). Fresh leaves (0.05 g) were homogenized with 3 mL 10% trichloroacetic acid (TCA) at 12000 g for 25 min. The supernatant (2 mL) was mixed with 2 mL 0.6% 2-thiobarbituric acid (TBA), and the mixture was heated at 100 °C for 15 min and then cool quickly and centrifuge. The absorbance of the supernatant was measured at 450 nm, 532 nm and 600 nm.

Soluble protein content was determined following the method of Bradford (1976).

### 2.5 Enzyme activities measurements and H\(_2\)O\(_2\) content

Fresh leaves (0.1 g) were homogenized with a phosphate buffer solution (50 mM, pH 7.8), containing NaH\(_2\)PO\(_4\), Na\(_2\)HPO\(_4\), and 1 % (m/v) polyvinylpyrrolidone (PVPP) at 4 °C. The mixture was centrifuged at 12000 g for 25 min at 4 °C (Eppendorf Centrifuge 5417 R, Hamburg, Germany). The supernatant was stored at 4 °C and used for the measurement of antioxidant enzyme activities, including CAT, POD and PPO activities.

The CAT activity was assayed according to Cang and Zhao (2013), where one enzyme activity unit (U/g·min FW) was defined as a decrease of 0.1 in absorbance at 240 nm in 1 min. The reaction mixture consisted of 0.2 M pH 7.8 PBS (1.5 mL), 0.1M H\(_2\)O\(_2\) solution (0.3 mL), crude enzyme solution (0.2 mL) and distilled water (1 mL). The activity of POD was measured according to Liu and Li (2007), with an absorbance change of 0.01 per minute at 470 nm representing one unit of enzyme activity (U/g·min FW). The reaction mixture consisted of 0.1 M pH 6.0 PBS (2.9 mL), 0.05 mM guaiacol (1.0 mL), 2 % H\(_2\)O\(_2\) (1.0 mL) and crude enzyme solution (0.1 mL). The activity of PPO was estimated following the guaiacol method described by Shi (2016), and one unit of enzyme activity (U/g·min FW) corresponded to an absorbance change of 0.01 per minute at 398 nm. The content of H\(_2\)O\(_2\) (ug/g FW) was examined following the method described by Shi (2016). The reaction mixture contained the enzyme extraction and 5 % titanium sulfate in 20% sulfuric acid.
2.6 Statistical analysis

The experiment utilized a randomized block design. All values were expressed as the mean±standard deviation. Homogeneity of the variance was analyzed by performing Levene's test. When necessary, the date was transformed and normalized to reduce the heterogeneity of variance. ANOVAs were performed to assess the variability of data and validity of results. Post hoc Duncan tests were done to separate differences between pairs of treatments. P-values ≤ 0.05 were considered significant. The statistical analysis was performed by SPSS 23.0 for Windows (IBM Inc., Chicago, IL, USA), and graphs were generated in SigmaPlot 12.5 for Windows (Systat Software, Inc., USA).

Results

3.1 Plant growth

As shown in Table 1, no significant effect on the RGR of V. natans was observed at up to 10 mg/L CTC/OTC after 7 D, beyond which it declined evidently (p < 0.05). The 100 mg/L CTC treatment yielded the minimum fresh weight growth (decreased by 32.76 % compared with the control), and the OTC concentration of 50 mg/L yielded the lowest fresh weight value (decreased by 35.56 % of the control).

Similar to the RGR of V. natans after 7 D, the RGR of the plants declined markedly beyond 10 mg/L with increasing concentrations of CTC/OTC after 14 D (p < 0.05). In addition, under 100 mg/L CTC and 50 mg/L OTC, the RGR of V. natans compared with the control were as low as 38.70% and 49.14%, respectively.

Table 1. Effects of different concentrations of CTC and OTC on the RGR of V. natans after 7 D and 14 D.

| concentrations (mg L⁻¹) | RGR (%)                          |
|------------------------|----------------------------------|
|                        | CTC 7 D | CTC 14 D | OTC 7 D | OTC 14 D |
| 0                      | 3.20±1.28a | 3.26±0.17ab | 3.05±2.10a | 3.42±1.09a |
| 0.1                    | 2.73±1.12ab | 2.89±0.54b | 2.66±1.26a | 3.18±0.48a |
| 1                      | 3.78±0.82a | 3.73±0.71a | 2.88±0.56a | 3.28±0.71a |
| 10                     | -0.07±0.49bc | 1.26±0.36c | 0.23±1.45b | 0.07±0.91b |
| 30                     | -0.80±2.03c | 0.26±0.54d | -0.14±1.10b | -0.99±1.17c |
| 50                     | -0.33±1.31bc | 0.73±0.21cd | -1.36±0.10b | -1.35±0.88c |
| 100                    | -1.26±3.46c | -0.01±0.78d | -0.82±0.86b | -0.69±0.63bc |
All values represent the mean of three replicates ± standard deviation. Means with different letters in the same columns are significantly different among different exposure concentrations \((p \leq 0.05, \text{ Duncan test})\).

### 3.2 Photosynthetic pigments

As shown in Fig. 1, no significant differences were found for the photosynthetic pigments when *V. natans* plants were exposed to CTC after 7 D. When the concentration of OTC was between 1.0 ~ 30 mg/L, the contents of photosynthetic pigments of *V. natans* decreased, then returned to the normal level. And the content of chlorophyll a and carotenoid of *V. natans* significantly decreased at 30 mg/L OTC.

Exposed to CTC treatment, the contents of chlorophyll a and total chlorophyll of *V. natans* showed a similar response to those of OTC treatment after 14 D, and both significantly decreased at 10 mg/L CTC/OTC (Fig. 2a and d). After 14 D, no significant differences were observed in the content of chlorophyll b of *V. natans* under various CTC concentrations, and when the concentration of OTC was exceeded 10 mg/L, the content of chlorophyll b was significantly suppressed (Fig. 2b). The carotenoid content of *V. natans* showed a concentration-dependent decrease, and 100 mg/L CTC and 50 mg/L OTC significantly reduced the carotenoid content to 40.30% and 39.87%, respectively (Fig. 2c).

### 3.3 MDA content

There was no significant difference in the MDA content of *V. natans* after 7 D of CTC/OTC treatment (Fig. 3a). After 14 D of CTC exposure, the maximum content of MDA was 11.00 ± 2.13 nmol/g FW obtained at 10 mg/L, and then returned to the normal level, while for the OTC exposure, the maximum value \((14.79 \pm 2.05 \text{ nmol/g FW})\) was noticed at 10 mg/L, beyond which the MDA content of *V. natans* still remained at a high level and was significantly higher than that of the control group (Fig. 3b).

### 3.4 Antioxidative enzymes

After 7 D, the CAT activity of *V. natans* hardly changed before the concentration of CTC/OTC reached 10 mg/L, when the concentration of CTC/OTC exceeded 30 mg/L, the CAT activity decreased significantly, and there were no remarkable differences between the high-concentration treatment groups (30~100 mg/L) (Fig. 4a). No significant differences were observed in the POD and PPO activities of *V. natans* compared with the control group \((p > 0.05)\) (Fig. 4c and e).

After 14 D, the CAT activity of *V. natans* was more obvious than that of 7 D. The maximum value was noticed at 1.0 mg/L CTC/OTC, beyond which the CAT activity decreased significantly as the concentration of CTC/OTC increased (Fig. 4b). The POD activity of *V. natans* significantly increased at 0.1 mg/L CTC/OTC, while the value returned to the normal level at higher concentrations of CTC/OTC \((\geq 30 \text{ mg/L})\) (Fig. 4d). The PPO activity of *V. natans* showed first a significant increase and then a decreasing trend, and the maximum value \((125\%\ \text{greater than the control})\) was noticed at 10 mg/L CTC. The effect of OTC on the PPO activity of *V. natans* was negligible \((p > 0.05)\) (Fig. 4f).
3.5 Soluble protein content

The effect of CTC on the soluble protein content of *V. natans* after 7 D was similar to that of 14 D, and both evidently decreased at 10 mg/L ($p < 0.05$). In addition, the 100 mg/L CTC treatment yielded the lowest soluble protein content, and decreased by 47.80% and 49.99%, respectively, compared with the control group of 7 D and 14 D (Fig. 5 a and b).

There was no significant difference in the soluble protein content of *V. natans* under 0.1-10 mg/L OTC treatment compared with the control group after 7 D ($p > 0.05$). Beyond 10 mg/L with increasing concentration of OTC, the soluble protein content of *V. natans* declined significantly ($p < 0.05$) (Fig. 5 a). After OTC treatment for 14 D, the soluble protein of *V. natans* showed a concentration-dependent decrease with increasing concentrations of OTC (Fig. 5 b).

3.6 $H_2O_2$ content

After 7 D, no significant differences in $H_2O_2$ content were observed between the control and the other experimental groups after CTC treatment. The $H_2O_2$ content did not change significantly when the OTC concentration was less than 10 mg/L, and when the OTC concentration was more than 10 mg/L, it increased significantly with the increasing of OTC concentration ($p < 0.05$) (Fig. 6a).

After 14 D, no significant differences were observed for the $H_2O_2$ content when *V. natans* plants were exposed to CTC. The OTC treatment resulted in a significant decrease in the $H_2O_2$ content at 0.1 mg/L, where a minimum decrease of 17.18% was observed, and when the OTC concentration $\geq$ 1 mg/L, the $H_2O_2$ content of *V. natans* increased significantly with the increasing concentration of OTC (Fig. 6b).

Discussion

After 7 D and 14 D of CTC/ OTC exposure, the RGR decreased significantly when the concentration of CTC/ OTC exceeded 10 mg/L, which indicated that CTC and OTC can harm the growth of *V. natans*. Our study was consistent with the result of Cui et al. (2008), Guo et al. (2020) and Liu et al. (2020). A possible reason for the negative effect may be because they inhibit the absorptions of water and trace elements by plants which are of great significance for photosynthesis, respiration and protein synthesis in plants (Cui et al., 2008; Munns, 2002). Other studies have reported that tetracyclines can complexes with metal ions such as $Cu^{2+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$ and $Zn^{2+}$, which may reduce the biomass and the absorption of trace elements by plants (Tongaree et al., 1999). When exposed to high concentration of CTC/ OTC ($\geq$ 30 mg/L) after 14 D, the inhibition degree of OTC on the fresh weight of *V. natans* was always stronger than that of CTC treatment, which was similar to the result of Ye et al. (2017) but different from what found in *Brassica campestris* (Zhu et al., 2018). It has been suggested that a degree of species-specific properties leads to differences in susceptibility to poisons (Brain et al., 2005; Hanson et al., 2006). Although tetracyclines have a similar structure, subtle differences in structure can lead to differences in toxicity to plants (Dong et al., 2012).
Soluble proteins are important macromolecules in organisms, which play an important role in maintaining cell structure and regulating physiological metabolic activities in organisms (Liu et al., 2020). Tetracyclines can enter into cells through active transfer and irreversibly bind to the cell ribosome 30s subunit, prevent ammonia radical transfer from binding to DNA and inhibit the synthesis of cell proteins, thus inhibiting their growth (Halling-Sørensen, 2000; Lu et al., 2015). After 7 D of CTC exposure, the soluble protein content of *V. natans* was significantly decreased at 10 mg/L, while it showed a mark decrease at 30 mg/L OTC. However, 0.1 mg/L OTC observably reduced the content of soluble protein in *V. natans* after 14 D, while CTC still required 10 mg/L. The chemical structure of CTC may be responsible for the result, the present of halogens can enhance the molecular polarity and lipid solubility of CTC, making it easier to integrate with enzyme system in a short-term (Dong et al., 2012). In addition, the observation indicated that CTC/ OTC could impede the synthesis of soluble proteins in *V. natans*, which was consistent with the results of Zhang et al. (2019) and Siedlewicz et al. (2020a). The content of soluble protein in plants depends on the kinetic equilibrium of their catabolism, and the decrease in soluble protein content may be due to the increased proteolysis in the organism to compensate for the loss of energy, or to the formation of lipoproteins to repair cells, tissues and organs (Singh et al., 2006). Analogously, other studies have reported that the synthesis rate of soluble protein in plants will slow down when exposed to adversity stress (Zhang et al., 2010; Zhu et al., 2020).

Photosynthetic pigment is a basic parameter for evaluating photosynthetic activity which is often used as an indicator of plant damage under adversity stress (Liu and Wu, 2018). Many studies have shown that tetracyclines can significantly reduce chlorophyll contents (Rydzyński et al., 2019; Siedlewicz et al., 2020b). Tetracyclines mainly affect chlorophyll contents by inhibiting chloroplast translation activity and the activities of enzymes related to chlorophyll molecule synthesis (Kasai et al., 2004). Moro et al. (2020) reported that 10 mg/L OTC reduced photosynthetic pigment contents by causing changes of chloroplast structure in *Isochrysis galbana* Parke. In addition, Jiao et al. (2008) found that exposure to 20 mg/L of TC in *V. natans* resulted in the plasmolysis of mitochondria and chloroplasts and disordered interlamellar structure of chloroplasts. After 7 D of CTC/ OTC, there was no significant difference or only a partial decrease in the chlorophyll contents. However, the chlorophyll contents of *V. natans* significantly decreased with the increase of CTC/ OTC concentration after 14 D. The results indicated that both antibiotics had a cumulative toxic effect, that was, the organism exposed to CTC/ OTC may not or showed a lighter toxic effect in a short time, but the toxic effect would increase in the later period. Studies have shown that chlorophyll degradation is related to ·OH and MDA produced by O$_2^-$ and H$_2$O$_2$ (Dhindsa et al., 1981). The increase of H$_2$O$_2$ content will cause a decline in chlorophyll contents, affect the integrity of thylakoid membrane, and inhibit the synthesis of PSI-related proteins (Kar and Choudhuri, 1987; Takahashi and Murata, 2008). In this experiment, the contents of MDA and H$_2$O$_2$ in *V. natans* both maintained a higher level under higher concentrations of OTC treatment after 14 D, which may be the reason for that the more obvious decreasing trend of the chlorophyll contents in *V. natans* under OTC treatment than that of CTC treatment. In addition, the reduction of chlorophyll a and carotenoid in *V. natans* were more pronounced than chlorophyll b content after 14 D of CTC/ OTC treatment, which was consistent with what found in *Trapa bispinosa* to TC stress (Liu et al., 2020) but different from the result
of Guo et al. (2020b). The different performance of photosynthetic pigments may indicate that the content of chlorophyll b in *V. natans* has a delayed response to CTC and OTC, and the effect of tetracyclines may be species-specific.

Pollutants in the environment can cause oxidative stress, leading to the accumulation of reactive oxygen species (ROS) in plants, and excessive ROS will cause membrane lipid peroxidation which leads to the accumulation of MDA (Zhong et al., 2018). No lipid peroxidation was observed in *V. natans* after 7 D of CTC/OTC treatment, which was similar to the findings in *Hydrocharis dubia* (Bl.) Backer (Liu et al., 2020). After 14 D, the content of MDA in *V. natans* significantly increased at 10mg/L CTC, and with the increase of CTC concentration, it showed no significant difference compared with the control. The results illustrated that the antioxidant system of *V. natans* worked well to scavenge free radicals and maintain the stability of cell membrane. Notably, the MDA content of *V. natans* decreased significantly at 1.0 mg/L CTC, contrary to what we expected because of oxidative stress in plants exposed to tetracyclines (Bártíková et al., 2016; Xie et al., 2019). The findings in *Trapa bispinosa* under TC exposure were consistent with ours (Liu et al., 2020). The decrease of MDA content may show an adaptation response of *V. natans* expose to CTC. It is possible to hypothesize that given the similar chemical structure to the antioxidant vitamin E, the decline may be related to the potential antioxidant properties of CTC (Kraus et al., 2005; Nunes et al., 2015). However, the contents of MDA and H$_2$O$_2$ in *V. natans* under high concentration of OTC were always significantly higher than those of the control, which confirmed the present of oxidative stress in *V. natans*. In general, high levels of H$_2$O$_2$ may induce lipid peroxidation. Many studies also demonstrated that the increase in lipid peroxidation in tetracyclines-exposed plants associated with high levels of H$_2$O$_2$ (Yonar, 2012).

The antioxidant system composed of antioxidant enzymes and antioxidants in the body can remove free radicals produced by cell growth and metabolism under normal circumstances (Zhou et al., 2018). However, when an organism is stimulated by the outside, there will be excessive production of ROS, such as superoxide radicals, hydroxyl radicals and H$_2$O$_2$ (Wu et al., 2010). The enzyme CAT oxidizes H$_2$O$_2$ to generate H$_2$O and O$_2$, POD catalyzes the decomposition of H$_2$O$_2$ through the oxidation of phenolic compounds and PPO can reduce the toxicity of phenol to plants through hydroxylation and oxidation of exogenous phenols (Kouka et al., 2018; Liu and Wu, 2018). After 7 D of CTC/OTC exposure, no significant differences were found for the POD and PPO activities, but the CAT activity of *V. natans* decreased significantly under high concentrations of CTC/CTC ($\geq$ 30 mg/L). Considering that the content of MDA did not change, the decrease of CAT content confirmed that the antioxidant enzyme system in *V. natans* responded well to the stress of CTC/OTC. The CAT activity still reduced markedly at high concentrations ($\geq$ 30 mg/L) after 14 D, which was a somewhat interesting result, since tetracyclines stress could cause an increase in CAT activity (Dong et al., 2012; Xie et al., 2011). Chi et al. (2010) found that OTC can interact with a binding site of CAT through van der Waals interaction and hydrogen bonding, and the microenvironment of tryptophan residues and the secondary structure of CAT change after combining with OTC. Other studies have reported that the CAT activity of ryegrass and maize decreased under tetracyclines stress which confirmed our result (Cui and Zhao, 2011; Han et al., 2019).
The work of POD activity at 0.1 mg/L CTC/ OTC indicated that the antioxidant system of *V. natans* was activated to remove ROS with the increasing substrate levels. In addition, the changes in membrane lipid permeability induced by CTC/ OTC could promote the uptake of environmental nutrients by the plants. Iron in nutrient solution is not only involved in the synthesis of chlorophyll, but also a component of POD, which may lead to the increase of POD activity (Liu et al., 2019). The contents of MDA and H$_2$O$_2$ of *V. natans* under high concentrations of OTC were always significantly higher than the control level, which indicated that the antioxidant enzyme system of *V. natans* was not enough to eliminate the ROS persecuted cells. The different responses of PPO activity of *V. natans* under CTC and OTC stress may be one reason why the content of H$_2$O$_2$ and MDA was still high at high concentration of OTC. PPO can participate in the oxidation of phenols, and the oxidation products (such as quinones) formed by the oxidation of phenolic compounds can combine with the side chain of amino acids to reduce the protein content (Mayer, 2006; Singh et al., 2008). These results suggest that the antioxidant defense system of plants is unstable and can change over time. Plants under external stress will constantly adjust the activity of antioxidant enzymes to strictly control the ROS level (Song et al., 2012).

**Conclusion**

The toxicity of CTC and OTC on *V. natans* was different. After 7 D, the content of H$_2$O$_2$, at higher concentrations of OTC treatment ($\geq$ 10 mg/L), was always significantly higher than the control group, while it hardly changed when exposed to CTC. High concentration of CTC and OTC both had significant damage to the RGR, soluble protein content and CAT activity of *V. natans*, which were consistent with the trend after 14 D. After 14 D, the activity of PPO increased significantly in the CTC concentration range from 1 mg/L to 30 mg/L and then decreased, while it remained unchanged when exposed to OTC. The soluble protein content significantly decreased at 10 mg/L CTC and 0.1 mg/L OTC, respectively. Different from the MDA and H$_2$O$_2$ contents of *V. natans* of CTC exposure, they were significantly higher than the control under high concentration of OTC after 14 D. Overall, the toxicity of OTC to *V. natans* is stronger than that of CTC.

**Declarations**

**Ethical Approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate**

No applicable.

**Consent for publication**

Written informed consent for publication was obtained from all participants.
Availability of date and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

the National Science Foundation of China (No. 31270410, No. 30970303)

the Scientific Research Project of Hubei Province Environmental Protection Department (2014HB07).

Authors’ contribution statements

Jing Li: Conceptualization; Methodology; Resources; Investigation; Writing-Original Draft.

Lu Yang: Resources; Validation.

Zhonghua Wu: Supervision; Project administration; Funding acquisition.

Acknowledgments

This work was supported by the National Science Foundation of China (No. 31270410, No. 30970303) and the Scientific Research Project of Hubei Province Environmental Protection Department (2014HB07).

References

Anchordoquy, J.M., Anchordoquy, J.P., Nikoloff, N., Gambaro, R., Padula, G., Seoane, A., Furnus, C., 2019. Doramectin induced cytotoxic and genotoxic effects on bovine peripheral lymphocytes and cumulus cells in vitro. J. Environ. Sci. Heal. Part B 54, 147–154. https://doi.org/10.1080/03601234.2018.1559569

Bártíková, H., Podlíná, R., Skálová, L., 2016. Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144, 2290–2301. https://doi.org/10.1016/j.chemosphere.2015.10.137

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. Anal. Biochem. 71, 248–254.

Brain, R.A., Wilson, C.J., Johnson, D.J., Sanderson, H., Bestari, K. (Jim), Hanson, M.L., Sibley, P.K., Solomon, K.R., 2005. Effects of a mixture of tetracyclines to Lemna gibba and Myriophyllum sibiricum evaluated in aquatic microcosms. Environ. Pollut. 138, 425–442. https://doi.org/10.1016/j.envpol.2005.04.021
Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A., Buschmann, A.H., 2013. Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ. Microbiol. 15, 1917–1942. https://doi.org/10.1111/1462-2920.12134

Cang, J., Zhao, H., 2013. Experimental Course of Plant Physiology. Higher Education press, Peking. pp. 151-153 (in Chinese).

Carusso, S., Juárez, A.B., Moretton, J., Magdaleno, A., 2018. Effects of three veterinary antibiotics and their binary mixtures on two green alga species. Chemosphere 194, 821–827. https://doi.org/10.1016/j.chemosphere.2017.12.047

Chen, H., Jing, L., Teng, Y., Wang, J., 2018. Characterization of antibiotics in a large-scale river system of China: Occurrence pattern, spatiotemporal distribution and environmental risks. Sci. Total Environ. 618, 409–418. https://doi.org/10.1016/j.scitotenv.2017.11.054

Cheng, D., Liu, X., Wang, L., Gong, W., Liu, G., Fu, W., Cheng, M., 2014. Seasonal variation and sediment–water exchange of antibiotics in a shallower large lake in North China. Sci. Total Environ. 476–477, 266–275. https://doi.org/10.1016/j.scitotenv.2014.01.010

Chi, Z., Liu, R., Zhang, H., 2010. Potential enzyme toxicity of oxytetracycline to catalase. Sci. Total Environ. 408, 5399–5404. https://doi.org/10.1016/j.scitotenv.2010.08.005

Christou, A., Agüera, A., Bayona, J.M., Cytryn, E., Fotopoulos, V., Lambropoulou, D., Manaia, C.M., Michael, C., Revitt, M., Schröder, P., Fatta-Kassinos, D., 2017. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: The knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes – A review. Water Res. 123, 448–467. https://doi.org/10.1016/j.watres.2017.07.004

Cui, X., Qiao, X., Han, C., Wang, Z., 2008. Uptake of Oxytetracycline and Its Phytotoxicity to Lettuce. J. Agro-Environment Sci. 27, 1038-1042 (in Chinese).

Cui, Y., Zhao, N., 2011. Oxidative stress and change in plant metabolism of maize (Zea mays L.) growing in contaminated soil with elemental sulfur and toxic effect of zinc. Plant, Soil Environ. 57, 34–39. https://doi.org/10.17221/193/2010-PSE

Dai, Y., Li, J., Shan, D., 2020. Adsorption of tetracycline in aqueous solution by biochar derived from waste Auricularia auricula dregs. Chemosphere 238, 124432. https://doi.org/10.1016/j.chemosphere.2019.124432

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. J. Exp. Bot. 32, 93–101. https://doi.org/10.1093/jxb/32.1.93
Ding, H., Wu, Y., Zhang, W., Zhong, J., Lou, Q., Yang, P., Fang, Y., 2017. Occurrence, distribution, and risk assessment of antibiotics in the surface water of Poyang Lake, the largest freshwater lake in China. Chemosphere 184, 137–147. https://doi.org/10.1016/j.chemosphere.2017.05.148

Dong, L., Gao, J., Xie, X., Zhou, Q., 2012. DNA damage and biochemical toxicity of antibiotics in soil on the earthworm Eisenia fetida. Chemosphere 89, 44–51. https://doi.org/10.1016/j.chemosphere.2012.04.010

Dutta, J., Mala, A.A., 2020. Removal of antibiotic from the water environment by the adsorption technologies: A review. Water Sci. Technol. 82, 401–426. https://doi.org/10.2166/wst.2020.335

Fukushima, Y., Tsuyuki, Y., Goto, M., Yoshida, H., Takahashi, T., 2019. Species Identification of β-Hemolytic Streptococci from Diseased Companion Animals and Their Antimicrobial Resistance Data in Japan (2017). Jpn. J. Infect. Dis. 72, 94–98. https://doi.org/10.7883/yoken.JJID.2018.231

Guo, X., Liu, M., Zhong, H., Li, P., Zhang, C., Wei, D., Zhao, T., 2020a. Responses of the growth and physiological characteristics of Myriophyllum aquaticum to coexisting tetracyclines and copper in constructed wetland microcosms. Environ. Pollut. 261, 114204. https://doi.org/10.1016/j.envpol.2020.114204

Guo, X., Liu, M., Zhong, H., Li, P., Zhang, C., Wei, D., Zhao, T., 2020b. Responses of the growth and physiological characteristics of Myriophyllum aquaticum to coexisting tetracyclines and copper in constructed wetland microcosms. Environ. Pollut. 261, 114204. https://doi.org/10.1016/j.envpol.2020.114204

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. Chemosphere 40, 731–739. https://doi.org/10.1016/S0045-6535(99)00445-2

Han, T., Liang, Y., Wu, Z., Zhang, L., Liu, Zhenwei, Li, Q., Chen, X., Guo, W., Jiang, L., Pan, F., Ge, S., Mi, Z., Liu, Zunchun, Huang, H., Li, X., Zhou, J., Li, Y., Wang, J., Zhang, Z., Tang, Y., Yang, L., Wu, M., 2019. Effects of tetracycline on growth, oxidative stress response, and metabolite pattern of ryegrass. J. Hazard. Mater. 380, 120885. https://doi.org/10.1016/j.jhazmat.2019.120885

Hanson, M.L., Knapp, C.W., Graham, D.W., 2006. Field assessment of oxytetracycline exposure to the freshwater macrophytes Egeria densa Planch. and Ceratophyllum demersum L. Environ. Pollut. 141, 434–442. https://doi.org/10.1016/j.envpol.2005.08.068

Hoagland, D.R., Arnon, D.I., 1950. Preparing the nutrient solution. Water-Culture Method Grow. Plants without Soil 347, 29–31.

Jampeetong, A., Brix, H., 2009. Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of Salvinia natans. Aquat. Bot. 91, 181–186. https://doi.org/10.1016/j.aquabot.2009.05.003
Jeong, J., Song, W., Cooper, W.J., Jung, J., Greaves, J., 2010. Degradation of tetracycline antibiotics: Mechanisms and kinetic studies for advanced oxidation/reduction processes. Chemosphere 78, 533–540. https://doi.org/10.1016/j.chemosphere.2009.11.024

Jiao, S., Dou, Y., Chen, L., Pu, H., Zheng, S., Yin, D., 2008. Effect of aqueous tetracycline on Vallisneria Natans in growth and ultrastructure. Environ. Chem. 27, 335-338 (in Chinese). https://doi.org/10.3321/j.issn:0254-6108.2008.03.013

Kar, R.K., Choudhuri, M.A., 1987. Possible mechanisms of light-induced chlorophyll degradation in senescing leaves of Hydrilla verticillata. Physiol. Plant. 70, 729–734. https://doi.org/10.1111/j.1399-3054.1987.tb04331.x

Kasai, K., Kanno, T., Endo, Y., Wakasa, K., Tozawa, Y., 2004. Guanosine tetra- and pentaphosphate synthase activity in chloroplasts of a higher plant: Association with 70S ribosomes and inhibition by tetracycline. Nucleic Acids Res. 32, 5732–5741. https://doi.org/10.1093/nar/gkh916

Kouka, P., Chatzieffraimidi, G.-A., Raftis, G., Stagos, D., Angelis, A., Stathopoulos, P., Xynos, N., Skaltsounis, A.-L., Tsatsakis, A.M., Kouretas, D., 2018. Antioxidant effects of an olive oil total polyphenolic fraction from a Greek Olea europaea variety in different cell cultures. Phytomedicine 47, 135–142. https://doi.org/10.1016/j.phymed.2018.04.054

Kraus, R.L., Pasieczny, R., Lariosa-Willingham, K., Turner, M.S., Jiang, A., Trauger, J.W., 2005. Antioxidant properties of minocycline: neuroprotection in an oxidative stress assay and direct radical-scavenging activity. J. Neurochem. 94, 819–827. https://doi.org/10.1111/j.1471-4159.2005.03219.x

Krzeminski, P., Tomei, M.C., Karaolia, P., Langenhoff, A., Almeida, C.M.R., Felis, E., Gritten, F., Andersen, H.R., Fernandes, T., Manaia, C.M., Rizzo, L., Fatta-Kassinos, D., 2019. Performance of secondary wastewater treatment methods for the removal of contaminants of emerging concern implicated in crop uptake and antibiotic resistance spread: A review. Sci. Total Environ. 648, 1052–1081. https://doi.org/10.1016/j.scitotenv.2018.08.130

Li, X., Cui, K., Guo, Z., Yang, T., Cao, Y., Xiang, Y., Chen, H., Xi, M., 2020. Heterogeneous Fenton-like degradation of tetracyclines using porous magnetic chitosan microspheres as an efficient catalyst compared with two preparation methods. Chem. Eng. J. 379, 122324. https://doi.org/10.1016/j.cej.2019.122324

Lichtenthaler Hartmut K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 350–382.

Lin, L., Yuan, K., Liang, X., Chen, X., Zhao, Z., Yang, Y., Zou, S., Luan, T., Chen, B., 2015. Occurrences and distribution of sulfonamide and tetracycline resistance genes in the Yangtze River Estuary and nearby coastal area. Mar. Pollut. Bull. 100, 304–310. https://doi.org/10.1016/j.marpolbul.2015.08.036
Liu, N., Wu, Z., 2018. Growth and antioxidant response in Ceratophyllum demersum L. under sodium dodecyl sulfate (SDS), phenol and joint stress. Ecotoxicol. Environ. Saf. 163, 188–195. https://doi.org/10.1016/j.ecoenv.2018.07.074

Liu, N., Zhong, G., Zhou, J., Liu, Y., Pang, Y., Cai, H., Wu, Z., 2019. Separate and combined effects of glyphosate and copper on growth and antioxidative enzymes in Salvinia natans (L.) All. Sci. Total Environ. 655, 1448–1456. https://doi.org/10.1016/j.scitotenv.2018.11.213

Liu, P, Li, M., 2007. Experimental Techniques of Plant Physiology. Science Press, Peking. pp. 123-125 (in Chinese).

Liu, Y., Pang, Y., Yang, L., Ning, S., Wang, D., Wu, Z., 2020. Responses of Hydrocharis dubia (Bl.) Backer and Trapa bispinosa roxb. to tetracycline exposure. Ecotoxicol. Environ. Saf. 202, 110890. https://doi.org/10.1016/j.ecoenv.2020.110890

Lu, L., Wu, Y., Ding, H., Zhang, W., 2015. The combined and second exposure effect of copper (II) and chlortetracycline on fresh water algae, Chlorella pyrenoidosa and Microcystis aeruginosa. Environ. Toxicol. Pharmacol. 40, 140–148. https://doi.org/10.1016/j.etap.2015.06.006

Maged, A., Iqbal, J., Kharbish, S., Ismael, I.S., Bhatnagar, A., 2020. Tuning tetracycline removal from aqueous solution onto activated 2:1 layered clay mineral: Characterization, sorption and mechanistic studies. J. Hazard. Mater. 384, 121320. https://doi.org/10.1016/j.jhazmat.2019.121320

Matsui, Y., Ozu, T., Inoue, T., Matsushita, T., 2008. Occurrence of a veterinary antibiotic in streams in a small catchment area with livestock farms. Desalination 226, 215–221. https://doi.org/10.1016/j.desal.2007.01.243

Mayer, A.M., 2006. Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry 67, 2318–2331. https://doi.org/10.1016/j.phytochem.2006.08.006

Moro, I., Trentin, R., Moschin, E., Dalla Vecchia, F., 2020. Morpho-physiological responses by Isochrysis galbana Parke to different concentrations of oxytetracycline. Environ. Pollut. 262, 114273. https://doi.org/10.1016/j.envpol.2020.114273

Munns, R., 2002. Comparative physiology of salt and water stress. Plant, Cell Environ. 25, 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x

Nkoom, M., Lu, G., Liu, J., 2018. Occurrence and ecological risk assessment of pharmaceuticals and personal care products in Taihu Lake, China: a review. Environ. Sci. Process. Impacts 20, 1640–1648. https://doi.org/10.1039/c8em00327k

Nunes, B., Antunes, S.C., Gomes, R., Campos, J.C., Braga, M.R., Ramos, A.S., Correia, A.T., 2015. Acute Effects of Tetracycline Exposure in the Freshwater Fish Gambusia holbrooki: Antioxidant Effects,
Neurotoxicity and Histological Alterations. Arch. Environ. Contam. Toxicol. 68, 371–381. https://doi.org/10.1007/s00244-014-0101-z

Pham, D.K., Chu, J., Do, N.T., Brose, F., Degand, G., Delahaut, P., De Pauw, E., Douny, C., Van Nguyen, K., Vu, T.D., Scippo, M.L., Wertheim, H.F.L., 2015. Monitoring Antibiotic Use and Residue in Freshwater Aquaculture for Domestic Use in Vietnam. Ecohealth 12, 480–489. https://doi.org/10.1007/s10393-014-1006-z

Rydzyński, D., Piotrowicz-Cieślak, A.I., Grajek, H., Wasilewski, J., 2019. Investigation of chlorophyll degradation by tetracycline. Chemosphere 229, 409–417. https://doi.org/10.1016/j.chemosphere.2019.05.035

Shi, H., 2016. Experimental Guidance of Plant Stress Physiology. Science Press, Peking. pp. 58-75 (in Chinese).

Siedlewicz, G., Żak, A., Sharma, L., Kosakowska, A., Pazdro, K., 2020a. Effects of oxytetracycline on growth and chlorophyll a fluorescence in green algae (Chlorella vulgaris), diatom (Phaeodactylum tricornutum) and cyanobacteria (Microcystis aeruginosa and Nodularia spumigena). Oceanologia 62, 214–225. https://doi.org/10.1016/j.oceano.2019.12.002

Siedlewicz, G., Żak, A., Sharma, L., Kosakowska, A., Pazdro, K., 2020b. Effects of oxytetracycline on growth and chlorophyll a fluorescence in green algae (Chlorella vulgaris), diatom (Phaeodactylum tricornutum) and cyanobacteria (Microcystis aeruginosa and Nodularia spumigena). Oceanologia 62, 214–225. https://doi.org/10.1016/j.oceano.2019.12.002

Singh, S., Eapen, S., D’Souza, S.F., 2006. Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, Bacopa monnieri L. Chemosphere 62, 233–246. https://doi.org/10.1016/j.chemosphere.2005.05.017

Singh, S., Melo, J.S., Eapen, S., D’Souza, S.F., 2008. Potential of vetiver (Vetiveria zizanoides L. Nash) for phytoremediation of phenol. Ecotoxicol. Environ. Saf. 71, 671–676. https://doi.org/10.1016/j.ecoenv.2007.10.023

Song, G., Gao, Y., Wu, H., Hou, W., Zhang, C., Ma, H., 2012. Physiological effect of anatase TiO2 nanoparticles on Lemna minor. Environ. Toxicol. Chem. 31, 2147–2152. https://doi.org/10.1002/etc.1933

Takahashi, S., Murata, N., 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13, 178–182. https://doi.org/10.1016/j.tplants.2008.01.005

Tongaree, S., Flanagan, D.R., Poust, R.I., 1999. The Interaction Between Oxytetracycline and Divalent Metal Ions in Aqueous and Mixed Solvent Systems. Pharm. Dev. Technol. 4, 581–591.

Wang, C., Zhang, S.H., Wang, P.F., Hou, J., Li, W., Zhang, W.J., 2008. Metabolic adaptations to ammonia-induced oxidative stress in leaves of the submerged macrophyte Vallisneria natans (Lour.) Hara. Aquat.
Wang, Z., Du, Y., Yang, C., Liu, X., Zhang, J., Li, E., Zhang, Q., Wang, X., 2017. Occurrence and ecological hazard assessment of selected antibiotics in the surface waters in and around Lake Honghu, China. Sci. Total Environ. 609, 1423–1432. https://doi.org/10.1016/j.scitotenv.2017.08.009

Wu, Z., Yu, D., Li, J., Wu, G., Niu, X., 2010. Growth and antioxidant response in Hydrocharis dubis (Bl.) Backer exposed to linear alkylbenzene sulfonate. Ecotoxicology 19, 761–769. https://doi.org/10.1007/s10646-009-0453-8

Xie, X., Zhou, Q., Lin, D., Guo, J., Bao, Y., 2011. Toxic effect of tetracycline exposure on growth, antioxidative and genetic indices of wheat (Triticum aestivum L.). Environ. Sci. Pollut. Res. 18, 566–575. https://doi.org/10.1007/s11356-010-0398-8

Xie, Z., Tang, J., Wu, X., Li, X., Hua, R., 2019. Bioconcentration, metabolism and the effects of tetracycline on multiple biomarkers in Chironomus riparius larvae. Sci. Total Environ. 649, 1590–1598. https://doi.org/10.1016/j.scitotenv.2018.08.371

Ye, J., Du, Y., Wang, L., Qian, J., Chen, J., Wu, Q., Hu, X., 2017. Toxin Release of Cyanobacterium Microcystis aeruginosa after Exposure to Typical Tetracycline Antibiotic Contaminants. Toxins (Basel). 9, 53. https://doi.org/10.3390/toxins9020053

Ye, J., Huang, C., Shang, A., Xu, C., Wu, L., 2020. Characteristics of toxin production and release in Microcystis aeruginosa exposed to three tetracycline antibiotics. Environ. Sci. Pollut. Res. 27, 16798–16805. https://doi.org/10.1007/s11356-020-08253-x

Yonar, M.E., 2012. The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (Oncorhynchus mykiss, W.). Fish Shellfish Immunol. 32, 994–1001. https://doi.org/10.1016/j.fsi.2012.02.012

Zargar, A., Taheri Mirghaed, A., Mirzargar, S.S., Ghelichpour, M., Yousefi, M., Hoseini, S.M., 2020. Dietary ginger administration attenuates oxidative stress and immunosuppression caused by oxytetracycline in rainbow trout (Oncorhynchus mykiss). Aquac. Res. 51, 4215–4224. https://doi.org/10.1111/are.14763

Zhang, D., Li, Y., Shen, C., Xiao, D., Liu, J., Zhao, F., Xiong, M., 2019. Understanding the toxic effects of chlortetracycline and its isomer degradation products on Scenedesmus obliquus. J. Agro-Environment Sci. 38, 756–764. https://doi.org/10.11654/jaes.2018-0964

Zhang, M., Cao, T., Ni, L., Xie, P., Li, Z., 2010. Carbon, nitrogen and antioxidant enzyme responses of Potamogeton crispus to both low light and high nutrient stresses. Environ. Exp. Bot. 68, 44–50. https://doi.org/10.1016/j.envexpbot.2009.09.003

Zhao, S., Liu, X., Cheng, D., Liu, G., Liang, B., Cui, B., Bai, J., 2016. Temporal–spatial variation and partitioning prediction of antibiotics in surface water and sediments from the intertidal zones of the
Yellow River Delta, China. Sci. Total Environ. 569–570, 1350–1358.
https://doi.org/10.1016/j.scitotenv.2016.06.216

Zhong, G., Wu, Z., Yin, J., Chai, L., 2018. Responses of Hydrilla verticillata (L.f.) Royle and Vallisneria natans (Lour.) Hara to glyphosate exposure. Chemosphere 193, 385–393.
https://doi.org/10.1016/j.chemosphere.2017.10.173

Zhou, J., Wu, Z., Yu, D., Pang, Y., Cai, H., Liu, Y., 2018. Toxicity of linear alkylbenzene sulfonate to aquatic plant Potamogeton perfoliatus L. Environ. Sci. Pollut. Res. 25, 32303–32311.
https://doi.org/10.1007/s11356-018-3204-7

Zhu, L., Xu, H., Xiao, W., Lu, J., Lu, D., Chen, X., Zheng, X., Jeppesen, E., Zhang, W., Wang, L., 2020. Ecotoxicological effects of sulfonamide on and its removal by the submerged plant Vallisneria natans (Lour.) Hara. Water Res. 170, 115354. https://doi.org/10.1016/j.watres.2019.115354

Zhu, X., Ding, D., Ru, S., Tan, X., Xie, Qilai, Deng, C., Xie, Qiaoyun, 2018. Effects of Ca2⁺tetracycline antibiotic monomers complex solution and soil desorption solution on seed germination and root bud elongation of Chinese cabbage. Jiangsu Agric. Sci. 46, 141-144 (in Chinese).
https://doi.org/10.15889/j.issn.1002-1302.2018.17.036

**Figures**
Figure 1

Chlorophyll a (a), chlorophyll b (b), total chlorophyll (a+b) (c) and carotenoid (d) contents of V. natans after 7 D of CTC and OTC treatments. All values represent the mean of three replicates ± stands deviation. ANOVA significant at p < 0.05. Bars with different letters are significantly different among different exposure concentration (p ≤ 0.05, Duncan test).
Figure 2

Chlorophyll a (a), chlorophyll b (b), total chlorophyll (a+b) (c) and carotenoid (d) contents of V. natans after 14 D of CTC and OTC treatments. All values represent the mean of three replicates ± stands deviation. ANOVA significant at p < 0.05. Bars with different letters are significantly different among different exposure concentration (p ≤ 0.05, Duncan test).
Figure 3

MDA content of *V. natans* after 7 D (a) and 14 D (b). All values represent the mean of three replicates ± stands deviation. ANOVA significant at \( p < 0.05 \). Bars with different letters are significantly different among different exposure concentration (\( p \leq 0.05, \) Duncan test).
Figure 4

CAT (a), POD (c) and PPO (e) activities of V. natans after 7 D CTC and OTC treatments. CAT (b), POD (d) and PPO (f) activities of V. natans after 14 D CTC and OTC treatments. All values represent the mean of three replicates ± stands deviation. ANOVA significant at $p < 0.05$. Bars with different letters are significantly different among different exposure concentration ($p \leq 0.05$, Duncan test).
Figure 5

Soluble protein contents of V. natans after 7 D (a) and 14 D (b) of CTC and OTC treatments. All values represent the mean of three replicates ± stands deviation. ANOVA significant at p < 0.05. Bars with different letters are significantly different among different exposure concentration (p ≤ 0.05, Duncan test).

Figure 6

H2O2 contents of V. natans after 7 D (a) and 14 D (b) of CTC and OTC treatments. All values represent the mean of three replicates ± stands deviation. ANOVA significant at p < 0.05. Bars with different letters are significantly different among different exposure concentration (p ≤ 0.05, Duncan test).