Ecological Processes

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Enrichment and toxic effects of triclosan on aquatic macrophytes *Eichhornia crassipes* and *Hydrilla verticillata* exposed to triclosan in sediments

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Abstract

Background Clarifying the enrichment and response processes of triclosan (TCS) in hydrophytes is crucial for assessing the ecological risk of TCS in aquatic environments. This study delves into the chronic toxic effects of TCS in floating plant *Eichhornia crassipes* (Mart.) Solms and submerged plant *Hydrilla verticillata* (L. f.) Royle exposed to TCS sediments through hydroponic experiments.

Results The absorption abilities of hydrophytes to TCS were species-dependent. The concentration of TCS in the roots of *E. crassipes* was significantly higher than that in its leaves, while the absorption capacities of the leaves of *H. verticillata* to TCS were stronger than that in its roots. Furthermore, the physiological indexes, including chlorophyll concentration, soluble protein concentration, and antioxidant enzyme activities, showed a significant decrease with the exposure concentration and time of TCS. Although the chlorophyll and soluble protein concentrations and the antioxidant enzyme activities in the leaves were initially increased at a low concentration of TCS (at 7 days of exposure), they decreased significantly over time. Compared to the leaves, the physiological indexes of the roots were more sensitive to the ecotoxicological effects of TCS. The inhibition effects of TCS on *H. verticillata* were significantly higher than those on *E. crassipes*, which may be associated with the absorbing abilities of TCS and the growth characteristics of the plants. Pearson's correlation analysis found a significant negative correlation between the TCS concentrations and the antioxidant enzyme activities in the plants.

Conclusions This study highlighted the differences in the uptake and enrichment process and toxic effects of TCS by different aquatic plants. Compared with *E. crassipes*, *H. verticillata* is more sensitive to TCS toxicity.

Keywords Triclosan, Aquatic macrophyte, Enrichment, Antioxidant defense system, Ecotoxicological effect

Introduction

Triclosan (2,4,4'-trichloro-2'hydroxydiphenyl ether, TCS) is a broad-spectrum antibacterial compound widely used in personal care products, such as medical soap, hand sanitizer and toothpaste (Gallego et al. 2021). It is reported that approximately 1500 tons of TCS are produced annually worldwide (Li 2021). TCS can enter surface water through various pathways and easily be adsorbed onto the sediment due to its high lipophilicity with a log K_{ow} of 4.8 (Chen et al. 2018). Recently,

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numerous studies have been conducted to investigate the occurrence and distribution of TCS in the aquatic environment, particularly in river sediments. Nag et al. (2018) reported concentration ranges of TCS in river sediments in India to be 5.11–50.36 μg/kg. In China, Zhao et al. (2013) detected mass concentrations of TCS in the sediments of various rivers, including Liaohe River, Haihe River, Yellow River, Zhujiang River and Dongjiang River were as high as 1329 μg/kg. It is worth noting that TCS has a half-life of up to 107 days in sediments, leading to its long-term persistence, especially under the anaerobic or sterile aerobic conditions (Pavlostathis et al. 2003). Although TCS is not considered highly toxic, prolonged exposure to TCS can result in chronic toxicological effects on aquatic organisms. Several studies have investigated the eco-toxicological effects of TCS on lower plants, such as algae. For example, Huang et al. (2016) found that the EC_{50} value (the concentration for half maximal effect) of TCS for algae *Microcystis aeruginosa* at 96 h was 9.2 μg/L. The EC_{50} of TCS for algae *Cymbella* sp. at 24, 48 and 96 h were 625.8, 240.3 and 324.9 μg/L, respectively (Ding et al. 2018; Bester 2005). Brausch and Rand (2011) reported that the EC_{50} of TCS for *Skeletonema costatum* at 96 h was greater than 66 μg/L. Besides the algae, the entry of TCS into the aquatic system and its higher persistence levels can have toxic effects on aquatic flora and fauna. For instance, Khatikarn et al. (2018) demonstrated that EC_{50} of TCS for *Macrobrachium lanchesteri* at 96 h was 962 μg/L. Capkin et al. (2017) found that the EC_{50} of TCS for rainbow trout at 96 h was 0.05 mg/L, and TCS caused significant DNA damage to rainbow trout.

It usually takes about 3–5 days to observe adverse effects on species in traditional chronic tests (Christakos et al. 2017). However, in the natural aquatic environment, aquatic organisms are exposed to TCS for longer periods, leading to irreversible ecotoxicological effects (Xin et al. 2019). Prolonged exposure to TCS can also result in its accumulation in organisms, posing threats to ecological safety and human health, including endocrine disruption, genotoxicity, carcinogenicity, and fetal malformations (Liu et al. 2020). Although the experiments have been conducted to investigate the ecotoxicological effects and accumulation of TCS on aquatic organisms from algae to fish in recent years, the chronic ecotoxicological effects of TCS enrichment on aquatic macrophytes are unclear.

Aquatic macrophytes play a vital role in maintaining the structure and function of the aquatic ecosystem. Moreover, some macrophytes are usually used as animal feed, medicinal materials, and even edible vegetables. The growth status of the macrophytes exposed to pollutants can affect human health through the food chain (Keerthanan et al. 2020). In recent years, aquatic plants, including free-floating and submerged species, have become typical materials for evaluating the toxic effect of organic pollutants (Pi et al. 2017; Chang et al. 2020). In addition, some macrophytes can take up and enrich antibiotics, microplastics and TCS (Azanu et al. 2020; Mao et al. 2023; Shao et al. 2019). For example, the roots of the typical aquatic plant, *Phragmites australis* can take up antibiotics, such as tetracycline, 4-epitetracycline, 4-epianhydrotetracycline and anhydrotetracycline at the concentrations of 31.5, 46.1, 27.0 and 16.5 μg/kg, respectively (Madikizela et al. 2018). The uptake of organic pollutants may also affect the growth and development of plants (Peng et al. 2021). It is proposed that phytoremediation may serve as a potential approach to decrease the TCS concentration in the aquatic environment (Ali et al. 2013). Previous studies have demonstrated that aquatic macrophytes can be effective remediation approaches for accumulating and removing a variety of waterborne contaminants (Wang et al. 2017). However, the research on ecotoxicological effects and bioaccumulation in aquatic macrophytes induced by TCS is still very limited, particularly with long time low dose TCS exposure in sediments.

In this study, two species of aquatic macrophytes were investigated: *Eichhornia crassipes* (Mart.) Solms (a free-floating species) and *Hydrilla verticillata* (L. f.) Royle (a submerged species). Both species are worldwide distributed and commonly used as aquatic ornamental plants (Srivastava et al. 2007; Tiwari et al. 2007). *E. crassipes* has a well-developed root system, while *H. verticillata* has adventitious root. Although *E. crassipes* and *H. verticillata* are different in habits, these two aquatic macrophytes share many common characteristics, such as fast growth and reproduction, strong adaptability, good water purification capacity, and are widely used as animal feed (Gao et al. 2018). The enrichment and toxic effects of TCS on aquatic macrophytes, specifically *E. crassipes* and *H. verticillata*, exposed to TCS sediments, are not well-understood. Therefore, the aims of this study were to (1) investigate the uptake process of TCS in *E. crassipes* and *H. verticillata* when exposed to low concentrations of TCS in sediments for a long period of time; (2) evaluate the dynamic changes of chlorophyll concentration, soluble protein concentration and antioxidant enzyme activity to understand the damage of the enrichment process to the antioxidant defense system; (3) clarify the correlation between the TCS concentration in plants and physiological indexes. The findings will provide valuable data on the toxicity of TCS to aquatic organisms and contribute to our understanding of the ecological risk posed by TCS. In addition, the study will provide insights into the potential use of aquatic macrophytes for the removal of micropollutants from water systems.

Materials and methods

Experiment materials

Chemicals

Triclosan (99.0% purity) was purchased from Shanghai Tengzhun Bio-Technology Co., Ltd. Acetonitrile methanol, acetonitrile, Coomassie brilliant blue were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals and organic solvents, including ethyl-acetate, dimethyl sulfoxide (DMSO), phosphoric acid, calcium carbonate, ethylenediaminetetraacetic acid disodium salt, used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). These reagents were of at least analytical grade.

Sampling location and sediment preparation

The sediment samples were collected from Dahuofang Reservoir in Liaoning Province (41.842°N, 124.154°E), which has no wastewater input and little anthropogenic contact. Surface sediments (at 0–10 cm depth) were taken by a sampler and transported to the laboratory. All samples were sieved through a 2-mm mesh to remove coarse debris. A small fraction of the initial sediment sample was freeze dried (FD-1C-50, Bo Yikang Experimental Instrument Co., Ltd., Beijing, China) and used for analyzing of TCS concentration. TCS was not detected in the tested sediment samples or was below the detection limit of the instrument (UPLC–MS/MS; Waters Acquity™ and Quattro Premier XE, Milford, MA, USA).

Aquatic plants preparation

The seedlings of *E. crassipes* were purchased from the Xiaojinqiao Aquatic Plant Planting Company in Shenyang. The seedlings of *H. verticillata* were purchased from Jizhong Aquatic Plant Growing Company, Anxin County, Liaoning Province. After acclimating in 30% Hoagland nutrient solution for 14 days, the seedlings of *E. crassipes* uniform in size with 3–4 leaves, were used for the experiment. After acclimating in 10% Hoagland nutrient solution for 14 days, seedlings of *H. verticillata* about 10 cm high were used for the experiment.

Plant cultivation experiment

The experiment was conducted with sediments (4 cm thick) on the bottom of the hydroponic tanks (35 cm × 20 cm × 25 cm). Then, 8 L of 100% Hoagland solution was added to each tank. To maintain the water level and nutrient supply, we regularly supplement the nutrient solution. TCS at varying concentrations (0, 0.05, 0.10 and 0.50 mg/kg) were employed for a complete experiment of four treatments. Each treatment was performed in triplicate. Ten well-grown *E. crassipes* were placed in water and 20 well-grown *H. verticillata* were planted in the sediments in each of the prepared hydroponic tanks, respectively. The experiment was conducted in a greenhouse at the Key Laboratory of Pollution Ecology and Environmental Engineering, Chinese Academy of Sciences. The experiment was conducted at 25 °C. Artificial light at 3600 lx was provided for each tank with a light cycle of 12 h light and 12 h dark. Aeration was performed during the experiment. The plants were removed from the tanks at 7, 14, 21 and 28 days for testing. After receiving the plants, they were washed with distilled water, wiped dry, divided into leaves and roots, and stored in the refrigerator at − 20 °C until analysis.

Analysis of plant physiological indexes

Chlorophyll concentration

The chlorophyll (CHL) concentration in leaves of fresh plants was determined by grinding 0.20 g fresh leaves in a mortar with a small amount of quartz sand, calcium carbonate powder and acetone (3 mL, 95%). The resulting homogenate was filtered through a funnel using a glass rod into a brown volumetric flask, and the volume was adjusted to 10 mL with acetone (95%). The absorbance of the solution was measured at 645 nm and 663 nm using a UV-Vis spectrophotometer (Varian Cary 50 Conc, Varian, Inc., USA). The CHL concentration was calculated according to the method described by Marr et al. (1995)

$$C_a(\mathrm{mg/L}) = 12.7A_{663} - 2.69A_{645} \quad (1)$$

$$C_b(\mathrm{mg/L}) = 22.9A_{645} - 4.68A_{663} \quad (2)$$

$$C_T(\mathrm{mg/L}) = 20.2A_{645} + 8.02A_{663} \quad (3)$$

where C_a is the concentrtion of chlorophyll *a*, C_b is the content of chlorophyll *b*, C_T is the concentration of total chlorophyll, A_{663} and A_{645} are the absorbance at 663 nm and 645 nm wavelength, respectively.

Soluble protein concentration

Fractions of fresh leaf and root samples (0.5 g *E. crassipes* and 0.2 g *H. verticillata*) were homogenized under liquid nitrogen. Then, 5 mL of extraction buffer (50 mM phosphate buffer, pH 7.8) and some quartz sands were added to the homogenate, which was ground in an ice bath. The homogenates were centrifuged at 12,000 r/min at 4 °C for 10 min using a centrifuge (5804R, Eppendorf Crop., Germany). To measure soluble protein concentration, 0.1 mL of the supernatant extract was mixed with 5 mL of Coomassie Brilliant Blue reagent. A blank was

prepared by mixing 0.1 mL of the extraction buffer with 5 mL of Coomassie Brilliant Blue reagent. The absorbance of the samples and blank was measured at 595 nm. The concentration of soluble protein in the supernatant was measured using the dye-binding method of Coomassie Brilliant Blue (Bradford 1976).

Antioxidant enzyme activity

Fresh root and leaf samples (0.5 g) were ground to homogenate using 0.05 M phosphate buffer (pH 7.0). The homogenate was then transferred to centrifuge tube and centrifuged at 12 000 r/min at 4 °C for 10 min. The resulting supernatant was used to measure the activity of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). The detection kits from JianCheng were used for these measurements.

Analysis of TCS concentration in plants

Aliquots of 3.0 g of vacuum freeze-dried crushed samples were transferred to a 50 mL polypropylene tube and 10 mL of acetonitrile was added. The mixture was then subjected to ultrasonic extraction for 30 min. After extraction, the mixture was centrifuged at 4000 r/min at 4 °C for 10 min, and the supernatant was collected in centrifuge tubes. This extraction process was repeated three times. The pH of supernatant was adjusted to 2.0 using 1%, H_2SO_4. Mixture after being shaken was filtered through a solid phase extraction (SPE) cartridge, which was pre-washed 5 mL of methanol and 10 mL of water in advance. After the removal of matrix interferences with 6 mL of water and methanol (methanol/water, 5:95, V/V), the SPE cartridge was dried completely and then loaded with 9 mL of methanol to elute TCS out. The extract was evaporated and then reconstituted to 1 mL by nitrogen and stored at − 20 °C until analysis.

The concentration of TCS in plants was analyzed using high-performance liquid chromatography–mass spectrometry (HPLC–MS/MS) with electrospray ionization (ESI) in the negative mode. The compounds were separated on a C18 column (2.1 × 150 mm, 3.5 μm, Akzo Nobel Kromasil Eternity-5). 5 mL ethyl acetate, 5 mL methanol and 10 mL Milli-Q water were used to activate through the C18 solid–liquid extraction column at the flow rate of 1 mL/min; then the sample solution was purified through the C18 solid–liquid extraction flow at the velocity of 5 mL/min. The mobile phase consisted of Milli-Q water and acetonitrile (10:90, V:V) at a flow velocity of 1.0 mL/min. The injection volume was 20 μL. The conditions were initially 20% acetonitrile gradient to 85% for 7 min, 85% acetonitrile sustained for 1 min, dropped to 20% acetonitrile at 9 min, then maintained for 1 min. The mean recovery of TCS in spiked samples was 92% ± 11%. To monitor instrument drift, a standard sample check was injected after every 10 samples. The instrumental calibration curve for TCS ranged from 0.2 to 100 ng/mL, with a regression coefficient (r) of ≥ 0.996. TCS was not tested in the procedural blanks and field blanks.

Data analysis

All mean values and standard deviations were calculated using Microsoft Excel 2016. The percentages of chlorophyll, soluble protein, SOD, CAT and POD were calculated as % control = (TCS treatment/control group) × 100. Significant differences among different treatments were determined using SPSS software version 25.0 (Chicago, Illinois, USA) according to the Least Significant Difference (LSD) test at $p < 0.05$. Figures were created using Origin 2023 software (OriginLab Corp., Northhampton, USA).

Results

Adsorption and enrichment of TCS by aquatic plants

The uptake of TCS in leaves and roots increased significantly with increasing TCS concentrations and exposure time (Table 1). The TCS concentration in the roots of *E. crassipes* was higher than that in the leaves. In *H. verticillata*, the TCS concentration in the leaves was greater than that in the roots. The maximum uptake of TCS was observed in *E. crassipes* roots and *H. verticillata* leaves under 0.5 mg/kg TCS at 28 days. Based on the mean values of concentrations in the plants, the concentrations of the TCS in different parts decreased in the following order: *E. crassipes* roots > *H. verticillata* leaves > *H. verticillata* roots > *E. crassipes* leaves.

Changes of chlorophyll concentration in the hydrophytes stressed by TCS

In the early exposure (7 days), the CHL concentrations of both plant species increased with 0.05 mg/kg TCS compared to the controls (Fig. 1). However, as the concentration of TCS in the sediments increased and the exposure time prolonged, the CHL concentrations of the plants gradually decreased. The CHL concentrations of the plants in the 0.1–0.5 mg/kg TCS treatments were significantly lower than those in the 0.05 mg/kg TCS treatment during in the exposure period. After 14 days of exposure, 0.05 mg/kg TCS had no inhibitory effects on the CHL concentration in *E. crassipes*, but the concentration of CHL decreased significantly in *H. verticillata*. After 28 days of exposure to 0.5 mg/kg TCS, the inhibition rate of CHL concentration in *E. crassipes* was 26.01%, while in the leaves of *H. verticillata*, it was 33.74%. The CHL concentrations in *H. verticillata* were more vulnerable than those in *E. crassipes* under TCS stress at the same exposure time.

Table 1 Concentrations of TCS in plant parts of *Eichhornia crassipes* and *Hydrilla verticillata* (mg/kg)

Plants	Treatments (mg/kg)	Plant uptake concentration (mg/kg)							
		7 d		14 d		21 d		28 d	
		Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
E. crassipes	0.05	0.54 ± 0.09c	1.96 ± 0.21c	0.99 ± 0.17b	4.35 ± 0.25b	1.08 ± 0.25b	5.53 ± 0.49b	1.43 ± 0.07b	6.02 ± 0.50c
	0.10	0.91 ± 0.11b	3.33 ± 0.56b	1.27 ± 0.16b	5.48 ± 0.67b	1.21 ± 0.18b	6.71 ± 0.73b	1.45 ± 0.08b	7.73 ± 0.56b
	0.50	1.22 ± 0.08a	5.18 ± 0.26a	1.47 ± 0.12a	6.27 ± 0.50a	1.86 ± 0.22a	9.41 ± 1.28a	1.97 ± 0.17a	11.37 ± 0.9a
H. verticillata	0.05	1.44 ± 0.14c	0.97 ± 0.11c	1.96 ± 0.07c	1.35 ± 0.12c	3.86 ± 0.26c	1.76 ± 0.25c	4.41 ± 0.43c	2.89 ± 0.44c
	0.10	2.50 ± 0.32b	2.26 ± 0.10b	3.02 ± 0.19b	2.65 ± 0.30b	5.00 ± 0.25b	2.86 ± 0.18b	5.83 ± 0.30b	5.46 ± 0.49b
	0.50	3.91 ± 0.23a	3.02 ± 0.25a	4.07 ± 0.07a	3.39 ± 0.18a	6.74 ± 0.37a	4.12 ± 0.10a	8.74 ± 0.63a	6.86 ± 0.28a

*The data in the table are means ± standards; Different lowercase letters indicate significant differences among different treatments in same plant part, $p < 0.05$

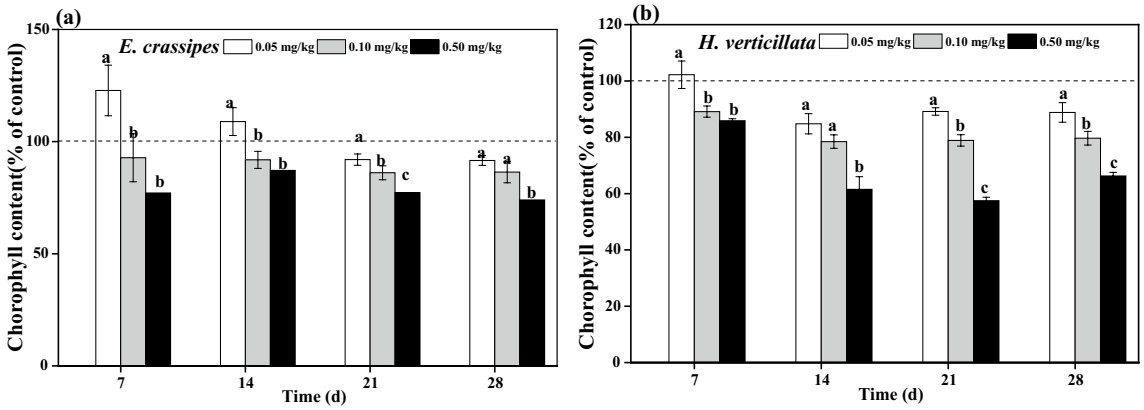

Fig. 1 Relative changes in the chlorophyl concentration of *E. crassipes* (**a**) and *H. verticillata* (**b**) exposed to different TCS concentrations and durations of exposure as compared to the control. Different lowercase letters indicate a significant difference at $p < 0.05$ among treatments for a particular duration

Changes of soluble protein concentration in the hydrophytes stressed by TCS

The soluble protein (SP) concentration in the leaves of both plant species increased during the initial exposure stage (7 days) for all concentration treatments (Fig. 2). In *E. crassipes*, the SP concentration in the leaves continued to increase at the 14th day exposure stage for the 0.05–0.1 mg/kg TCS treatments. However, it decreased with the 0.05–0.5 mg/kg TCS treatments during the exposure period (21–28 days). In *H. verticillata*, the SP concentration in the leaves showed an increasing trend on the 7th day, and decreased with increasing exposure time to TCS in sediments. After 28 days of exposure to 0.5 mg/kg TCS, the inhibition rate of SP concentration in the leaves of *E. crassipes* was 45.82%, while in leaves of *H. verticillata* it was 78.30%. The SP concentrations in the roots of *E. crassipes* were significantly reduced ($p < 0.05$) by 0.05–0.5 mg/kg TCS during the exposure period (Fig. 2). However, the SP concentrations in the roots of *H. verticillata* increased at the 7 day exposure period for TCS treatments and at the 14 day exposure stage for the 0.05 mg/kg TCS treatment. Then, SP concentration decreased with 0.05–0.5 mg/kg TCS treatments during the exposure period of 21–28 days. After 28 day exposure to 0.5 mg/kg TCS, the inhibition rate of SP concentration in the roots of *E. crassipes* and *H. verticillata* was 73.77% and 66.51%, respectively.

Changes of antioxidant enzyme activities in the hydrophytes stressed by TCS

Compared to control, the SOD activity in the leaves of the two species of plants increased at the initial exposure stage (7 days) for all concentration treatments (Fig. 3a). Then, it decreased with an increase in the exposure time under TCS treatments, except for *E. crassipes* at 14 day exposure stage for 0.05 mg/kg TCS treatment. The SOD activity in the roots of *E. crassipes* was significantly reduced ($p < 0.05$) at 0.05–0.5 mg/kg TCS stress during the exposure period of 21–28 days. However, compared to control, the SOD activity in the roots of *H. verticillata* increased at 7 day exposure period for TCS treatments and at the 14 day exposure stage for the 0.05 mg/

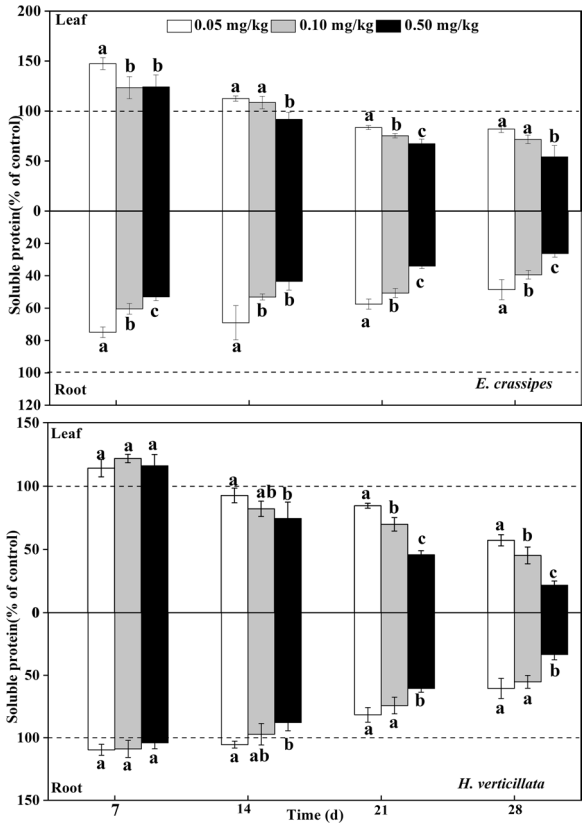

Fig. 2 Relative changes in the soluble protein concentration of *E. crassipes* and *H. verticillata* exposed to different TCS concentrations and durations of exposure as compared to the control. Different lowercase letters indicate a significant difference at $p < 0.05$ among treatments for a particular duration

kg TCS treatment. TCS exhibited a higher inhibitory rate of SOD in the roots of *E. crassipes* and the leaves of *H. verticillata*.

Compared to control, the CAT activity in the leaves of *E. crassipes* increased at 14 day exposure stage for all TCS treatments and even at the 21 day exposure stage for the 0.05 mg/kg TCS treatment (Fig. 3b). The CAT activity in the leaves of *H. verticillata* increased at the 7 day exposure stage for all TCS treatments, then decreased at 14–28 day exposure stage for all TCS treatments. The CAT activity in the roots of *E. crassipes* increased at 7 day exposure stage for 0.05–0.1 mg/kg TCS treatments. The CAT activity in the roots of *H. verticillata* increased at 7 day exposure stage for all TCS treatments, then, decreased at 14–28 day exposure stage for all TCS treatments. TCS exhibited a higher inhibitory rate of CAT in the leaf of submerged species, *H. verticillata*.

The POD activity in *E. crassipes* leaves increased with 0.05–0.1 mg/kg TCS treatments during the exposure period (Fig. 3c). The POD activity in the leaves of *H. verticillata* increased at 7 day exposure stage for only the 0.05 mg/kg TCS treatment, then decreased at the exposure stage for all TCS treatments. The POD activity in the roots of *E. crassipes* increased at the 7 day exposure stage for 0.05–0.1 mg/kg TCS treatments. However, the POD activity in the roots of *H. verticillata* increased at the 7–14 day exposure stage for all TCS treatments, and even at 21 day exposure stage for 0.05–0.1 mg/kg TCS treatments. TCS exhibited a higher inhibitory rate of POD in the roots of the free-floating species, *E. crassipes*, and the leaves of the submerged species, *H. verticillata*.

Pearson's correlation between TCS concentration in plants and physiological indexes

We conducted Pearson's correlation analysis between TCS concentration in plants and physiological indexes of plants stressed by 0.5 mg/L TCS during the exposure time (Fig. 4). In both plants, the concentration of TCS in leaves and roots was significantly positively correlated each other. In *E. crassipes*, CHL concentration and SP concentration in leaves of *E. crassipes* were significant positively correlated with TCS concentration in roots. The SP concentration in *E. crassipes* leaves was positively correlated with TCS in leaves. SP concentration in roots, SOD activity in leaves, CAT activity in leaves and POD activity in roots were significantly negatively correlated with TCS concentration in roots of *E. crassipes*. CAT activity in leaves and POD activity in roots were negatively correlated with TCS in leaves. However, in *H. verticillata*, SP concentration, SOD activity and CAT activity were all significantly negatively correlated with TCS concentrtaion. The activities of antioxidant enzymes of *H. verticillata* were more directly affected by TCS stress compared to *E. crassipes*.

Discussion

Aquatic macrophytes, such as floating plants (*E. crassipes*) and submerged plants (*H. verticillata*), are crucial components of aquatic ecosystems. They play a significant role in carbon cycling and nutrient regulation (Arts et al. 2008). However, there is a lack of information regarding enrichment process and dynamic toxic effects of TCS on aquatic macrophytes exposed to TCS sediments. Therefore, we used floating (*E. crassipes*) and submerged (*H. verticillata*) plants to assess the enrichment process of TCS and dynamic toxic effects of TCS.

Organic contaminants can enter aquatic ecosystems through various pathways and their fate and transport depend on their partitioning between particulate and dissolved phases. Due to their lipophilic nature, these contaminants tend to accumulate in sediments through adsorption onto solid particle surfaces (Pizzini et al. 2021; Venturini et al. 2015). In our study, we compared the uptake of TCS by submerged and floating plants.

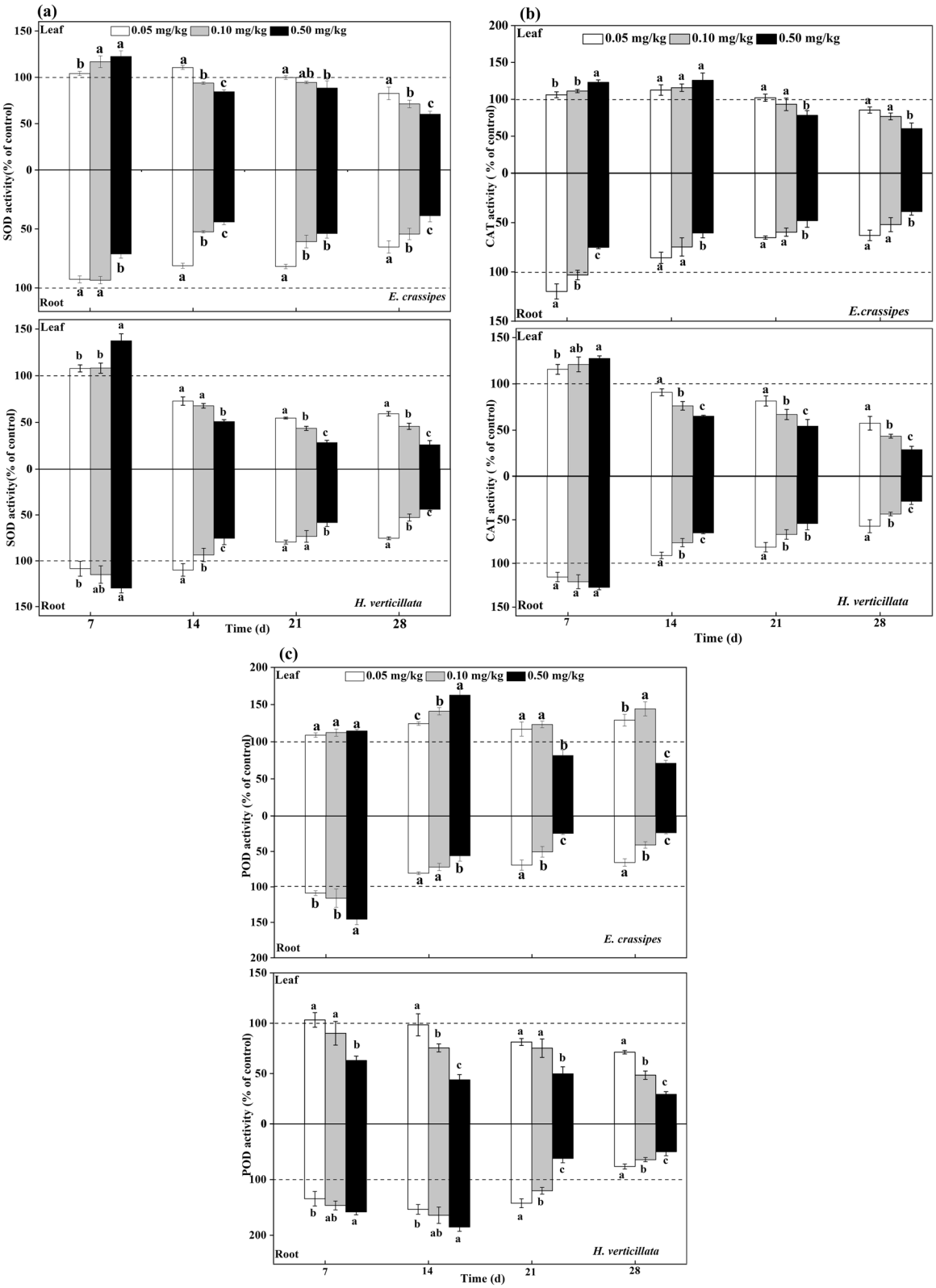

Fig. 3 Relative changes in the POD, SOD, and CAT activities in leaves and roots of both species exposed to different TCS concentrations and durations of exposure as compared to the control. The data represent the means ± SDs ($n = 3$). Different letters indicate a significant difference at $p < 0.05$ among treatments for a particular duration

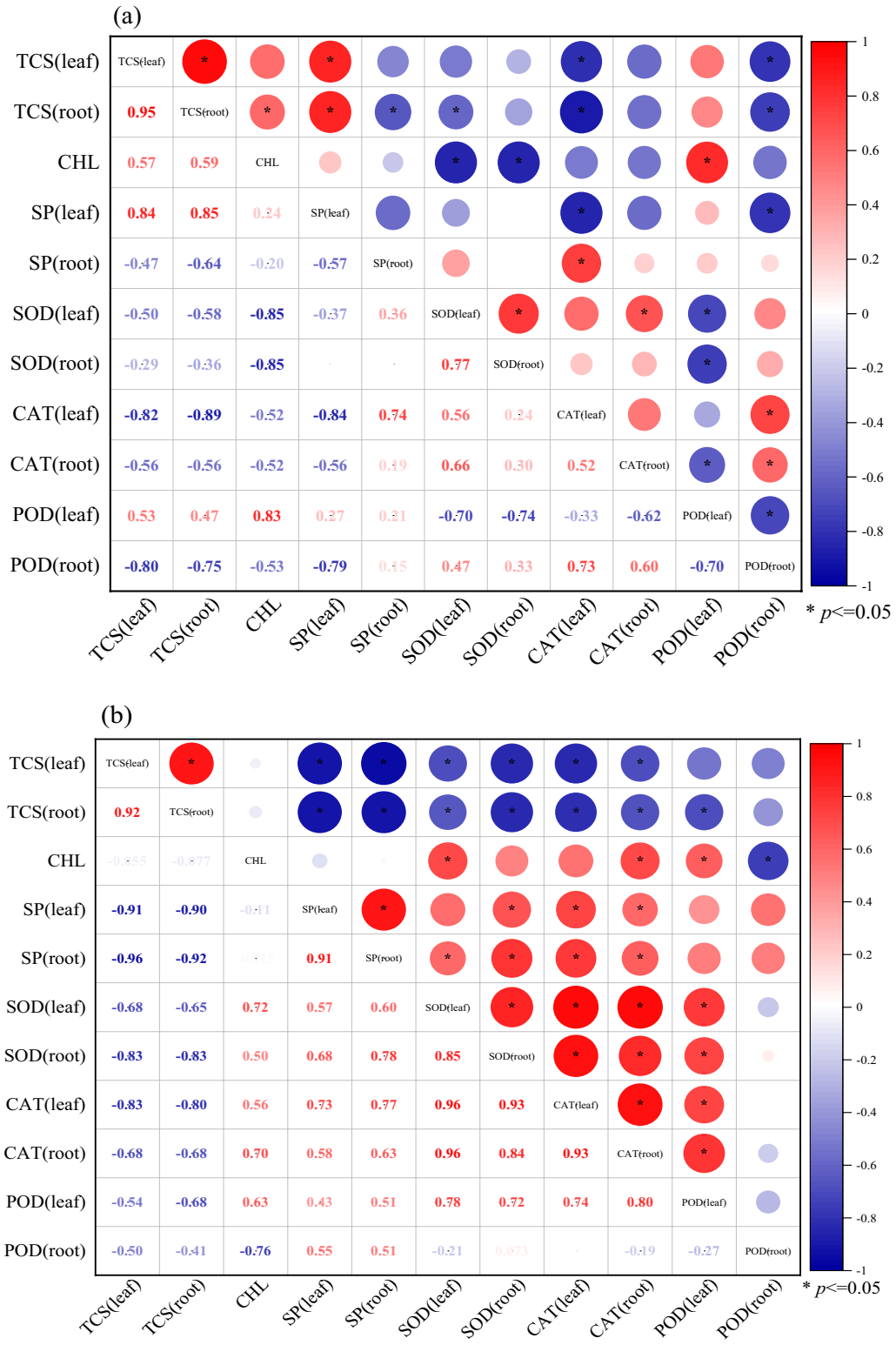

Fig. 4 Pearson's correlation analysis between TCS concentration in plants and physiological indexes of plant stressed by 0.5 mg/L TCS during exposure time. **a** *E. crassipes*, **b** *H. verticillata*

We found that the roots of *E. crassipes* had higher TCS concentration than the leaves. This is consistent with the general trend of pollutant uptake being stronger in roots than in leaves (Yan et al. 2022a, b). Studies have shown that TCS has a significant cumulative effect in plant roots. It can be transported as a solute through the epidermis on the surface of plant roots, further transported to vascular tissue, and then transported to aboveground sites through xylem or phloem (Al-Farsi et al. 2017). The predominance of TCS in plant roots may be related to its high hydrophobicity (Chu and Metcalfe 2007; Nghiem and Coleman 2008; Bedoux et al. 2012) and relatively high lipid concentration in roots (Liu and Schnoor 2008). Collins et al. (2011) found that plant roots have relatively higher lipid concentration than other tissues, while Chiou et al. (2001) showed that even a small amount of lipids can become a major concentration of highly hydrophobic substances. Therefore, the TCS absorbed by plants are mainly concentrated in the roots. However, we observed that the enrichment of TCS in leaves of *H. verticillata* was stronger than that in roots. This could be attributed to the fact that *H. verticillata* leaves in direct contact with the water containing TCS. The water-to-leaf pathway is a major route for the phytoaccumulation of pollutants in aquatic plants (Porra et al. 1989; Fan et al. 2018). Meeks (1968) also reported similar findings and suggested that the great surface area of submerged plants in contact with the water accounts for such results. In addition, the TCS found in the leaves may be partly transported from the roots in addition to its own uptake. Therefore, the uptake process of TCS in the two plants is different.

The CHL concentration of plants is an important parameter for assessing photosynthetic activity and can indicate pollutant-induced plant stress (Zhou and Leul 1999; Huang et al. 2004). In our study, the results indicate that the CHL concentration in the leaves of two plant species increased under relatively low concentrations of TCS, but decreased with higher concentration of TCS in sediments and longer exposure time. This could be attributed to the low concentration of TCS promoting the synthesis of CHL in a short period of time. However, Peng et al. (2021) found that TCS at certain concentrations can have adverse effects on plant growth, including disrupting balance of reactive oxygen species (ROS) and damaging the photosynthetic system of plants. ROS plays a crucial role in various life process and stress responses in plants (Dietz et al. 2016). With the increase in exposure time, there was a significant inhibition of CHL concentration in both plant species, and there was a significant difference between the test groups ($p < 0.05$). This decrease in CHL concentration can be attributed to the ability of TCS to reduce photosynthetic efficiency and cause damage to the photosynthetic apparatus, including uncoupling of oxidative phosphorylation, inhibition of non-photochemical quenching, and damage to photopigments (Almeida et al. 2017; Xin et al. 2019). This decrease in photosynthetic efficiency can be an early indication of toxic effects occurring at the structural level (Juneau et al. 2001). In terms of CHL concentration, *H. verticillata* is more sensitive to TCS-induced stress compared to *E. crassipes*, mainly because *E. crassipes* is a free-floating species with stronger photosynthesis than the submerged species, *H. verticillata*. Correlation analysis found that there is a correlation between CHL concentration and antioxidant enzyme activities (Fig. 4). The disturbance of photosynthetic system, caused by lower concentration of TCS and longer exposure time treatments, can potentially disrupt the metabolic process of aquatic macrophytes.

SP concentration is regarded as an indicator of both oxidative stress and growth status in plants (Song et al. 2017). The SP concentration in the leaves decreased with increasing exposure time of TCS. The adverse effect of TCS on SP in *E. crassipes* leaves was lower than that in *E. crassipes* roots, possibly due to the degradation of TCS in leaves through photolysis (Ozaki et al. 2021), which reduced its toxic effect on SP. However, the impact of TCS on SP concentration in the leaves of *H. verticillata* was significantly higher than that in the roots. This could be attributed to the double stress of TCS from water and the TCS transferred from the roots in *H. verticillata*. The SP concentration generally declined in the roots of both species with increasing TCS concentration. TCS can affect proteins in plants by combing with or disrupting specific proteins, leading to oxidative damage to SP (Perozzo et al. 2002). In addition, TCS can induce the formation of ROS, which can further contribute to oxidative damage to SP (Xin et al. 2019). Both the concentration and exposure time of TCS are important factors that affect the SP concentration in plants (An et al. 2009). Correlation analysis showed that the SP concentration of *H. verticillata* was more directly affected by TCS stress compared to *E. crassipes*.

The antioxidant enzymes play a crucial role in protecting plants from oxidative damage caused by ROS (Liu et al. 2019; Chen et al. 2016; Singh et al. 2010). One such enzyme is SOD, which acts as the first line of defense in the antioxidant defense system. SOD catalyzes the conversion of superoxide radicals into H_2O_2 and O_2, thereby inhibiting the formation of hydroxyl radicals, and terminating the free radical chain reaction to protect plants from oxidative damage (Zikic et al. 1996). In the case of TCS contamination, plants initially experience stress, leading to the production of large amounts of superoxide radicals. As a result, SOD activity increases to maintain homeostasis and protect the plants from oxidative

damage. Therefore, compared with the control group, the SOD activity first increased and then decreased during the uptake and enrichment of TCS in plants. Correlation analyses revealed that the TCS concentration was significantly negatively correlated with SOD activity in the plants.

CAT and POD are viewed as the second line of defense against damage caused by reactive oxygen species damage. They can act synergistically to convert H_2O_2 produced by SOD into H_2O and O_2 (Song et al. 2018). POD and CAT showed relatively high activity to maintain the balance of the antioxidant defense system's enzyme activity. In addition, in the process of plant absorption and enrichment of TCS, the activity of CAT and POD in plants increased for a longer time than that of SOD. POD activity is more stable than CAT. Through correlation analyses, it was also found that TCS concentration was significantly negatively correlated with CAT and POD activity in the plants.

Therefore, in the process of plant absorption and enrichment, there was a direct negative correlation between the concentration of TCS in plants and the activities of antioxidant enzymes.

Conclusion

This study aims to investigate the uptake processes and dynamic toxic effects of sediment exposure TCS on the submerged macrophyte *H. verticillata* and the floating macrophyte *E. crassipes*. The enrichment of TCS in these macrophytes was dependent on the exposure time and TCS concentration. The uptake of TCS was mainly observed in the roots of *E. crassipes* and in the leaves of *H. verticillata*. However, *H. verticillata* leaves showed higher sensitivity to TCS toxicity compared to *E. crassipes* leaves. This difference in susceptibility may be attributed to the different absorption capacities and growth characteristics of the two species. Pearson's correlation analysis revealed the activities of antioxidant enzymes were negatively affected by TCS exposure in both species. There was a significant negative correlation between TCS concentration and physiological indexes in both species under TCS stress. *H. verticillata* was more susceptible to toxicity than *E. crassipes* under TCS stress.

Abbreviations

TCS	Triclosan
CHL	Chlorophyll
SP	Soluble protein
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase

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Author contributions

XXY: conceptualization, methodology, software, analyzed the results, writing—original draft. FYH: conceptualization, purchase materials, analyzed the results. JA: conceived and designed, data curation, methodology, supervision, writing—review and editing. YCY: investigation, data curation, providing language help. LYZ: conducted plant seedling and chemicals purchase. SHW: validation, review and editing.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable in this section.

Consent for publication

Not applicable in this section.

Competing interests

The authors declare that they have no competing interests in this section.

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