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Integrative transcriptomic analysis reveals a broad range of toxic effects of triclosan on coral *Porites lutea*

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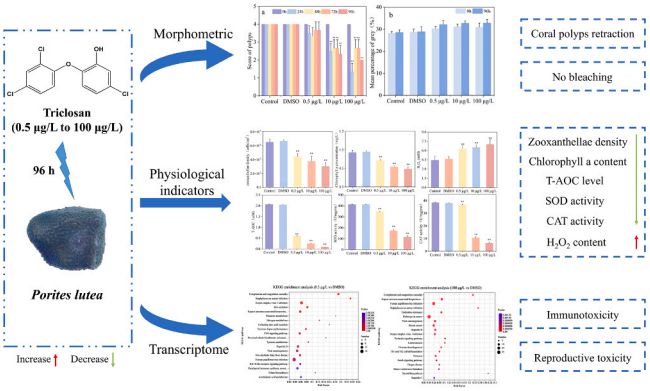
HIGHLIGHTS

- Limited bioaccumulation potential of TCS in the coral was reported.
- Exposure to 100 μg/L TCS does not cause bleaching in *Porites lutea*.
- SOD and CAT activities of *Porites lutea* were inhibited by TCS exposure.
- TCS disrupted the immune system and reproductive system in coral.

GRAPHICAL ABSTRACT



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ABSTRACT

Triclosan (TCS) is an antimicrobial agent commonly used in personal care products. However, little is known about its toxicity to corals. Here, we examined the acute toxic effects (96 h) of TCS at different levels to the coral *Porites lutea*. Results showed that the bioaccumulation factors (BAFs) of TCS in *Porites lutea* decreased with increasing TCS exposure levels. Exposure to TCS at the level up to 100 μg/L did not induce bleaching of *Porites lutea*. However, by the end of the experiment, both the density and chlorophyll a content of the symbiotic zooxanthellae were 19–52 % and 19.9–45.6 % lower in the TCS treatment groups than in the control, respectively. For the coral host, its total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT) activities were all significantly lower in the TCS treatment groups than the control. Transcriptome analysis showed that 942 and 1077 differentially expressed genes (DEGs) were identified in the coral host in the 0.5 and 100 μg/L TCS treatment groups, respectively. Meanwhile, TCS can interfere with pathways related to immune

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system and reproductive system in coral host. Overall, our results suggest that environmentally relevant concentrations of TCS can impact both the coral host and the symbiotic zooxanthellae.

1. Introduction

Coral reefs are one of the most important and biodiverse ecosystems on Earth [1]. They provide significant economic benefits to humans and play an important role in coastal protection [2]. However, coral reefs are facing threats from rising sea temperature, chemical pollution, ocean acidification as well as overfishing [3]. In fact, it has been well documented that various organic pollutants including microplastics and organic ultraviolet absorbers were found in coral reef areas [4,5]. However, toxicological studies have demonstrated that some of such organic pollutants were able to reduce the density of the symbiotic zooxanthellae [6] and disrupt the immune system, antioxidant system and metabolic activity of coral [7]. In addition, exposure to pollutants can reduce coral reproductive function, recruitment, growth rate, and survival of larvae and adults, while increasing disease susceptibility [8]. For coral reefs, pollutants in the ocean are a significant threat and can cause coral reef degradation over time.

Triclosan (TCS) is an antimicrobial agent widely used in personal care products such as soap, detergent, sponges, and deodorants, with an estimated annual global production of 10.5 million pounds in 2015 [9]. Consequently, TCS has been extensively detected in rivers, lakes, seawater, and aquatic organisms [10]. Additionally, TCS has also been found in urine, blood, and breast milk samples from human populations worldwide [11]. Due to the potential risks of TCS to human health, the US Food and Drug Administration (FDA) banned its use in antibacterial products in 2016 [12], and the European Commission stopped its use in products for general hygiene in 2017 [10]. However, there are no restrictions on the use of TCS in other products or in other countries. For example, TCS is still widely used in hand sanitizers in China, Brazil, and India [13]. Due to insufficient restrictions on the use of TCS, the concentration of TCS in the marine environment may have reached alarming levels. A study in 2023 showed that the highest concentration of TCS detected in coastal areas of India reached 494 ng/L [14]. The widespread presence of TCS may pose a significant threat to the marine environment.

Numerous studies have demonstrated the potential toxicity of TCS to aquatic organisms, including algae, fish, mussels, sea cucumbers, and sea urchins [10]. For instance, TCS concentrations ranging from 20 to 500 μg/L have been shown to inhibit the growth of the green algae *Chlamydomonas reinhardtii* and increase the content of malondialdehyde (MDA) [15]. Additionally, exposure to 10–50 μg/L of TCS has inhibited the growth of *Microcystis aeruginosa* and *Microcystis viridis*, significantly increasing reactive oxygen species (ROS) content and promoting microcystin release [16]. In the green algae *Chlorococcum sp.*, exposure to 100 μM TCS has been found to inhibit fatty acid synthesis and cause protein aggregation [17]. These studies collectively indicate that TCS can inhibit algal growth and induce oxidative stress. Furthermore, the toxic effects of TCS on marine organisms have also been documented. For example, exposure to 100 μg/L of TCS has led to physiological damage, oxidative stress, lipid peroxidation, and neurotoxicity in Mediterranean mussels [18]. Moreover, exposure to 0.3 and 0.375 mg/L of TCS significantly reduced sperm motility in *Strongylocentrotus nudus* by 13 % and 22 %, respectively. At 0.3 mg/L, fertilization rates decreased by 82.7 ± 1.57 %, and at 0.375 mg/L, fertilization rates dropped to 0 % [19]. These findings underscore that TCS exposure induces oxidative stress and causes reproductive toxicity in marine organisms. Other aquatic organisms, including corals, have received limited attention compared to common organisms, although there is sufficient evidence that TCS has been detected in coral reefs and the maximum concentration reached 142 ng/L [20]. Further, given the increasing usage of TCS, a rise in TCS levels could be expected in the coral reefs. However, to our knowledge, no studies have so far investigated potential toxicological effects of TCS exposure on corals.

Porites lutea is a widely distributed reef-building coral in the Indo-Pacific region and is one of the dominant species in high-latitude coral reefs, including locations such as Daya Bay, Weizhou Island, and Sanya in China [21]. Among coral species, *Porites lutea* has been recognized for its resilience to both anthropogenic and natural disturbances [22]. Consequently, *Porites lutea* has been utilized in studies to assess the impacts of various environmental stressors, such as pollutants and elevated temperatures, on coral reef ecosystems [7,23]. Furthermore, prior researches have demonstrated that TCS can inhibit the growth of fish and algae, induce oxidative stress, and alter gene expression [10,16]. Based on these findings, we hypothesized that TCS affects the health and physiological state of *Porites lutea*. In this study, our objectives were: i) to investigate whether environmentally relevant levels of TCS can induce bleaching in *Porites lutea*; ii) to evaluate the effects of TCS on *Porites lutea* by assessing endpoints such as zooxanthellae density, chlorophyll a concentration in zooxanthellae, and antioxidant activities; and iii) to elucidate the molecular responses and related mechanisms of both the coral host and its symbiotic zooxanthellae under TCS stress.

2. Materials and methods

2.1. Chemicals and materials

TCS (purity > 99 %) and mass labelled TCS (D_3-TCS) (purity > 98 %) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and TLC Pharmaceutical Standards (Ontario, Canada), respectively. The solubility of TCS is 12 g/L. LC-MS grade methanol, ethanol, and dimethyl sulfoxide (DMSO) were all provided by Merck Corporation (Darmstadt, Germany). Oasis HLB Cartridges (500 mg, 6 mL) were bought from Waters Corporation (Milford, MA, USA). Commercial kits of total antioxidant capacity (T-AOC), hydrogen peroxide (H_2O_2) content, superoxide dismutase (SOD) and catalase (CAT) assay were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Bicinchoninic acid (BCA) protein assay kit was purchased from Sangon Biotech (Shanghai, China). Ultrapure water was produced by a Milli-Q purification system of Merck Millipore (Darmstadt, Germany), while Reverse Osmosis (RO) water was provided by a Reverse Osmosis Water System of Novartis Qingyuan (Tianjin, China). Sea salt was purchased from Aquarium Systems (Sarrebourg, French). Artificial seawater (ASW) with salinity of 32 ‰ was prepared with sea salt and RO water. The stock solution of TCS was prepared in DMSO at the concentration of 1000 mg/L.

2.2. Test organism

Porites lutea was collected using diving equipment with hammers and pliers from Weizhou Island, Guangxi Province (China). After collection, corals were quickly transported to a 100-L aquarium at the Coral Reef Research Centre of Guangxi University, and maintained in the Berlin system with lighting (250 W metal halide lamps and 4 T5-fluorescent high output tube (T5HO) lamps), which mimics natural light with a 12 h:12 h light-dark cycle. The water quality in the aquarium was similar to that of natural seawater at the sampling site (salinity: 32 ± 1 ‰, temperature: 26 ℃, bicarbonate concentration index (KH): 7.8 ± 0.2, and pH: 8.2 ± 0.1). For experiment conducted in the present study, a piece of *Porites lutea* was randomly collected from the aquarium and cut into small nubbins of approximately 3 cm^2 each using a cutting machine. These nubbins were acclimated in the aquarium for one month prior to the experiment.

2.3. Experimental design

Sixty coral nubbins with similar size and in good growth condition were used for the experiment. Five groups were set up: ASW control, DMSO control (0.01 % (v/v)) and three TCS treatment groups (0.5 μg/L, 10 μg/L and 100 μg/L), with three replicates per group, resulting in 4 coral nubbins per replicate. The selection of low concentrations of TCS was based on the concentration of TCS in the ocean [14], and the selection of high concentrations was to better understand the stress response of coral exposed to TCS [24]. Exposure experiment was conducted in 16-L glass tanks filled with 12 L ASW each. During the exposure period, water parameters, temperature and lighting were kept the same as the acclimation period. To minimize water evaporation, the top of each tank was covered with a transparent plastic wrap. The exposure was lasted for 96 h. At both the beginning and end of exposure (0 and 96 h), 100 mL of seawater was collected from each tank to analyze TCS concentration, which were extracted and analyzed as soon as possible. At 96 h, coral nubbins were also collected using forceps for analyses of transcriptome, physiological parameters and TCS bioaccumulation. Specifically, coral nubbins for transcriptome analysis were rapidly frozen with liquid nitrogen and stored at −80 °C until analysis and coral nubbins for physiological parameter analyses were processed immediately once collected; and coral nubbins for TCS analysis were stored at −20 °C and processed as soon as possible.

2.4. Sample pretreatment and analysis

The method used to extract TCS from seawater was based on a previous study with minor modification [25]. Briefly, the seawater sample was filtered using glass fiber filter (GF/F) and concentrated on an Oasis HLB solid phase cartridge (500 mg, 6 mL, Waters). The coral tissue and skeleton were separated using a Waterpik (Ultra Water Flosser, Jiebi Limited, China) with 0.5 L filtered ASW, and the resulting suspension containing the coral tissue was transferred to a beaker. The beakers were stored at −20 ℃ overnight, freeze-dried and subsequently homogenized. Thereafter, coral tissue samples were extracted to examine TCS bioaccumulation using a previous method [25]. See Text S1 in the supplementary material for detailed procedures of TCS extraction.

TCS concentrations were analyzed by Agilent 1290 ultra-high performance liquid chromatography in conjunction with a 6460 triple quadrupole mass spectrometer (UPLC-MS/MS, Agilent Technologies, Santa Clara, CA). Details on the instrumental method are described in Text S2.

2.5. Calculation of bioaccumulation factors (BAFs)

BAFs of TCS in coral species were calculated using the following formula [26]:

$$BAFs = Ccoral/Cwater \quad (1)$$

where C_{water} (ng/L) and C_{coral} (ng/g ww) represent the measured concentration of TCS in seawater and in *Porites lutea*, respectively. In general, a log BAF greater than 3.3 (corresponding to a BAF of 2000) represents bioaccumulation, while a log BAF greater than 3.7 (corresponding to a BAF of 5000) indicates significant bioaccumulation [27].

2.6. Morphological observations of corals

Coral color and polyp activity were recorded daily using an Olympus Tough TG-5 camera under the identical lighting conditions. Photos were analyzed to examine coral color change using Image-Pro Plus (version 6.0, Media Cybernetics, USA), following the method reported by previous study [28]. Details on the method are provided in Text S3. The degree of coral bleaching was expressed as a mean percentage of grey (MPG) and was calculated using the following formula:

$$MPG = \text{intensity of all random points across the coral sample}/255 \times 100\% \quad (2)$$

The average intensity of random points on the coral was used for calculation. When corals are completely bleached and have a whiteness equivalent to the reference standard, the average grey intensity is close to 100 %; and when the coral color is comparable to the black reference point, the average grey intensity is 0 % theoretically.

The polyp activities of corals under TCS stress were assessed according to a scoring rule, which is shown in Table 1. The activities of corals polyp were divided into six grades, with polyp fully extended being rated as five while polyp fully retracted or corals completely bleached being rated as zero [29]. Moreover, in order to determine the maximum extent of the polyp activities, disturbance to corals was avoided when taking photographs.

2.7. Quantification of physiological parameters of corals

2.7.1. Density of the symbiotic Zooxanthellae

Coral nubbins were washed using a recirculating Waterpik containing a certain amount of filtered seawater. The resultant suspension was mixed evenly using a homogenizer (IKA T10 basic) and the initial volume was recorded. A sub-sample of 2 mL was then collected and centrifuged at 4000 rpm for 5 min. The resulting precipitate was collected, resuspended in filtered seawater, and centrifuged at 4000 rpm for 5 min. The number of zooxanthellae was counted under a microscope (Olympus, Japan), and the surface area of the coral nubbin was determined with the aid of aluminum foil [30], which were used to calculate the density of zooxanthellae.

2.7.2. Chlorophyll a (chl a) concentration of the symbiotic Zooxanthellae

Another portion of 15 mL of the homogenized suspension was dispensed and centrifuged at 4000 rpm for 5 min. The resultant precipitate was collected, resuspended in 95 % (v/v) ethanol, and stored at 4 °C for 24 h. Afterwards, they were centrifuged at 4000 rpm for 5 min, and the absorbance of the resulting supernatant was measured at wavelengths of 665 nm and 649 nm. The chl a content was calculated through the following equation [31]:

$$\text{chl a} = 13.95\ A665 - 6.88\ A649 \quad (3)$$

2.7.3. Maximum photochemical quantum yield (Fv/Fm) of the symbiotic zooxanthellae

Fv/Fm is a reliable approximation of the maximum photochemical efficiency of photosystem II (PSII). The Fv/Fm was determined for zooxanthellae by the pulse-amplitude modulated fluorescence (Diving PAM) method. Specifically, at the same time point on each day during the exposure period, after 30 min of dark acclimation, every coral nubbin was measured by a Diving PAM fluorometer (Wolz, Germany) under complete darkness [32].

2.7.4. H_2O_2 content, SOD and CAT activities and T-AOC levels of corals

A third portion of 2 mL of the homogenized suspension was collected and centrifuged at 4000 rpm for 5 min. The supernatant was collected to

Table 1
The score criteria of coral polyp activity.

Score	Polyp activity
5	All fully extended
4	Mostly extended, few withdrawn
3	Polyps extended 50 %
2	Polyps extended 10 %
1	No polyps extended
0	Dead nubbin

determine SOD and CAT activities, H_2O_2 content, T-AOC levels and the total protein content of corals following the standard procedure of the commercial kit. The T-AOC, H_2O_2 content, SOD, and CAT activities were quantified using spectrophotometry at wavelengths of 593 nm, 405 nm, 550 nm, and 405 nm, respectively.

2.8. Transcriptome analysis

RNA was extracted from coral symbiont using Trizol reagents (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The quality of the extracted RNA was assessed using the 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA), and then quantified using ND-2000 (NanoDrop Technologies). A total of 5 μg RNA per sample was used for RNA-seq transcriptome library preparation with the TruSeqTM RNA sample preparation kit (Illumina) according to the manufacturer's protocol. Briefly, messenger RNA was isolated using oligonucleotide (dT) beads through the polyA selection method, and subsequently fragmented with fragment buffers. The double-stranded cDNA was synthesized using the SuperScript double-stranded cDNA synthesis kit (Invitrogen) along with random hexameric primers from Illumina. Following this step, end-repairing, phosphorylation, and addition of 'A' base were carried out as per Illumina's library construction protocol. Size-selective libraries targeting cDNA fragments of 200–300 base pair were generated on a 2 % low range agarose gel followed by PCR amplification for 15 cycles using Phusion DNA polymerase (New England Biolabs Inc., Ipswich MA). After quantification with a TPS-380 fluorometer (Turner Biosystems Sunnyvale CA) [32], the double-ended RNA seq library underwent sequencing on an Illumina NovaSeq 6000 platform.

After sequencing and aligning the sequences, we conducted transcriptome assembly, differential expression analysis, and functional enrichment. The assembled unigenes were annotated in six databases (Non-redundant (Nr), Swiss-prot, Pfam, orthologous Cluster (COG), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG)) using BLAST [33]. BLASTx was used to distinguish coral hosts or zooxanthellae. Transcript expression level was measured in transcript fragments per million mapped reading segments per thousand bases [34]. Differentially expressed genes (DEGs) were identified using DESeq2 software, with significance determined by a p-value < 0.05 and $|\log_2$ fold change$| > 0.585$. KEGG annotation and enrichment analysis were performed using the Majorbio cloud platform. The functional enrichment analysis of DEGs involved gene ontology (GO) and KEGG pathway analyses. RT-PCR was used to validate transcriptomic findings for genes C3, TRAF3, Notch1, CA, CCoAOMT, and RPL21. Specific experimental details can be found in Text S4 and Table S1.

2.9. Statistical analysis

Data were expressed with mean ± standard deviation (mean ± SD). Before analysis, data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and Levene's test, respectively. Independent T-tests were used to analyze 0 h and 96 h concentrations in seawater. Differences in physiological parameters (the concentration of TCS in *Porites lutea*, algal cell density, photosynthetic system, H_2O_2 content and antioxidant enzyme activity) were determined by one-way analysis of variance (ANOVA). The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. TCS exposure and bioaccumulation

Table 2 provides the concentrations of TCS in the seawater and corals and the corresponding BAFs after 96 h of exposure. In the seawater, at the start of exposure, the actual TCS concentrations were about 90–96 % of the nominal concentrations; after 96 h of exposure, the TCS mean concentrations decreased to 0.13 μg/L, 6.24 μg/L, and 64.2 μg/L in the 0.5, 10 and 100 μg/L TCS treatment groups, respectively. For corals, after 96 h of exposure, the mean concentrations of TCS in 10 μg/L and 100 μg/L TCS treatment groups were 0.36 ng/g ww and 0.63 ng/g ww, respectively. Since all BAFs reported here are below 3.3, our results indicate that the accumulation of TCS in *Porites lutea* was limited during the 96-hour exposure period. A previous study has demonstrated that TCS can be metabolized into OH-TCS in hepatocytes through the action of cytochrome P450 (CYP450) enzymes [35]. Under TCS stress, increased CYP450 enzyme activity may facilitate the biotransformation of TCS in *Porites lutea*, thereby reducing its accumulation. As observed, the BAF of TCS decreased with increasing TCS concentrations, ranging from 0.01 in the 100 μg/L TCS treatment group to 3.16 in the 0.5 μg/L TCS treatment group. This phenomenon can be attributed to a non-linear adsorption mechanism, where limitations in binding sites restrict the amount of chemical that can be adsorbed to target sites, which may become progressively saturated with increasing exposure concentrations [36]. Additionally, saturated uptake and elimination processes, as well as biological metabolic processes, also influence BAF values [37,38]. Previous studies have also reported that the bioconcentration factors of sulfadiazine (SDZ) and organophosphate flame retardants (OPFRs) in *Cyprinus carpio* decreased with increasing exposure concentrations [39,40].

Table 2
The concentration of TCS in seawater (μg/L), *Porites lutea* (ng/g ww) and BAF.

Treatment	Seawater		*Porites lutea*	BAF
	0 h	96 h	96 h	
0.5 μg/L	0.45 ± 0.04	0.13 ± 0.02*	0.52 ± 0.08	3.16 ± 0.60
10 μg/L	9.28 ± 0.32	6.24 ± 0.31*	0.36 ± 0.18	0.06 ± 0.03
100 μg/L	95.78 ± 1.67	64.23 ± 1.30*	0.63 ± 0.09	0.01 ± 0.00

* Indicates significant differences in seawater concentration between 0 h and 96 h in water samples ($p < 0.05$).

3.2. Morphometric alterations of corals under TCS stress

Results from the analysis of coral polyp activity showed that there was no significant difference in the polyp score between the two groups of controls ($p > 0.05$). Specifically, polyp scores in the 0.5 μg/L TCS treatment group decreased over the course of the experiment, indicating a slight contraction of coral polyps at this concentration. In contrast, polyp scores in the 10 μg/L and 100 μg/L TCS treatment groups decreased significantly throughout the experimental period ($p < 0.01$), suggesting a more pronounced contraction of polyps at higher TCS concentrations (Fig. 1a). These results demonstrate that exposure to different TCS concentrations leads to varying degrees of coral retraction, consistent with findings from a previous study on the biocide 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one, which may be related to the corals' energy conservation strategy [41]. Additionally, it has been shown that expanded polyps require more energy than retracted ones [42]. In our study, we observed a reduction in zooxanthellae density in the three TCS treatment groups, and since most of the corals' energy is derived from zooxanthellae [43], the differences in zooxanthellae density among treatment groups likely account for the varying degrees of polyp contraction observed.

In addition to polyp activity, coral bleaching level is another important indicator of coral health. In our study, neither the control nor the TCS-treated corals exhibited bleaching by the end of the experiment (Fig. S1) and no significant differences in bleaching rates were observed between the control and TCS treatment groups (Fig. 1b). Similarly, another study found that exposure of *Acropora tenuis* to comparable levels of benzophenone did not induce bleaching [44]. Coral bleaching occurs when the loss of symbiotic zooxanthellae reaches a significant level. A previous study indicated that bleached corals lost 72 %−90 % of their zooxanthellae, while non-bleached corals also lost 31 %−53 % of their zooxanthellae [22]. In our study, zooxanthellae densities were

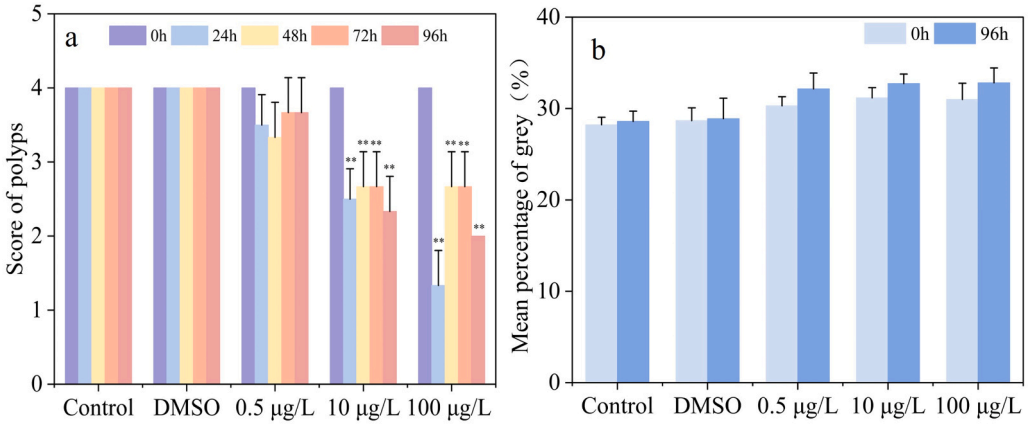

Fig. 1. Morphology of *Porites lutea* under TCS exposure. (a) Polyp activity score of *Porites lutea* at different times; (b) Average percentage of grey at 0 h and 96 h. * and * * indicate different significance ($p < 0.05$, $p < 0.01$).

reduced by 52 % at the highest concentrations of TCS exposure. Additionally, multiple regulatory mechanisms can be activated in coral under external stress to maintain homeostasis. For example, when *Pocillopora damicornis* was subjected to microplastics and heat stress, an upregulation of the *HSP70* gene was observed, indicating an adaptive response to maintain cellular protein homeostasis [45]. Additionally, short-term exposure of corals to nitrate enrichment and prometryn led to increased superoxide SOD activity, which helps counteract excessive ROS production [46]. Through regulatory mechanisms, corals can resist physiological stress and avoid bleaching [47].

3.3. Photosynthetic parameters of the symbiotic zooxanthellae

The presence of the symbiotic zooxanthellae is crucial for coral health. There was no significant difference in the zooxanthellae density between the two groups of controls ($p > 0.05$) (Fig. 2a). The density of zooxanthellae in the TCS treatment groups were 19–52 % lower than the solvent control group, ranging from 1.48×10^6 cells/cm^2 in the 0.5 μg/L TCS treatment group to 9.74×10^5 cells/cm^2 in the 100 μg/L TCS group. Moreover, such difference reached statistical significance in the two higher TCS treatments (i.e., 10 and 100 μg/L treatment groups), suggesting that TCS was able to impact *Porites lutea* through reducing the density of its symbiotic zooxanthellae. Similarly, it has been reported that 100 μg/L pentachlorophenol reduced zooxanthellae density in *Porites lutea* [7]. The reduction of the zooxanthellae density caused by pollutant exposure could be a result of the stress response in the internal mechanisms of the coral system. Indeed, some biological mechanisms such as cytotoxicity, cellular necrosis, and immune responses were found to be activated when corals were under external stresses, accompanied by reduced density of zooxanthellae [48–50].

Chl a content is one of the indicators to evaluate coral health [30]. In the present study, Chl a content was significantly lower in all TCS treatment groups than the control groups ($p < 0.05$), and decreased with increasing TCS concentration, with the lowest level (3.96 ± 0.33 μg/cm^2) measured in the 100 μg/L TCS treatment group (Fig. 2b). Fv/Fm was used to evaluate the accumulation of PSII photoinactivation [51]. There were no significant differences in Fv/Fm among groups ($p > 0.05$, Fig. 2c), which suggests that exposure to TCS under the examined levels did not significantly influence the photosynthetic system of the symbiotic zooxanthellae. Likewise, a previous study found no effects of copper exposure (1.9–6.7 μg/L) on Fv/Fm for *Mussismilia harttii*, another coral species [52]. In contrast, exposure to 5 μg/L of prometryn (a herbicide) for 96 h caused an approximately 10 % decrease in Fv/Fm for the coral *Acropora hyacinthus* [53]. Taken together, these data suggest that different pollutants have different toxic effects on corals, which could be attributed to the differences in chemicals' modes of toxic action, the sensitivity of coral species, and the tissues/genes of coral species attacked by pollutants [54,55].

3.4. The antioxidant capacity of corals

The protein concentrations of corals were 44.95, 64.37 and 85.59 μg/mL in the 0.5, 10 and 100 μg/L TCS treatment groups, respectively, which were significantly higher than the control groups (Fig. S2). These results demonstrate that exposure to environmentally relevant levels of TCS can lead to increased protein levels in the coral hosts, which may be an adaptive response to environmental changes [56]. Similar results have been found for the coral species *Pocillopora verrucosa* when it was exposed to a polyphosphate scale inhibitor [57]. In contrast to protein, the levels of T-AOC were significantly lower in the

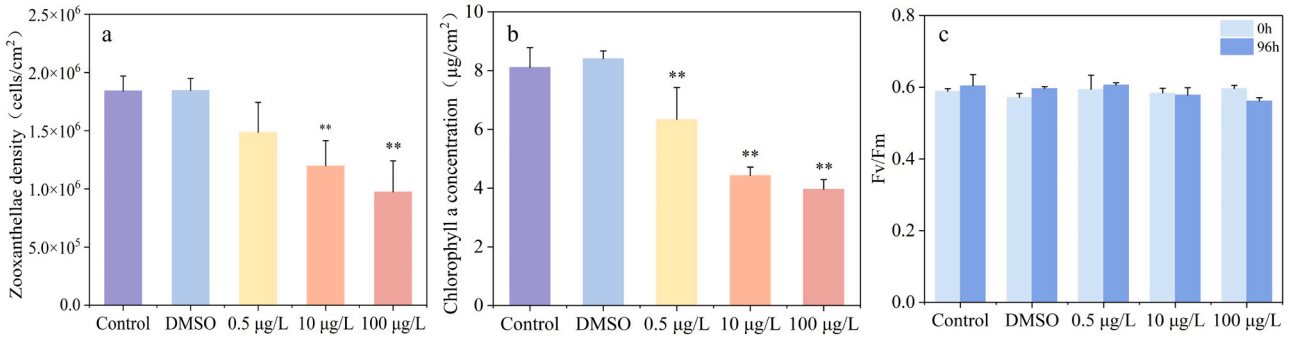

Fig. 2. Response of symbiotic zooxanthellae under TCS exposure. (a) Zooxanthellae density; (b) Chlorophyll a content. (c) Fv/Fm. * and * * indicate different significance of $p < 0.05$, $p < 0.01$.

TCS treatment groups than the controls ($p < 0.01$), and decreased with increasing TCS concentration, with the lowest level reaching 0.09 ± 0.004 mmol/gprot in the 100 μg/L TCS treatment group (Fig. 3a). These results suggest potential depletion of the non-enzymatic antioxidant reserves of corals [58].

H_2O_2 is the main component of ROS and is widely used as an inducer in oxidative stress model [59]. Compared with control group, the H_2O_2 content in *Porites lutea* increases with increasing TCS concentration, suggesting enhanced oxidative stress in *Porites lutea* (Fig. 3b). SOD and CAT are enzymes involved in neutralization of ROS [60]. Here, the SOD and CAT activities of corals in the TCS treatment groups were approximately 16–72 % and 4–83 % lower than the control groups, respectively, with all reaching statistical significance ($p < 0.01$) (Fig. 3c-d), which demonstrates that exposure to TCS at environmentally relevant concentrations can impact the antioxidant enzyme system of corals through inhibiting the activities of SOD and CAT. These inhibitory effects might be related to the excessive production of ROS [61].

3.5. Transcriptome responses of Porites lutea to TCS exposure

3.5.1. Transcriptomic responses of the coral host

There were 942 and 1077 DEGs for coral hosts under the exposure of 0.5 and 100 μg/L TCS treatment groups compared with the control groups, respectively (Fig. 4a-b). PCA analysis showed good clustering among all groups (Fig. S3a). The results of the GO enrichment term analysis indicated that the GO terms were primarily associated with heme binding, peptidase regulator activity, extracellular region, extracellular space, negative regulation of angiogenesis and complement activation (Fig. S4). The results of qRT-PCR were in good agreement with those of RNA-seq (Fig. S5). KEGG enrichment pathway analysis showed that the enriched pathways were mainly related to complement and coagulation cascade pathway, TNF signaling pathways, Notch signaling pathway, thiamine metabolism, nitrogen metabolism, prolactin signaling pathway and ovarian steroidogenesis (Fig. 4c-d). Specifically, in the complement and coagulation cascade pathway, TCS significantly inhibited the expression of the immune-related gene *C3* ($p < 0.05$), which is involved in activating phagocytosis [62]. This suggests potential suppression of the coral host's immune defense. Additionally, in the TNF signaling pathway, the expression of *TRAF3* was significantly downregulated in the 100 μg/L TCS treatment group ($p < 0.01$). *TRAF3* is an important inflammatory regulatory factor, and its suppression by bisphenol A (BPA) has been linked to exacerbated tracheal inflammation in chickens [63]. The downregulation of *TRAF3* in our study suggests that TCS exposure might induce coral inflammation. Moreover, the expression of *Notch1* was significantly downregulated ($p < 0.05$) in the Notch signaling pathway in the 100 μg/L TCS treatment group. *Notch1* plays a crucial role in regulating the development and function of immune cells [64]. and its downregulation disrupts coral immune regulation. Additionally, the expression of gene *alkaline phosphatase* (*E3.1.3.1*) was significantly reduced in the thiamine metabolism pathway in the 0.5 μg/L TCS treatment group ($p < 0.05$). Alkaline phosphatase is vital for recognizing and clearing pathogens, playing a crucial role in the coral immune system [65]. A prior study indicated that microplastic stress decreases alkaline phosphatase activity in corals [66]. Reduced expression of alkaline phosphatase would impair the coral's ability to clear pathogens. Our results suggest that TCS exposure disrupts coral immune system function, interfering with immune regulation and cause potential immunotoxicity. In corals, the immune system is essential for homeostasis and for coordinating relationships between the host, symbionts, and pathogens [67]. Exposure to external stressors can prompt the immune system to regulate the symbiotic relationship and maintain coral homeostasis. Consistent with findings in *Acropora cervicornis*, which showed a negative correlation between bleaching and disease [68], the decrease in zooxanthellae density observed in our study may have been modulated by the coral's immune system to enhance resistance to disease. The process of apoptosis is also an

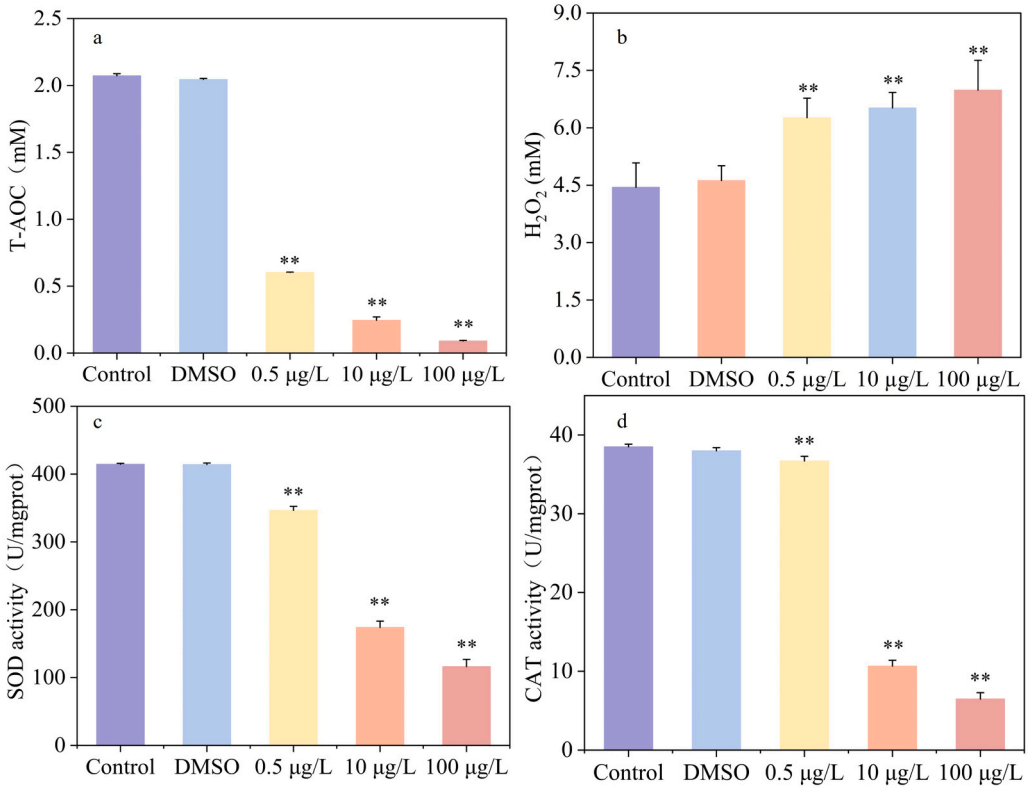

Fig. 3. Biochemical indices of *Porites lutea* under TCS exposure. (a) T-AOC; (b) H_2O_2 content; (c) SOD activity; (d) CAT activity. * and * * represent different significance of $p < 0.05$, $p < 0.01$.

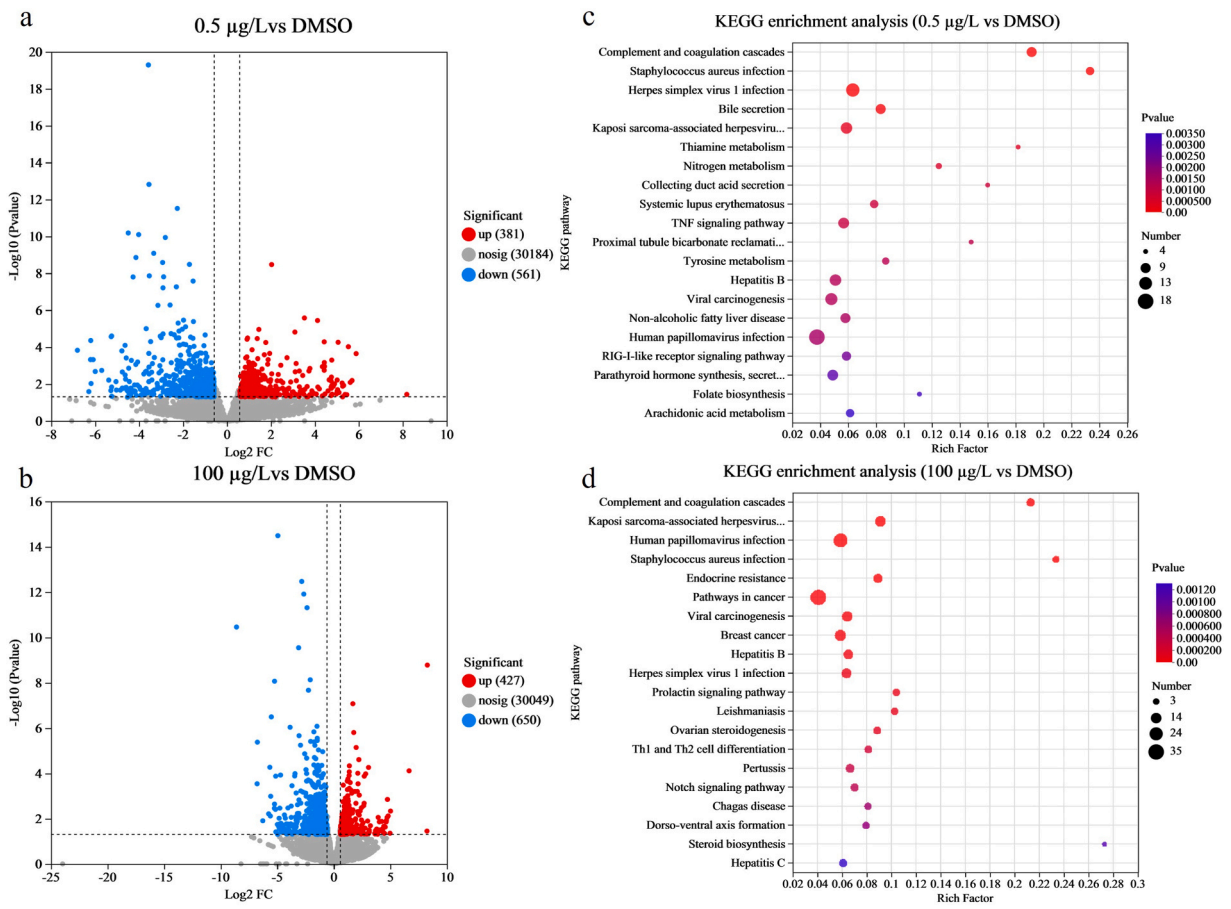

Fig. 4. Transcriptomic analysis of coral host exposed to TCS. (a) Volcano map of DEGs in 0.5 μg/L TCS treatment group; (b) Volcano map of DEGs in 100 μg/L TCS treatment group; (c) KEGG pathway diagram of DEGs in 0.5 μg/L TCS treatment group; (d) KEGG pathway diagram of DEGs in 100 μg/L TCS treatment group.

important part of the coral immune pathway and is thought to play an important role in helping corals defend themselves against pathogens [69]. Our result showed that apoptosis-related genes (*casp8* and *casp3*) were significantly down-regulated in the 0.5 μg/L TCS treatment group ($p < 0.05$), suggesting the inhibition of apoptosis in the coral host. The down-regulation of *casp8* and *casp3* genes expression could effectively help corals resist the pressure of TCS, thus avoiding the bleaching of corals. Besides, the expression of gene *carbonic anhydrase (CA)* was significantly up-regulated in nitrogen metabolism pathway in the 0.5 μg/L TCS treatment group ($p < 0.05$), which may affect the calcification of coral because *CA* plays an important role in coral calcification. A prior study revealed that carbonic anhydrase can catalyze the conversion of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) via a reversible hydration reaction, which not only provides dissolved inorganic carbon (DIC) for calcification, but also plays a key role in photosynthesis [70]. The up-regulated expression of *CA* gene can increase the supply of DIC in *Porites lutea* to enhance zooxanthellae photosynthesis to obtain more energy. A previous study found that under short-term heat stress, zooxanthellae would fix more carbon and transfer it to coral host for tissue growth and energy storage. The enhancement of carbon supply of zooxanthellae may effectively help corals reduce the risk of bleaching [71].

In the present study, the expression of gene *Foxo3* was significantly down-regulated ($p < 0.05$) and the expression of gene *CYP17* was significantly up-regulated in the prolactin signaling pathway related to reproductive system in the 100 μg/L TCS treatment group ($p < 0.01$). The *Foxo3* gene is associated with the development of follicles, and knocking out the *Foxo3* gene leads to oocyte death and early depletion of functional ovarian follicles in mice [72]. The downregulation of *Foxo3* expression could impair the reproductive system of corals. The expression of the *CYP17* gene plays an important role in coral spawning. It was reported that the enzyme activity of *CYP17* in reproductive corals *Pocillopora damicornis* remained consistent with the lunar phase cycle in the absence of thermal stress [73]. The upregulation of *CYP17* may lead to spawning dysregulation in *Porites lutea*, adversely affecting coral reproduction. Furthermore, the expression of gene *IGF1* was significantly up-regulated ($p < 0.05$) in the ovarian steroid-producing pathway in the 100 μg/L TCS treatment group. The *IGF1* gene is known to play a crucial role in sexual maturation and the regulation of gonadal function. It has been shown to increase the secretion of follicular estradiol [74]. The rise in estradiol (E_2) content can enhance its binding to estrogen receptor (ER), leading to the activation of vitellogenin gene expression and subsequent increase in vitellogenin content. A previous study demonstrated that prolonged exposure to TCS resulted in significantly elevated levels of E_2 and vitellogenin (VTG) in male fish, while VTG levels were notably reduced in female fish, indicating estrogenic effects [75]. Up-regulation of *IGF1* gene expression can stimulate estradiol secretion and an increase in vitellogenin content, potentially causing TCS to act as an estrogen-like substance *Porites lutea* and impact coral reproduction. Therefore, our study suggests that TCS exposure may damage the reproductive system of corals and disrupt coral reproduction, causing potential reproductive toxicity.

3.5.2. *Transcriptomic responses of the symbiotic zooxanthellae*

For zooxanthellae, 453 and 338 DEGs were detected in the 0.5 and 100 μg/L TCS treatment groups compared with the control groups, respectively (Fig. S6a-b). PCA analysis showed good clustering among all groups (Fig. S3b). The results of the GO enrichment term analysis

indicated that the GO terms were primarily associated with structural constituent of ribosome, 3′−5′ exonuclease activity, ribosome, exosome (RNase complex), integrin-mediated signaling pathway and macromolecule 5biosynthetic process (Fig. S7). The results of the KEGG enrichment pathway analysis showed that the enrichment pathways mainly included flavonoid biosynthesis pathway, stilbenoid, diarylheptanoid and gingerol biosynthesis pathway and ribosome pathway (Fig. S6c-d). Here, the expression of gene *caffeoyl-CoA O-methyltransferase* (*CCoAOMT*) involved in the flavonoid biosynthesis pathway and the stilbenoid, diarylheptanoid and gingerol biosynthesis pathway was significantly up-regulated in 0.5 μg/L TCS treated group ($p < 0.05$). The gene *CCoAOMT* can effectively help plants resist external stress [76]. Up-regulation of gene *CCoAOMT* expression might relief the toxicity of TCS to zooxanthellae. It was found that the expression of gene *RPL21* was significantly down-regulated in the ribosomal pathway ($p < 0.05$), which might lead to impaired protein synthesis in zooxanthellae, because *RPL21* gene plays an important role in protein synthesis [77].

4. Conclusions

This study investigates the effects of TCS on the coral *Porites lutea* and assesses its potential environmental risks. Our results show that TCS bioaccumulation in *Porites lutea* is relatively low, with the BAF decreasing as TCS concentration increases. TCS exposure significantly reduces the density of symbiotic zooxanthellae and causes coral polyp contraction. Additionally, TCS inhibits the activities of SOD and CAT, leading to increased H_2O_2 levels and oxidative stress. Transcriptome analysis and qPCR results reveals that TCS exposure may impair coral immune and reproductive functions, potentially affecting long-term ecological adaptability and reproductive success. These findings highlight the extensive environmental risks associated with chemical pollutants and call for more proactive conservation measures to mitigate the adverse effects of pollutants on these crucial ecosystems.

Environmental implication

The COVID-19 pandemic has significantly increased triclosan (TCS) usage, raising concerns about its environmental impact. Coral reefs, crucial to marine ecosystems, are particularly vulnerable to TCS contamination. This study demonstrates that even low, environmentally relevant concentrations of TCS can negatively affect *Porites lutea*, a key reef-building coral. These adverse effects could lead to broader ecological consequences, including diminished reef health and resilience, which in turn threaten marine biodiversity and human livelihoods. These findings highlight the urgent need for TCS to be prioritized in global monitoring programs and for stricter regulatory controls to protect vulnerable ecosystems.

CRediT authorship contribution statement

Jun-Jie Hu: Software, Investigation. **Feng-Jiao Peng:** Writing – review & editing, Methodology, Data curation. **Changgui Pan:** Writing – review & editing, Funding acquisition, Conceptualization. **Hao Liang:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Kefu Yu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Zhen-Zhu Liu:** Investigation. **Chao-Yang Zhou:** Data curation. **Rong-Gui Zhu:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136033.

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