Toxicity effects of ciprofloxacin on biochemical parameters, histological characteristics, and behaviors of Corbicula fluminea in different substrates

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Received: 15 March 2021 / Accepted: 9 November 2021 / Published online: 23 November 2021
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Abstract
Antibiotic toxicity and antibiotic resistance have become significant challenges to human health. However, the potential ecotoxicity of sediment-associated antibiotics remains unknown. In this study, biochemical responses, histological changes, and behavioral responses of Corbicula fluminea exposed to sediment-associated ciprofloxacin (CIP) were systemically investigated. Special attention was paid to the influence of different substrate types. Biochemical analyses revealed that the balance of the antioxidant system was disrupted, eventually leading to oxidative damage to the gills and digestive gland with increasing CIP concentration. Severe histopathological changes appeared along with the oxidative damage. An enlargement of the tubule lumen and thinning of the epithelium in the digestive gland were observed under exposure to high CIP concentrations (0.5 and 2.5 μg/g CIP). In a behavioral assay, the filtration rate of C. fluminea in high concentration exposure groups was clearly inhibited. Moreover, from the integrated biomarker response (IBR) index, the toxicity response gradients of the digestive gland (no substrate--NOS > Sand > Sand and kaolinite clay--SKC > Sand, kaolinite clay, and organic matter--SCO) and gills (NOS > SCO > SKC > Sand) were different among substrate exposure groups. The most serious histopathological damage and highest siphoning inhibition were observed in the NOS group. The changes in the morphological structure of digestive gland cells in C. fluminea were similar in the other three substrate groups. The inhibition of the filtration rate in the higher concentration groups decreased in the order Sand > SKC > SCO.

Keywords Asian clam · Sediment · Oxidative stress · Cell morphology alterations · Filtration response

Introduction
Sediments not only serve as a habitat for many aquatic organisms (e.g., benthic invertebrates) but also are a major sink of contaminants in aquatic systems (Ho et al. 2002; Allen Burton 2002). Extensive research has demonstrated the occurrence and distribution of heavy metals (Feng et al. 2014; Feng et al. 2017), organic pollutants, polar pesticides (Herrero et al. 2018; Rusina et al. 2019), emerging contaminants (microplastics, pharmaceuticals, and steroids) (Yang et al. 2010; Xu et al. 2014; Matić Bujagić et al. 2019), plastic-derived contaminants (Lubecki and Kowalewska 2019), and various organic micro-pollutants (Qi et al. 2014) in surface sediments. The contaminated sediments pose a threat to aquatic organisms and even to human beings. However, due to the complexity of sediment composition, there are still limited reports on toxicity of sediment-associated pollutants (Guo and Feng 2018). This has resulted in a lack of standard sediment pollution controls (Wilson et al. 2005).
Fluoroquinolone antibiotics (FQs) are heavily used worldwide and are frequently detected in aquatic environment (Wang et al. 2020). Ciprofloxacin (CIP) is one of the most commonly used FQs (Huang et al. 2020). Due to the existence of ionizable chemical functional groups, CIP is susceptible to adsorption in sediments through cation bridging (Luo et al. 2011; Riaz et al. 2018). It has been reported that concentrations of CIP in sediment reached up to 2118.9 ng/g in China (Jiang et al. 2014). Due to the wide distribution and the pseudo-durability (many antibiotics are being degraded at a certain rate, most of them, however, are replaced by ongoing wide use, resulting in their pseudo persistent characteristics in the environment) of CIP in sediments, the potential risk of this kind of pharmaceutical to the benthos is cause for serious concern (Fu et al. 2017). However, the potential aquatic eco-toxicity and threat to human health of CIP via sediment exposure routes remain unidentified (Carvalho and Santos 2016). The eco-toxicity of CIP in sedimentary environments thus needs to be further explored.

As an important medium for the continual buildup and loading of CIP, sediments provide a direct source of exposure for benthos organisms (Chen and Zhou 2014). Across invertebrate species, bivalves are important sedentary organisms and are often used as bio-indicators for multiple environmental toxins (Boening 1999). As a typical freshwater benthic bivalve, *Corbicula fluminea* is dispersed extensively throughout water-sediment boundaries worldwide (Li et al. 2018). The species is a sedimentary filter feeding clam with a very high capacity to bio-accumulate the pollutants dissolved in water (Doherty 1990; Santos and Martinez 2014) or bound to suspended particles (Fournier et al. 2005). Such advantages allow the use of *C. fluminea* as a bioindicator of organic contaminants (Wang et al. 2018), metals (Saidani et al. 2019) and some other emerging contaminant (e.g. microplastics) (Guilhermino et al. 2018). However, as summarized in our previous research (Guo and Feng 2018), the investigation of *C. fluminea*’s response to organic contaminants has focused on organic pesticides (e.g., chlorpyrifos and diazinon), POPs (e.g., polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)). As a kind of widely used pharmaceutical, toxicity of CIP to *C. fluminea* is potentially important. However, research on the toxic effects of this kind of pharmaceutical on *C. fluminea* is still imperfect and needs to be further explored.

Moreover, based on our previous review (Guo and Feng 2018), most research investigating the toxicity of pollutants to *C. fluminea* was conducted in the aqueous phase. Only a few previous studies have investigated the effects of sediment-associated pollutants (i.e., the metals and POPs) to *C. fluminea* (Guo and Feng 2018). Meanwhile, research concerning sediment-associated toxins has mostly focused on the accumulated content of pollutants in the soft tissues of the *C. fluminea* (Guo and Feng 2018). However, the actual pollution status is more likely to be reflected by whole-sediment exposure assessments (US EPA 2007). Studies related to the sediment-associated antibiotic risk to benthic bivalves are urgently needed. In addition, numerous studies have suggested that different sediment characteristics will influence the transfer of contaminants between the overlying water and sediment phases, thereby altering the bioavailability of the contaminants (Graney et al. 1984; Naylor and Rodriguez 1995; Zhou and Broodbank 2014; Crawford and Liber 2015; Liu et al. 2020). However, only very few studies have investigated the effect of sediment composition on the bioavailability of metals (cadmium and copper) to the benthos in whole sediment (Graney et al. 1984; Roman et al. 2007; Crawford and Liber 2015). To our knowledge, the potential mechanisms of antibiotic toxicity to benthic bivalves in different substrates (e.g., using multi-parameter estimation of CIP toxicity) have not yet been explored.

Therefore, in this study, a common antibiotic (CIP) with high binding capacity for sediment was selected as the test pharmaceutical, and *C. fluminea* was selected as the test organism. The main objectives were to explore the oxidative stress, histological changes, and behavioral response following the exposure to sediment-associated CIP and to explore the influence of diverse substrate composition on the toxicity of antibiotics to the freshwater bivalve *C. fluminea*.

**Material and methods**

**Chemicals**

The commonly used antibiotic CIP was acquired from CNW (Technologies GmbH, Germany). The 10mg/L stock solution of CIP used for experiments was prepared in Milli-Q® water and stored in brown bottles, then placed in a refrigerator at 4°C. And it was replaced every three days. The concentration of the CIP stock solution was quantified immediately after preparation and three days later using ultra-performance liquid chromatography tandem mass spectrometry according to a previously described method (Guo et al. 2019). It should be noted that CIP was selected as ciprofloxacin hydrochloride monohydrate (CAS No.85721-33-1, soluble in water (35mg/ml), pKa = 6.09 (carboxylic acid group); pKa = 8.74 (nitrogen on piperazinyl ring), Log Kow=2.5, purity Log Kow=, as this compound can be easily dissolved in water. The neutral red indicator (CAS No.553-24-2), formalin solution (CAS No.1032570-98-1), and anhydrous ethanol (CAS No.64-17-5) were of analytical grade (purity ≥ 99.7%).
Sediment preparation and spiking

In the current work, the formulated sediment was prepared using quartz sand (0.05 mm–0.2 mm), kaolinite clay (< 0.002 mm) and organic matter (sphagnum moss peat). All of the components of the formulated sediments were commercially available substrates. The proportions of sediment components were based on OECD protocol 218, “sediment–water chironomid toxicity using spiked sediment” (OECD, 2004). Four substrate groups were prepared after proper adjustment (adjustment was based on Graney et al.’s research (Graney et al. 1984)) of the proportions of components listed in the OECD according to the natural sediment types (by weight): (1) the sand group consisted of 100% quartz sand; (2) the SKC group consisted of 75% quartz sand, 25% kaolinite clay; (3) the SCO group was formulated by 75% sand, 20% kaolinite clay and 5% organic matter (4); and the no substrate (NOS) group was composed of 100% dechlorinated tap water.

In this study, for each of the exposure concentrations (0.01, 0.5, 2.5 μg/g) 1000g of dry sediments of each group (Sand, SKC, SCO, NOS) were added, separately, to 4 L glass containers, in triplicate. The experimental concentrations of CIP were designed based on actual concentrations in natural water observed in our previous studies and the possible extremes (Guo et al. 2019). The concentration of CIP (0.01, 0.5, 2.5 μg/g) was diluted by stock solution (10mg/L), and the solution was then mixed for 15 min with the different substrates in the test container using an automatic mixer. Then, the overlying water (dechlorinated tap water) was added to obtain a ratio (by volume) of sediment to water of 1:4. The depth of the sediment layer in each vessel was 2–3 cm. After spiking, the sediment plus overlying water systems (testing group and control group) were stored in 23 ± 0.5°C constant temperature incubator in the dark to equilibrate for 24 h.

C. fluminea collection and acclimatization

Healthy mature C. fluminea (25.84 ± 5.21 mm) were collected with small rake from the beach in Chongming Island (31°30′N, 121°40′E, Shanghai, China). The collection area was near the drinking water source, and the initial residual concentrations of CIP in the area were lower than the detection limit. About One thousand C. fluminea individuals were collected each time and then returned to laboratory for domestication. The C. fluminea were acclimatized at constant temperature (23 ± 0.5°C), pH (7.57 ± 0.2), salinity (0.15 ± 0.03‰) and dissolved oxygen (4.53 ± 0.35) in a clean 300L aquarium containing bottom sand and aerated circulating filtration tap water for at least two weeks (on a 12 h: 12 h (light: dark) cycle) in the laboratory. The C. fluminea were fed with Spirulina powder (30 mg organism) every day during the acclimatization period.

Exposure assays

The exposure experiments with C. fluminea were conducted after two weeks of laboratory acclimatization. The control and CIP exposure groups were all performed in triplicate. Fifteen healthy C. fluminea individuals were added to each test container. The exposure period lasted 10 days. In order to reduce interference with the results, the C. fluminea were not fed during the exposure period, and the test substrate and overlying water were replaced on a daily basis (Aguirre-Martínez et al. 2015). The water temperature was maintained at 23 ± 0.5°C during the 10 days of exposure. Physical and chemical factors such as pH, salinity, and dissolved oxygen of the overlying water were measured every day and these parameter values were similar to acclimatization period. It should be noted that none of the C. fluminea died during the 10 days’ exposure. All of the C. fluminea specimens were removed from the test container and rinsed with Milli-Q® water at the end of the experiment. Then all the samples were prepared for further analysis.

Endpoints

Biochemical parameters measurements

Prior to the biochemical measurements, six C. fluminea of each treatment group were rinsed with Milli-Q® water, and the organs (gills and digestive glands) were dissected using clean forceps and a scalpel. The entire dissection was carried out on an ice plate. Afterwards, the soft tissues were homogenized using ultra-sonication in pH 7.4, 0.1 mol/L PBS buffer solution (137mM NaCl, 2.7 mM KCl, 10mM Na2HPO4, 1.8mM KH2PO4) under an ice water bath. Afterwards, the samples were centrifuged (4°C) at 8000 × g for 15 minutes, and the supernatant was saved for further analysis.

The biochemical parameters were the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), the content of glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione S-transferases (GST), and the content of the lipid peroxidation product malondialdehyde (MDA). All the biochemical indices employed were measured following the instructions of the respective assay kits (Nanjing Jiancheng Bioengineering Institute, China). The MDA content are expressed as nmol/mg protein. The SOD activity is expressed in units (U) per minute per mg protein. CAT activity was measured by monitoring the decrease in absorbance of hydrogen peroxide (H2O2) at 240 nm, with units of U/mg protein. Results of GSH-Px, GR, GST activities were expressed as U/mg protein. The GSH content was expressed in mmol GSH /mg protein.
protein. The results from biochemical analyses were normalized to sample total protein. A protein assay kit (Coomassie brilliant blue method, Nanjing Jiancheng Bioengineering Institute, China) was used for the analysis of protein content in all of the samples.

**Histological analysis**

The digestive gland samples of *C. fluminea* were processed for morphological observation according to the techniques detailed by Cid et al. (2015). After the experiment, four *C. fluminea* were rinsed with Milli-Q® water and immobilization in 10% formalin solution for at least 48 h. Briefly, after being immobilized, the samples were cleaned for 24 h using deionized water and then dehydrated using a graded ethanol series (70–100%). After that, the digestive gland was embedded in paraffin for histological sectioning. Sections of 5–7 μm were cut using a microtome (Leica ATC-RM2235, Germany) and stained with hematoxylin and eosin (H&E). An optical microscope (Leica DMi8) was used for performing histological observations.

**Siphoning behavior observations**

The filtration rate was measured by the method described by Coughlan (1969) and modified by Chen et al. (2015), which is based on the reduction of the concentration of neutral red solution due to filtration by *C. fluminea*. At the end of the exposure experiment, five *C. fluminea* individuals from each experimental and control group were rinsed with Milli-Q® water and placed in beakers with 100 mL neutral red solution (1 mg/L, diluted by Milli-Q® water) and were allowed to siphon for 2 h. The concentration of neutral red solution was tested by measuring the optical density at 530 nm using a spectrophotometer before and after the two hours’ siphoning. The filtration rate was calculated using the following equation from Chen et al. (2015):

\[
m = \left[ \frac{M}{nt} \right] \log \left( \frac{C_0}{C_t} \right)
\]

where M is the volume of the neutral red solution; n is the number of *C. fluminea* used; t is the time (hrs); C₀ is the dye initial concentration; Cₜ is the dye concentration at time t, and m is the filtration rate (mL/animal/h).

**Statistical analysis**

Each treatment group was conducted in triplicate and the results are presented as the means ± standard deviations. To evaluate the differences of each parameter between different treatment (control, 0.01, 0.5, and 2.5 μg/g) in four substrates treatments groups, two-way analysis of variance (ANOVA) followed by Tukey’s post hoc tests was performed using the Data Processing System Version 13.01 (Zhejiang University, Hangzhou, China). Values of p < 0.05 were considered significant. All data were tested for homogeneity of variance and normality. To summarize all of the biomarker responses from different concentrations of CIP, the integrated biomarker response indexes were calculated by the integrated biomarker response index version 2 (IBRv2) as described by Beliaeff and Burgeot (2002) and modified by Sanchez et al. (Sanchez et al. 2012). The details concerning the calculation method for the IBR are given in the supplementary information.

**Results and discussion**

**Biochemical parameters responses of C. fluminea**

**Sediment-associated CIP effects on biochemical parameters**

The antioxidant defense system is an important reactive oxygen species (ROS) scavenging system in aerobic organisms, as it prevents the organism from oxidative damage (Su et al. 2016). The antioxidant defense system of *C. fluminea* has been successfully employed in the bioassessment of various environmental pollutants (Ren et al. 2013; Chen et al. 2015; Yan et al. 2017). However, research is limited concerning the effect of sediment-associated antibiotics on the antioxidant defense system of *C. fluminea*. The functions and relationships among the biomarkers and the responses of the biochemical parameters (SOD, CAT, GSH-Px, GR, GST, GSH, and MDA) in the gills and digestive glands of *C. fluminea* after 10 days’ CIP exposure are shown in Fig. 1 (A-N). The detailed high-resolution figures about the response of each biomarker in the gills and digestive glands were shown in supplementary information (Fig. s1 and Fig. s2).

As the first barrier to oxidative stress, SOD is the fundamental antioxidant enzyme used to clear excess ROS by transforming the ROS to H₂O₂ (Wu et al. 2019). Compared with the control group, a significant increase of SOD activity in the gill and digestive gland was observed with 2.5 μg/g CIP exposure (p < 0.05, Fig. 1-C, D) in almost all of the substrate groups. Similar results were reported by other researchers, who found that SOD activity in organisms usually increases under stress conditions (Sajjad. Zare, H.P. 2012). The increase of SOD activity shown in Fig. 1-C, D indicates that the sediment-associated CIP exposure promoted the generation of ROS. The production of excess ROS increased the SOD activity to protect tissues and cells from oxidative damage.

Similar to SOD, CAT is essential for organism defense under oxidative stress. As shown in Fig. 1(E, F), CAT activities in gills and digestive glands of all of the substrate groups
were all decreased from the 0.01μg/g treatments and significantly decreased (P<0.05) in the 2.5 μg/g CIP exposure groups. This is due to the functions of SOD and CAT in antioxidant defense systems being collaborative. SOD catalyzes ROS to produce excessive H₂O₂, which increases the consumption of CAT. CAT catalyzes a redox reaction in which dismutation of H₂O₂ converts it to O₂ and H₂O (Trchounian et al. 2016). In this study, the decrease of CAT in Fig. 1(E, F) showed that the CAT was unable to eliminate excess H₂O₂ in time, leading to excess H₂O₂ exceeding the range of regulation of the organism, ultimately affecting the synthesis of CAT and decreasing its enzymatic activity (Suzuki et al. 2019). In addition, glutathione peroxidase enzymes (GSH-Px) were also involved in the detoxification of H₂O₂. The GSH-Px activity in the gills and digestive glands was slightly increased in the 0.01 μg/g CIP exposure group, and it was significantly increased in the 2.5 μg/g CIP exposure group (P<0.05, Fig. 1-G, H). This also indicate the production of excess ROS in C. fluminea after exposure to CIP in different substrates.

GSH plays a significant role in the process of removing H₂O₂ and ·OH. As shown in Fig. 1, two forms of glutathione exist in the organism: reduced glutathione (GSH) and oxidized glutathione (GSSG). In this study, the GSH content in gill tissue (Fig. 1-I) of C. fluminea decreased with the increase of CIP concentration, while the GSH content in the digestive gland increased significantly (P<0.05, Fig. 1-J). At the same time, GSH-Px and GR activity in gill tissue and digestive gland were increased by all three of the CIP concentrations (Fig. 1-G, H, K, L). Under the action of GSH-Px, the reduced glutathione (GSH) was constantly being oxidized to a disulfide form (GSSG) that is recycled to GSH by NADPH-dependent glutathione reductase (GR) (Csiszár et al. 2016). The decrease of GSH content in gill tissue shown in Fig. 1-I may be due to the production of excessive ROS in C. fluminea cells after the invasion of CIP. Under the catalysis of GSH-Px, ROS and H₂O₂ are removed, and GSH in gill tissue is converted into GSSG, and thus the content of GSH is decreased. This was confirmed by the increase of GSH-Px activity, as shown in Fig. 1-G, H.
The increase of GSH content in the digestive gland (Fig. 1-J) may be due to the earlier invasion of CIP in gill tissue than in the digestive gland. Gills are gas-exchange organs and openly exposed to water pollutants (Arini et al. 2014). Although filtration is the central ingestion route of C. fluminea, the intake of granular contaminants into the digestive gland arises primarily through swallowing (Ortmann 2003). GSH deficiency will aggravate the oxidative damage of CIP to the organism. Therefore, when GSH in the gill tissue (Fig. 1-I) was transformed into GSSG under the action of GSH-Px, the GR was activated. As shown in Fig. 1- K, L, the GR activity levels in gill and digestive gland were induced after 0.5 and 2.5 μg/g CIP exposure. At the same time, GSSG was transformed into GSH. As an important cellular antioxidant, GR is responsible for the transformation of glutathione disulfide (GSSG) to the sulfhydryl form GSH (Yan et al. 2017). Thus, although the antioxidant defense system of C. fluminea was stimulated by the antibiotics, the GSH content in the digestive gland was increased (Fig. 1-J).

GSTs constitute a family of phase II enzymes that are responsible for the removal and decontamination of peroxide (Sheehan et al. 2001). In the current study, the trend of GST activity in gill and digestive gland of C. fluminea was opposite to that of the content of GSH (Fig. 1). In the gills, the GSH content (Fig. 1-I, J) decreased with the increase of CIP concentration, while the activity of GST (Fig. 1-M, N) was induced with the increase of CIP concentration. This may be due to the excessive production of ROS, which promotes activation of phase II enzymes pathway. GST can conjugate electrophilic compounds with GSH (Fig. 1) and take part in cellular defense against the harmful effects of xenobiotics and oxidative metabolic byproducts (Silva et al. 2009). Thus, the activity of GST in the gills (Fig. 1-M) is promoted to catalyze the combination of GSH and CIP to form a complex (glutathione-S-conjugate (GSX)) to be discharged from the body. In the digestive gland, the GSH content increased (Fig. 1-J) with the increase of CIP concentration, while the activity of GST (Fig. 1-N) was decreased with the increase of CIP concentration. The reason might be that the CIP stress conditions led to excessive ROS accumulation, resulting in the decrease of GST in the process of catalytic removal of excessive xenobiotics by GSH.

Enzyme and non-enzyme in the antioxidant defenses can reflect the condition of the free radical scavenging system, while lipid peroxidation (LPO) is often used to reflect the degree of free radical reactions in organisms and to indirectly reflect the degree of cell damage (Chen et al. 2014; Chen et al. 2015). As shown in Fig. 1(A, B), the contents of MDA in the gills and digestive glands revealed significant increases at 2.5 μg/g CIP (P<0.05), further supporting the perception of an increase in ROS. MDA is produced from the decomposition of unsaturated fatty acid peroxides under the rapidly degraded by ROS (Yan et al. 2017). Thus, if the ROS content exceeded the elimination capacity of the antioxidant enzymes, the excess ROS could stimulate MDA production. Similar results have been reported in many studies (Legeay et al. 2005; Chen et al. 2014; Chen et al. 2015), MDA contents in the gills and digestive glands of C. fluminea were increased after exposure to organic pollutants and metals.

Through analysis of the above biomarkers, it can be concluded that sediment-associated CIP exposure significantly stimulated the antioxidant defense system of C. fluminea. The findings above confirm that the balance of the antioxidant system was disrupted and was incapable of removing excess reactive oxygen species. Exposure to CIP eventually led to oxidative damage in the gills and digestive gland.

Malondialdehyde (MDA), the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione S-transferases (GST), and the content of glutathione (GSH)

**Influence of different substrates on the integrated biomarker responses**

The integrated biomarker response (IBR) approach is often used to identify impacts of environmental stresses on organisms (Sanchez et al. 2012; Beliaeff and Burgeot 2002). To clarify toxicity differences of the CIP exposure groups with different substrates, IBR was applied to perform an integrated assessment of all of the biochemical parameters considered in this study. Seven parameters (i.e., SOD, CAT, MDA, GSH, GSH-Px, GR, and GST) were chosen to obtain an integrated evaluation of the stress level of C. fluminea after exposure to CIP in four substrates. The results of IBR are presented as a radar chart in Fig. 2.

Fig. 2 shows that IBR values increased in a concentration-dependent manner in all four substrate types after exposure to CIP. This indicates that the sediment-associated CIP did have an adverse health effect on C. fluminea, and the effect was gradually enhanced with increase of exposure concentration. Meanwhile, different substrate compositions had certain effects on the biochemical parameters of the C. fluminea antioxidant defense system after the exposure to CIP (Fig. 2). For gill and digestive gland, the values of IBR at all of the exposure concentrations were significantly higher in the NOS group than in other substrate groups (Fig. 2). This may have been due to the fact that the bioavailability of the CIP in NOS group was not affected by the sediment.

As shown in Fig. 2, the IBR values in the digestive gland in different substrate exposure groups were in the order of: NOS group > Sand group > SKC group > SCO group. The results also indicated that the bioavailability of CIP in the no-substrate group was the strongest, and the bioavailable antibiotic concentrations in the sand group, SKC group,
and SCO group decreased gradually. Previous studies have shown that adsorption is the most important means of antibiotic transport in water and sediment (Tolls 2001). The adsorption effect depends on the characteristics of the antibiotics and the sediment. CIP is susceptible to adsorption in sediments through cation bridging due to the existence of ionizable chemical functional groups (Luo et al. 2011; Riaz et al. 2018). Otherwise, the composition of the sediment has a large influence on the antibiotics adsorption, and the content of organic matter in the sediment is positively correlated with the adsorption capacity (Kaeseberg et al. 2018). In addition, smaller sediment particles with more adsorption sites have been regularly connected with greater pollutant adsorption (Crawford and Liber 2015). The downward trend of IBR values was completely consistent with the adsorption characteristics of antibiotics in the four substrates. That is, with the rise of silt content, clay, and organic matter in the substrate composition, the concentration of antibiotics in the overlying water decreased gradually.

The IBR values in the gills in different substrate exposure groups were in the order of: NOS group > SCO group > SKC group > Sand group in all of the treatments (0.01, 0.5, and 2.5μg/g). This may be due to the bioturbation effect of *Corbicula fluminea* on the sediment that caused filtering and swallowing by the clam of organic matter, clay, and other fine sediment particles that adsorbed antibiotics. Filtration and swallowing of suspended particles and plankton are the principal energy supply pathways of this clam species (Wu et al. 2019). Gills, as a gas exchange organ, are directly exposed to water contaminants. Compared to the digestive glands, organic matter, clay, and other fine particles adsorbing antibiotics in sediment were more likely to have adverse effects on gills. Therefore, the IBR values for the gills in the SCO group and SKC group were higher than in the sand group.

**Histological alterations of the digestive gland**

**Sediment-associated CIP effects on cell morphological of the digestive gland**

Cell morphological observation is the most basic approach in toxicological studies (Wu et al. 2019). Such observation can indicate the histopathological changes in an organism after exposure to xenobiotics. As a major organ for metabolism and bio-accumulation, the bivalves’ digestive gland plays a significant role in the regulation and eradication of xenobiotics (Wu et al. 2019). The epithelial cells lining the tubules of the digestive gland are responsive to the negative effects (e.g. enlargement of the tubule lumen and thinning of the epithelium) of numerous contaminants (Vale et al. 2014). However, there are few reports of histological observations of freshwater bivalves under the...
effect of antibiotics (Guo and Feng 2018). Therefore, the digestive gland of the freshwater bivalve *C. fluminea* was chosen to analyze cell morphology modifications following CIP exposure.

The normal structure of the digestive gland comprises a single layer of cells with a virtually occulted lumen (Vale et al. 2014; Cid et al. 2015). As shown in Fig. 3 (a1, a2, a3, a4), the digestive gland tubules have an epithelium surrounded by a distinctive basal lamina and a well-defined Y-shaped lumen. After the exposure to CIP in different substrates, the microscopy observations revealed that almost no alterations of the digestive gland cells (Fig. 3 b1, b2, b3, b4) were observed in the 0.01 μg/g exposure group. The clams exposed to concentrations higher than 0.01 μg/g were clearly affected, and enlargement of the tubule lumen and thinning of the epithelium in the digestive gland were observed. With the increase of exposure concentration, severe pathological changes in the digestive glands of *C. fluminea* were observed, including increase of vacuolation, the epithelial layer of the digestive gland becoming thinner, and irregular dilation of the lumen. The histopathological damage after exposure to 2.5 μg/g CIP was the most serious (Fig. 3 d1, d2, d3, d4).

**Influence of different substrates on the cell morphology responses of *C. fluminea***

Morphological alterations of cells are the result of profound changes at the physiological and biochemical levels (Zhang et al. 2020). As the IBR values shown in Fig. 2 indicate, the biochemical response of *C. fluminea* increased in a concentration-dependent manner in all four substrate types after exposure to CIP. Combined with the morphological damage shown in Fig. 3, these results revealed that the increased production of ROS after CIP exposure increased the oxidative damage to the organism, changed the expression of antioxidant enzymes, reduced the ability to scavenge free radicals, and caused severe tissue damage to the digestive glands of *C. fluminea*. There were differences in the changes of cell morphology between NOS (water) group and sediment groups in the 2.5 μg/g CIP treatment (Fig. 3 d1-d4). The thinnest epithelial cell layer with the most irregular changes and dilated lumina was observed in the NOS (water) group compared to the other three substrate groups. The trend of morphological structure changes of digestive gland cells in *C. fluminea* similar in the other three substrate groups. These results further suggest that the response of *C. fluminea* to toxins in the NOS group was the strongest.

![Fig.3 Morphology of digestive gland cells of *Corbicula fluminea* after 10 days of ciprofloxacin exposure in four test substrates: a1-a4—control, b1-b4—0.01 μg/g CIP, c1-c4—0.5 μg/g CIP, d1-d4—2.5 μg/g CIP; NOS: no substrate (water); sand (100%); SKC: sand (75%), kaolinite clay (25%); SCO: sand (75%), kaolinite clay (20%), and organic matter (5%). Yellow signals (Y-shaped lumen of the digestive gland tubules), signals of black stars (vacuolation).](image)
Behavioral responses reveal the most direct impact of pollutants on organisms and have strong significance for biological monitoring (Zhou et al. 2008). Owing to the feeding behavior of C. fluminea mainly depending on the siphon, siphoning behavior has become an important indicator of general health or stress (Wu et al. 2019). The test results of siphoning behavior of C. fluminea are shown in Fig. 4. The filtration rate was slightly inhibited in the low concentration (P<0.05, 0.01 μg/g CIP) treatment (Fig. 4). The adverse effects of CIP increased (P<0.05) with increasing concentration (from 0.5 μg/g to 2.5 μg/g). Previous studies have shown that many pollutants (such as bisphenol A, imidacloprid, and bifenthrin) result in reduced filtration rates by causing C. fluminea valves to close (Esperanza et al. 2020; Shan et al. 2020; Zhang et al. 2020). Valve closure regulation represents a trade-off between protection from the stressor and physiological performance (Esperanza et al. 2020). The reduction of filtration rate in this study may be related to cellular oxidative damage. With the increase in CIP concentration, balance of the antioxidant system was disrupted and rendered incapable of removing excess reactive oxygen species. At the same time, C. fluminea protects the cells from oxidative damage by closing the valve and reducing its siphonage efficiency.

The filtration rates were significantly influenced under the different substrate compositions by exposure to the high concentration of CIP (P<0.05, 0.5 and 2.5 μg/g, Fig. 4). The highest inhibition rate of siphoning behavior (filtration rate) was observed in the no substrate (NOS) group. The inhibitory degree of filtration rates in the higher concentration exposure groups (0.5 and 2.5 μg/g) decreased in the order of Sand > SKC > SCO. This phenomenon may be attributed to the complete contact of C. fluminea with the pollutant in aqueous solution. Previous studies demonstrated Cd accumulation in C. fluminea to be highest when exposed in water and to decrease under exposure in sand and mud substrates (Graney et al. 1984). The results of our study may be well confirmed by this conclusion. Moreover, in the current study, the filtration rate of C. fluminea in the sand substrate group was least compared with the SKC and SCO substrate groups. Other researchers have found that in formulated sediments, compared to clay and organic matter, minimal adsorption of pollutant was appeared to the sand (Crawford and Liber 2015). The minimal binding amount of the pollutant to the sand will result in the enhanced content of pollutants in the overlying water. Thus, C. fluminea in the sand group would swallow more pollutants through siphonage of the overlying water.
Conclusions

The adverse influence of ambient concentrations CIP on *C. fluminea* in different substrates was examined. The biochemical parameters response results revealed that the balance of the antioxidant system was disrupted, and oxidative damage appeared in the gills and digestive gland after sediment-associated CIP exposure. In terms of cell morphology and behavioral responses, CIP induced clear histopathological damage to the digestive gland and produced behavioral inhibition in high concentration exposure groups. In addition, the substrate composition significantly influenced the activity of antioxidant enzymes and the siphoning behavior of *C. fluminea* after CIP exposure. Compared to the sediment group, the histopathological damage was greater in the group without substrate addition. These results indicate that exposure to CIP can provoke significant alterations on antioxidation, histopathological characteristics and behaviour of *C. fluminea*. And the presented data reveal the sensitivity of *C. fluminea* to the presence of CIP in the sediment environment. The current study has established a basis for future research on the toxic effects of CIP in *C. fluminea* in the sedimentary environment. The impacted pathways and genes expressed by *C. fluminea* after exposure to CIP need further exploration to characterize the toxicity mechanism.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-17509-z.

Author contribution Xiaoyu Guo: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing - original draft. Chenghong Feng: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing - review & editing, supervision, project administration, funding acquisition. Yanpeng Cai: review & editing. Akhtar Islam: review & editing. William Wilson: review & editing.

Funding This work was supported by the National Natural Science Foundation of China (42007368), the Natural Science Foundation of Guangdong Province (2021A1515011863), and the National Key Research and Development Program of China (2016YFF0204502, 2016YFF0204500).

Data availability The datasets in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Approval was obtained from the ethics committee of Beijing Normal University. The benthic bivalve *Corbicula fluminea* used in this study was not listed as a protected species in China.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Arini A, Daffe G, Gonzalez P, Feurte-Mazael A, Baudrimont M (2014) Detoxification and recovery capacities of *Corbicula fluminea* after an industrial metal contamination (Cd and Zn): a one-year depuration experiment. Environ Pollut 192:74–82

Aguirre-Martinez GV, DelValls AT, Laura Martin-Diaz M (2015) Yes, caffeine, ibuprofen, carbamazepine, novobiocin and tamoxifen have an effect on Corbicula fluminea (Müller, 1774). Ecotoxicol Environ Saf 120:142–154

Beliaeff B, Burgeot T (2002) Integrated biomarker response: a useful tool for ecological risk assessment. Environ Toxicol Chem 21:1316–1322

Boening D (1999) An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ Monit Assess 55:459–470

Wilson C, Clarke R, D’Arcy BC et al (2005) Persistent pollutants urban rivers sediment survey: implications for pollution control. Water Sci Technol 51(3-4):217–224

Carvalho IT, Santos L (2016) Antibiotics in the aquatic environments: a review of the European scenario. Environ Int 94:736–757

Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. Mar Biol 2:356–358

Chen H, Zha J, Liang X, Li J, Wang Z (2014) Effects of the human antiepileptic drug carbamazepine on the behavior, biomarkers, and heat shock proteins in the Asian clam *Corbicula fluminea*. Aquat Toxicol 155:1–8

Chen H, Zha J, Yuan L, Wang Z (2015) Effects of fluoxetine on behavior, antioxidant enzyme systems, and multidrug resistance in the Asian clam *Corbicula fluminea*. Chemosphere 119:856–862

Chen K, Zhou JL (2014) Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95:604–612

Cid A, Picado A, Correia JB, Chaves R, Silva H, Caldeira J, de Matos APA, Diniz MS (2015) Oxidative stress and histological changes following exposure to diamond nanoparticles in the freshwater Asian clam *Corbicula fluminea* (Müller, 1774). J Hazard Mater 284:27–34

Crawford SE, Liber K (2015) Effects of clay minerals and organic matter in formulated sediments on the bioavailability of sediment-associated uranium to the freshwater midge, Chironomus dilutus. Sci Total Environ 532:821–830

Csiszár J, Horváth E, Bela K, Gallé Á (2016) Glutathione-Related enzyme system: glutathione reductase (GR), glutathione transferases (GSTs) and Glutathione peroxidases (GPXs). In: Gupta S, Ray A (eds) Cell Stress and Plant Cell Stress Responses. Springer International Publishing, Cham, pp 137–158

Doherty FG (1990) The Asiatic clam, Corbicula spp., as a biological monitor in freshwater environments. Environ Monit Assess 15:143

Esperanza M, Seoane M, Servia MJ, Cid Á (2020) Effects of Bispheno-A on the microalgae Chlamydomonas reinhardtii and the clam *Corbicula fluminea*. Ecotoxicol Environ Saf 197:11069

Feng C, Guo X, Yin S, Tian C, Li Y, Shen Z (2017) Heavy metal partitioning of suspended particulate matter–water and sediment–water in the Yangtze Estuary, Chemosphere 185:717–725

Feng C, Zhao S, Wang D, Niu J, Shen Z (2014) Sedimentary records of metal speciation in the Yangtze Estuary. Chemosphere 185:717–725

Fournier E, Adam C, Massabuau JC, Garnierlaplace J (2005) Bioaccumulation of waterborne selenium in the Asiatic clam *Corbicula fluminea*: influence of feeding-induced ventilatory activity and selenium species. Aquat Toxicol 72:251–260

Fu L, Huang T, Wang S, Wang X, Su L, Li C, Zhao Y (2017) Toxicity of 13 different antibiotics towards freshwater green algae...
Naylor C, Rodrigues C (1995) Development of a test method for Chi-
Matić Bujagić I, Grujić S, Laušević M, Hofmann T, Micić V (2019)
Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez PJJ (2011) Occur-
Liu Y, Junaid M, Xu P, Zhong W, Pan B, Xu N (1987) (2020) Sus-
Li D, Wang P, Wang C, Fan X, Hu B (2018) Combined toxicity of
Legeay A, Achard-Joris M, Baudrimont M, Massabuau J, Bour-
Herrero A, Vila J, Eljarrat E, Ginebreda A, Jaramillo D, Barceló D (2018) Transport of sediment borne contaminants in a Mediterranean river during a high flow event. Sci Total Environ 633:1392–1402
Huang Y, Wang Y, Huang Y, Zhang L, Ye F, Wang J, Shang J, Liao Q (2020) Impact of sediment characteristics on adsorption behavior of typical antibiotics in Lake Taihu Chinau. Sci Total Environ 718:137329
Jiang Y, Li M, Guo C, An D, Xu J, Zhang Y, Xi B (2014) Distribution and ecological risk of antibiotics in a typical effluent-receiving river (Wangyang River) in north China. Chemosphere 112:267–274
Jr. G. Allen Burton (2002) Sediment quality criteria in use around the world. Jpn J Limnol 3, 65–76
Kaeselberg T, Zhang J, Schubert S, Oertel R, Siedel H, Krebs P (2018) Sewer sediment-borne antibiotics as a potential environmental risk: adsorption and desorption affinity of 14 antibiotics and one metabolite. Environ Pollut 239:638–647
Legeay A, Achard-Joris M, Baudrimont M, Massabuau J, Bourdineau J (2005) Impact of cadmium contamination and oxygenation levels on biochemical responses in the Asiatic clam Corbicula fluminea. Aquat Toxicol 74:242–253
Li D, Wang P, Wang C, Fan X, Hu B (2018) Combined toxicity of organophosphate flame retardants and cadmium to Corbicula fluminea in aquatic sediments. Environ Pollut 243:S59762460
Liu Y, Junaid M, Xu P, Zhong W, Pan B, Xu N (1987) (2020) Suspended sediment exacerabates perfluorooctane sulfonate mediated toxicity through reactive oxygen species generation in freshwater clam Corbicula fluminea. Environ Pollut 267:115671
Lubecki L, Kowalewska G (2019) Plastic-derived contaminants in sediments from the coastal zone of the southern Baltic Sea. Mar Pollut Bull 146:255–262
Luo Y, Xu L, Rysz M, Wang Y, Zhang H, Alvarez PJJ (2011) Occurrence and Transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environ Sci Technol 45:1827–1833
Matić Bujagić I, Grujić S, Laušević M, Hofmann T, Micić V (2019) Emerging contaminants in sediment core from the Iron gate i reservoir on the Danube River, Sci Total Environ 662:77–87
Naylor C, Rodrigues C (1995) Development of a test method for Chironomus riparius using a formulated sediment. Chemosphere 31:3291–3303
Ottmann C (2003) (2003) Energy metabolism and valve closure behaviour in the Asian clam Corbicula fluminea. J Exp Biol 206(22):4167–4178
Qi W, Müller B, Pernet-Coudrier B, Singer H, Liu H, Qu J, Berg M (2014) Organic micropollutants in the Yangtze River: seasonal occurrence and annual loads. Sci Total Environ 472:789–799
Ren J, Luo J, Ma H, Wang X, Ma LQ (2013) Bioavailability and oxidative stress of cadmium to Corbicula fluminea. Environ Sci Process Impacts 15:86–89
Riaz L, Mahmood T, Khalid A, Rashid A, Ahmed Siddique MB, Kamal A, Coyne MS (2018) Fluorquinolones (FQs) in the environment: a review on their abundance, sorption and toxicity in soil. Chemosphere 191:704–720
Roman YE, De Schampheelaere KAC, Nguyen LTH, Janssen CR (2007) Chronic toxicity of copper to five benthic invertebrates in laboratory-formulated sediment: sensitivity comparison and preliminary risk assessment. Sci Total Environ 387:128–140
Rusina TP, Smidh F, Brborić M, Vrana B (2019) Investigating levels of organic contaminants in Danube River sediments in Serbia by multi–ratio equilibrium passive sampling. Sci Total Environ 696:133935
Saïdani W, Sellami B, Khazri A, Mezni A, Dellali M, Joubert O, Sheehan D, Beyrem H (2019) Metal accumulation, biochemical and behavioral responses on the Mediterranean clams Ruditas decussatus exposed to two photocatalyst nanocomposites (TiO2 NPs and AuTiO2NPs). Aquat Toxicol 208:71–79
Sajjad Zare, H.P. (2012) Changes activities of antioxidant enzymes in oilseed rape in response to salinity stress. Int J Agricultural Crop Sci 7:398–403
Sanchez W, Burgeot T, Porcher JM (2012) A novel “integrated biomarker response” calculation based on reference deviation concept. Environ Sci Pollut Res 20:2721–2725
Santos KCD, Martinez CBR (2014) Genotoxic and biochemical effects of atrazine and Roundup ®; alone and in combination, on the Asian clam Corbicula fluminea. Ecotoxicol Environ Saf 100:7–14
Shan Y, Yan S, Hong X, Zha J, Qin J (2020) Effect of imidacloprid on the behavior, antioxidant system, multixenobiotic resistance, and histopathology of Asian freshwater clams (Corbicula fluminea). Aquat Toxicol 218:105333
Suzuki J, Nakano D, Imamura M, Yamamoto R, Fujita M (2019) Assessing a polluted river environment by oxidative stress biomarker responses in caddisfly larvae. Sci Total Environ 696:134005
Sheehan D, Meade G, Foley VM, Dowd CA (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily, Biochem J 360:1–16
Silva SN, Azevedo AP, Teixeira V, Pina JE, Rueff J, Gaspar JF (2009) The role of GSTA2 polymorphisms and haplotypes in breast cancer susceptibility: a case-control study in the Portuguese population. Oncol Rep 22:593–598
Su T, Shao Q, Wang P, Ma C (2016) Oxidative Stress and its role in peroxisome homeostasis in plants. In: Gupta DK, Palma JM, Corpas FJ (eds) Redox state as a central regulator of plant-cell stress responses. Springer International Publishing, Cham, pp 117–136
Tollis J (2001) Sorption of veterinary pharmaceuticals in soils: a review. Environ Sci Technol 35:3397–3406
Trchounian A, Petrosyan M, Sahakyan N (2016) Plant Cell redox homeostasis and reactive oxygen species. In: Gupta DK, Palma JM, Corpas FJ (eds) Redox State as a Central Regulator of Plant-Cell Stress Responses. Springer International Publishing, Cham, pp 25–50
US Environmental Protection Agency (2007) Sediment toxicity identification evaluation (TIE) phases I, II, and III guidance document. EPA 600/R-07/080. Final Report, Washington DC
Vale G, Franco C, Diniz MS, Santos MMCD, Domingos RF (2014) Bioavailability of cadmium and biochemical responses on the freshwater bivalve *Corbicula fluminea*–the role of TiO2 nanoparticles. Ecotoxicol Environ Saf 109:161–168

Wang D, Ning Q, Dong J, Brooks BW, You J (2020) Predicting mixture toxicity and antibiotic resistance of fluoroquinolones and their photodegradation products in Escherichia coli. Environ Pollut 262:114275

Wang Q, Hong X, Chen H, Yuan L, Zha J (2018) The neuropeptides of Asian freshwater clam (*Corbicula fluminea*) as new molecular biomarker basing on the responses of organophosphate chemicals exposure. Ecotoxicol Environ Saf 160:52–59

Wu Y, Gu E, Li H, Tian C, Feng C (2019) Oxidative stress and histological changes in *Corbicula fluminea* exposed to nano-Al13 and monomeric Al coagulants. Environ Sci Nano 6:2736–2748

Xu J, Zhang Y, Zhou C, Guo C, Wang D, Du P, Luo Y, Wan J, Meng W (2014) Distribution, sources and composition of antibiotics in sediment, overlying water and pore water from Taihu Lake, China. Sci Total Environ 497-498:267–273

Yan S, Wu H, Qin J, Zha J, Wang Z (2017) Halogen-free organophosphorus flame retardants caused oxidative stress and multixenobiotic resistance in Asian freshwater clams (*Corbicula fluminea*). Environ Pollut 225:559–568

Yang JF, Ying GG, Zhao JL, Tao R, Su HC, Chen F (2010) Simultaneous determination of four classes of antibiotics in sediments of the Pearl Rivers using RRLC-MS/MS. Sci Total Environ 408:3424–3432

Zhang H, Hong X, Yan S, Zha J, Qin J (2020) Environmentally relevant concentrations of bifenthrin induce changes in behaviour, biomarkers, histological characteristics, and the transcriptome in *Corbicula fluminea*. Sci Total Environ 728:138821

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606:135–150

Zhou J, Broodbank N (2014) Sediment-water interactions of pharmaceutical residues in the river environment. Water Res 48:61–70

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