Acute Toxic Effects of Petroleum Hydrocarbons in Sediment on *Maoctra veneriformis* and Its Antioxidant Enzyme Activities

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**Abstract.** In order to clarify the toxic effects of petroleum hydrocarbons in sediments on bivalve mollusks, the acute toxicity and effects of petroleum hydrocarbons on antioxidant enzyme activities were studied using *Maoctra veneriformis* as a test organism. Our results showed that the health status of the *Maoctra veneriformis* gradually weakened with the increase of exposure time and concentration of petroleum hydrocarbon, and mortality occurred in the high-concentration group at the end of the exposure. The activities of peroxidase (POD) showed a trend of first decrease and then increase with the prolong of exposure time, and POD activity was significantly induced in some concentration groups at the beginning and the end of the exposure (P < 0.01). The activities of superoxide dismutase (SOD) generally decreased first and then increased as the petroleum hydrocarbons concentration increased. With the prolong of exposure time, the SOD activity decreased first and then increased and then decreased. Our results showed that petroleum hydrocarbons in the sediment produced a certain toxic effect on *Maoctra veneriformis*, and there was a certain time-dose-effect relationship between anti-oxidase activity and petroleum hydrocarbons. Meanwhile, petroleum hydrocarbons can produce significant induction or inhibition effect on anti-oxidase activity.

1. Introduction
With the development of the coastal economy, marine oil spills and ship oil spills have frequently occurred, and marine pollution has become increasing serious. Petroleum hydrocarbons are listed as one of the main pollutants in marine environment, which seriously affect the quality of the marine ecological system[1, 2]. Most of the petroleum hydrocarbons in the ocean are absorbed by solid particles and dispersed in water suspensions and sediments. *Mactra veneriformis* is a bivalve shellfish, mainly distribute in the intertidal zone and bury in 5-10 cm of sediment for a long time. Petroleum hydrocarbons are enriched in the marine organism by breathing, feeding, surface penetration and food chain transmission which will inevitably affect the health of bivalve shellfish [3, 4] and produce toxic effect on the organism [5, 6].
After petroleum hydrocarbons enter into the organism, they participate in the redox reaction through their own substances or intermediate metabolites, which will generate a large number of reactive oxygen free radicals, such as superoxide anion radicals (O$_2^-$•), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (•OH). These reactive oxygen radicals have serious damage to the organism[7]. For example, benzo[a]pyrene was converted into dihydroxyepoxy benzo pyrene (BPDE) by enzymatic hydrolysis, and BPDE could form adducts with specific amino acid residues of the protein and caused enzyme inactivation[8]. The oxidative stress response will occur in organism during the process of petroleum hydrocarbon pollution, but the organism can self-mediate the stress. Antioxidant enzymes such as POD and SOD have the function of scavenging active oxygen free radicals and peroxides.

Recently, it has become evident that petroleum hydrocarbons pollution has toxic effects on marine organisms at home and abroad, and most of the previous researches focus on the toxic effects of petroleum hydrocarbon pollution in water environments. However, there are few studies on the acute toxicity of petroleum hydrocarbon pollution in sediments, especially the study on the stress and biological response of marine bivalve antioxidant defense systems to low-level petroleum hydrocarbon exposure. In order to clarify the toxicity and toxicity mechanism of petroleum hydrocarbon in sediments on bivalve shellfish, the acute toxicity and the effects on antioxidant enzyme activities of Maoctra veneriformis exposed to petroleum hydrocarbon in sediment were studied. These results will provide references for further studies on toxicological mechanism and marine ecological restoration.

2. Materials and methods

2.1. Experimental materials

Adult individuals (mean shell length = 30 mm) were collected from Dongjiang Port, Tianjin, China (117°816782′N, 39°040082′E), and then domesticated in aquaria for 7 days. The aquaria (40 cm × 35 cm × 30 cm) covered with 3-5 cm clean sediment, and then covered with 3-5 cm artificial seawater (salinity 26‰). The artificial sea water were changed every 2 days, and temperature was 15-20 °C. Feeding chlorella bait once a day and stopping feeding 24 hours before experiment. After 7 days of domestication, the mortality is less than 5%, which can be used for toxicity experiments.

The sediments were collected from wetland core area in Tianjin Ancient Coast and Wetland National Nature Reserve (117°641545′N, 39°306893′E). And then sediments were naturally dried, crushed, decontaminated, sieved (60 mesh), and stored in sealed bags. The crude oil originate from Tianjin Dagang Oilfield. The determination of the petroleum hydrocarbons concentration in the sediment was based on the ultraviolet spectrophotometry method according to the "The specification marine monitoring" [9].

Anhydrous ethanol, n-hexane, and glacial acetic acid were all analytical grade; total protein quantification test boxes, POD and SOD activity test boxes were purchased from the Nanjing Institute of Biological Engineering.

2.2. Experimental methods

The experimental methods was based on a literature[10]. The experimental results were statistically analyzed with SPSS software.

3. Results and analysis

3.1. Acute toxic of petroleum hydrocarbons in sediments

When the experiment was conducted for 24 hours during acute toxicity exposure, the high-concentration (800 mg/kg) group had weakened external stimuli, and part of the Maoctra veneriformis secreted colorless and transparent substances. At 48 hours of experiment, the response of each concentration group was delayed, and the 800 mg/kg concentration group showed slight breathing, and the trachea was clipped to reduce external stimulation to itself. When the experiment was conducted for 72 hours, the Maoctra veneriformis of each concentration group showed strong
breathing. At 800 mg/kg group, the shells showed slight shell expansion, could not maintain a closed state, and died. The mortality rate was 8.3%. Finally, at 96 hours, the mortality rate in the 800 mg/kg group increased to 16.7%, but there was no death in other groups. And the Maoctra veneriformis in the control group were in good health during all the experiment.

3.2. Effects of petroleum hydrocarbons on antioxidant enzyme activities

3.2.1. POD activity.
Dynamic variations of POD activity of Maoctra veneriformis exposed to petroleum hydrocarbons was shown in Figure 1. With the exposure time prolonged, the POD activity generally decreased first and then increased, and there was a certain time-effect relationship. The dose-response curve of the effect of petroleum hydrocarbons on POD activity exposure to petroleum hydrocarbons for 12 hours and 72 hours was shown in Figure 2. With the increase of POD, the change trend of POD was first rising, then falling and then rising, there was a certain dose-response relationship.

POD activity was induced by the influence of petroleum hydrocarbons at the initial stage of exposure. The 100 mg/kg and 800 mg/kg concentration groups were induced significantly (P < 0.05) and extremely significant (P < 0.01), respectively, and the POD activity values were 1.86 times and 2.13 times that of the control group. With the increase of the exposure time, the POD activity of each concentration group was significantly reduced, even the POD activity of some concentration groups was inhibited. But at the end of exposure, it was extremely significantly induced (P < 0.01), especially after 72 hours of exposure, 400 mg/kg and 800 mg/kg concentration groups were induced, the induction was 3.85 times and 4.10 times that of the control group. After 96 hours of exposure, the 100 mg/kg, 200 mg/kg, and 400 mg/kg concentration groups were extremely significantly induced, and the induction were 3.16 times, 3.95 times, and 3.55 times respectively that of the control group (Figure 1).

Figure 1. Effect of petroleum hydrocarbons on POD activity of Maoctra veneriformis
Note: The data in the figure are mean ± standard deviation (n = 2), "a" indicates that the difference between the treatment group data and the control group data is significantly different (P < 0.05), and "aa" indicates that the difference is extremely significant (P < 0.01).
3.2.2. SOD activity.

Dynamic variations of SOD activity of *Maoctra veneriformis* exposed petroleum hydrocarbons was shown in Figure 3. With the exposure time prolonged, the SOD activity of the *Maoctra veneriformis* generally decreased first, then increased and then decreased. And there was a certain time-effect relationship. The dose-response curve of the effect of petroleum hydrocarbons on SOD activity exposure to petroleum hydrocarbons for 24 hours and 96 hours was shown in Figure 4. Dose-effect indicates that the change trend of SOD was first rising, then falling, and then rising, finally falling with the increase of the petroleum hydrocarbons concentration. The effect of different concentrations of petroleum hydrocarbons on SOD activity was relatively unstable.

At the beginning of exposure to petroleum hydrocarbons, the SOD activity of each concentration group was significantly induced at 12 hours ($P < 0.01$). The SOD activity was 1.74 times, 1.42 times, 1.38 times, and 1.44 times that of the control group respectively. With the exposure time prolonged, the overall activity of the every concentration groups showed a downward trend at 24 hours, but the 100 mg/kg and 400 mg/kg concentration groups were still significantly induced ($P < 0.01$). The SOD activity values were 1.33 times and 1.19 times that of the control group, while the 200 mg/kg concentration group was extremely significantly inhibited ($P < 0.01$), but still 62% of the control group. By 48 hours, the POD activity showed a slight upward trend, and all concentration groups were induced. All the concentration groups were 1.36 times, 1.26 times, 1.42 times, and 1.58 times that of the control group respectively. After exposure for 72 hours, the SOD activity of each concentration group showed a clear upward trend, and it (except 200 mg/kg) was extremely significantly induced ($P < 0.01$), which were 1.94 times, 2.01 times, and 2.47 times that the control group, respectively. The viability was significantly increased over 48 hours. At the end of the 96 hours exposure, the SOD activity of each concentration group showed a significant downward trend. Among them, the 200 mg/kg concentration group had an inhibitory effect on the SOD activity, and the SOD activity of individual concentration groups was extremely significantly induced ($P < 0.01$), in general, the enzyme activities declined (Figure 3).
Figure 3. Effect of petroleum hydrocarbons on SOD activity of Maoctra veneriformis

Note: The data in the figure are mean ± standard deviation (n = 2), "a" indicates that the difference between the treatment group data and the control group data is significantly different (P < 0.05), and "aa" indicates that the difference is extremely significant (P < 0.01).

Figure 4. Dose-effect curve of the effects of petroleum hydrocarbon exposure on SOD activity of Maoctra veneriformis

4. Conclusion
In this study we found that petroleum hydrocarbons in the sediment have a toxic effect on the Maoctra veneriformis. Maoctra veneriformis was weaken gradually with the increase of exposure time and the petroleum hydrocarbons concentration, and mortality occurred in the high-concentration group. The results of time-effect indicated that POD tended to decrease first and then increase with the increase of exposure time, SOD showed a downward trend and then rises and then decreases.

Our results showed that petroleum hydrocarbons in the sediment produced a certain toxic effect on Maoctra veneriformis, and there was a certain time-dose-effect relationship between anti-oxidase activity and petroleum hydrocarbons. Meanwhile, petroleum hydrocarbons can produce significant induction or inhibition effect on anti-oxidase activity.

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