Toxic effects of combined effects of anthracene and UV radiation on *Brachionus plicatilis*

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**Abstract.** Anthracene is a typical polycyclic aromatic hydrocarbon, with photo activity, can absorb ultraviolet light a series of chemical reactions, aquatic organisms in the ecosystem has a potential light induced toxicity. In this paper, the effects of anthracene and UV radiation on the light-induced toxicity of *Brachionus plicatilis* were studied. The main methods and experimental results were as follows: (1) The semi-lethal concentration of anthracene in UV light was much lower than that in normal light, The rotifers have significant light-induced acute toxicity. (2) Under UV irradiation, anthracene could induce the increase of ROS and MDA content in *B. plicatilis*, and the activity of antioxidant enzymes in *B. plicatilis* significantly changed, Where SOD, GPx activity was induced within 24 hours of the beginning of the experiment. And the content of GPX and CAT was inhibited after 48 hours. Therefore, the anthracite stress induced by UV radiation could more strongly interfere with the ant oxidative metabolism of *B. plicatilis*, and more seriously cause oxidative damage, significant light-induced toxicity.

1. **Introduction**

Polycyclic aromatic hydrocarbons are difficult to degrade in nature, can exist for a long time in the environment, and with the atmosphere, such as migration around the way. At present, PAHs are not only present in the environment of human activities, but also in the Arctic, deep sea and other remote ecosystems can also find its traces [1]. Because of its strong fat-soluble and high n-octanol /water partitioning coefficients, PAHs are easily absorbed and accumulated, and are enriched by the food chain, resulting in a threat to human health [2]. PAHs have photo activity, can absorb ultraviolet light to produce a series of chemical reactions, the aquatic organisms in the ecosystem there is a potential light induced toxicity [3]. At present, there have been some researches on the role of PAHs in bio-light-induced toxicity at home and abroad [4-6]. The results show that the photo-sensitization is one of the important mechanisms of PAHs light-induced toxicity, and the photo-sensitization reaction causes a large number of active oxygen clusters (ROS) [7]. When the ROS production rate exceeds the rate of removal of antioxidant system in the body, it will cause anti-oxidation reaction, it will cause damage to the biofilm, but also and the body of protein, nucleotides and other biological macromolecules interaction, Damage and death of living cells [8-10].

It has an important role in the circulation and energy flow of marine ecosystems because of its advantages such as extensive distribution, short life cycle, fast breeding speed, easy cultivation and
sensitive reaction to exogenous toxicants [11, 12]. However, there is almost no study of PAHs on light-induced toxicity of rotifers. In this experiment, the most active anthracene was used as the stress factor under UV irradiation, and the effect of anthracene on the light induced toxicity of *B. plicatilis* was studied under the condition of ultraviolet radiation. In this study, the blanks of anthracene on the light-induced toxicity of *C. mongolicus* were studied, which provided a basic theoretical basis for comprehensively evaluating the effect of anthracene on the photoinduced toxic effects of marine zooplankton.

This study fills up the blank of the photoinduced toxicity effect of anthracene on the rotifer, and provides a theoretical basis for the comprehensive evaluation of the photoinduced toxicity of anthracene to marine zooplankton.

2. Experimental materials and methods

2.1. Experimental materials

In this experiment, we selected the lively and robust *B. plicatilis* from the Ocean University of China. The container was cultured with 1.5 L beaker and placed in a GXZ-3008 intelligent light incubator. The incubation temperature was maintained at 20 ± 1 °C and the light intensity was 1313.8 ± 54.8 μW/(cm²•s⁻¹), the illumination period was 12 h: 12 h. Before the start of the formal experiment, *B. plicatilis* had been acclimated for three months under experimental ecological conditions.

2.2. Light source settings and experimental devices

The acute toxicity test was carried out in a constant temperature light incubator. The UV radiation system mainly consisted of two UV-A fluorescent tubes and one UV-B fluorescent tube with visible light irradiance of 1313.8 ± 54.8 μW/cm²; UV-A irradiation UV-B and UV-B can effectively simulate the sunlight in the natural environment. The UV-B and UV-B can be used to simulate the sunlight in the natural environment.

2.3. Experimental design

2.3.1. Determination of the lethal concentration of anthracene in *B. plicatilis* under different light conditions. A total of 480 healthy larvae of *B. plicatilis* were selected from the pre-cultured *B. plicatilis* and were exposed to anthracene solutions at different concentrations of different light sources (Table 2.3.1) The Each concentration gradient takes 20 larvae, each with three parallel samples. 24 hours to record the number of dead insects in each group of rotifers.

2.3.2. Effects of anthracene on the microstructure of *B. plicatilis* induced by UV radiation. The morphological structure of rotifers in 10 μg/L anthracene-treated group and 10 μg/L anthracene-treated group induced by UV radiation was observed in the control group after 24 hours of stress.

2.3.3. Determination of antioxidant enzyme activity. In this part, the concentration of two anthracene solutions was 0.4 μg/L and 4 μg/L, respectively. After pre accumulation of anthracene, transfer to the new medium, with / without UV radiation treatment. The larvae of Brachionus plicatilis were exposed to UV radiation and UV-free radiation respectively, and quantified to each experimental concentration. Each concentration was three parallel, the rotifer density was 100/mL, and no bait was given during the application. Each parallel had a total of 10,000 fold Brachionus (about 0.2 g). Exposure experiments were carried out at 24 h and 48 h, respectively, and the Brachionus plicatilus was collected. Add the concentration of 0.86% saline, in the ice bath under the conditions of ultrasonic cell breaker broken, every 5 s, interval 10 s, 5 times after the break completely. After crushing, centrifuge at 0 °C and 2500 r / min for 10 minutes. The supernatant was then used to determine the total protein TSP and MDA contents and the activity of SOD, CAT and GPX in the crude enzyme solution.
(1) Determination of protein content
The protein concentration of \textit{B. plicatil} was determined by BCA Protein Concentration Kit. The operation is carried out according to the product brochure. The procedure is as follows: ① 50 volume BCA reagent A plus 1 volume BCA reagent B (50: 1) preparation of BCA working solution 5mL, is now with the use; ② Take 10μL protein standard diluted to 100μL, the final concentration of 0.5 mg/mL; ③ the standard by 0,1,2,4,8,12,16,20 μL added to the 96-well plate of the standard hole, plus the standard for the dilution of the standard set to 20μL; ④ add appropriate volume of sample to the sample hole of 96-well plate, dilute the standard to 20mL; ⑤ each hole to join 200μL BCA working fluid, room temperature placed 10min; ⑥ Determination of absorbance at wavelength 562nm, according to the standard curve to calculate the protein concentration. The protein concentration of each group of samples was diluted with PBS buffer to determine the protein concentration in each sample.

(a) MDA content detection
Use the Nanjing Institute of Bioengineering Institute of the determination of the kit. Principle: MDA in lipid peroxides can be condensed with thiobarbituric acid (TAB) to form a red product with a maximum absorption peak at 532 nm and then quantitatively determined by colorimetric method. Defined as per mg of tissue protein MDA in the nanomolar number.

(b) Determination of SOD activity
Use the Nanjing Institute of Bioengineering Institute of SOD kit determination. When the sample contains SOD, xanthine and xanthine oxidase reaction system produced by the superoxide anion radicals have a specific inhibitory effect, so that the oxidation of hydroxylamine formed nitrite reduced, can be colorimetric determination, thus SOD activity was determined. The amount of SOD corresponding to the amount of SOD per mg of tissue protein in a 1 ml reaction solution was 50%, and the activity was defined as a viable unit (U).

(c) Determination of POD activity
Refer to Nanjing Institute of Bioengineering POD kit. POD activity determination principle: in the presence of H2O2, POD can catalyze the oxidation of guaiacol to produce brown material, and then by measuring its absorbance at 420 nm to obtain POD activity. The POD activity in the tissue is defined as the activity of 1μg of guaiacol substrate per mg of protein per minute at 37°C for a POD activity unit.

(d) Determination of CAT activity
CAT determination using catalase test box, purchased from Nanjing built bioengineering research institute. The main principle is that the addition of ammonium molybdate in the process of H2O2 formation of H2O to H2O2, the rapid termination of the reaction, the remaining H2O2 and ammonium molybdate action to produce a light yellow complex, at 405 nm to detect the absorption of light Value, can calculate the vitality of CAT. A viable unit is defined as the amount of H2O2 in the tissue homogenate that decomposes 1μmol per second of tissue protein per second.

2.4. Data processing
The data were analyzed by software SPSS19.0, and the data were analyzed by software Sigmaplot12.5. The data of the experimental group and the blank group were analyzed by one-way ANOVA. Use multiple comparisons (LSD) to analyze the variance of the data. Use Dunnett’s T3 to analyze variance data. When P <0.05, the difference was significant, P <0.01, the difference was significant. The change of each index is given by mean ± standard error (mean ± SE) (n = 3).
3. Experimental results

As shown in Figure 1, with the increase in anthracene concentration, the number of dead individuals of the B. plicatilis increased and the mortality rate increased. Linear regression analysis of anthracene concentration and mortality at 24 hours of rotifers under normal culture conditions (Chen Jianhong et al., 2005). The regression equation is: \( Y = 5.450X - 12.871, R^2 = 0.976 \) where \( P < 0.01 \), half of the lethal concentration LC50 = 229.97 \( \mu g/L \).

![Figure 1](image_url)

**Fig 1** Effect of different concentrations of ANT on the dead rate of B. plicatilis individuals at 24 h

It can be seen from Figure 2 that the acute toxicity of anthracene to B. plicatilis has a significant concentration-effect relationship under UV-A and UV-B radiation conditions. The regression equation was obtained as follows: \( Y = 10.63X - 17.43, R^2 = 0.944 \) where \( P < 0.01 \), half of the regression concentration LC50 = 43.642 \( \mu g/L \).

![Figure 2](image_url)

**Fig 2** Effect of different concentrations of ANT on the death rate of B. plicatilis individuals under UV-A and UV-B radiation at 24 h
equation for the death rate and mortality of rotifers induced by UV radiation (Chen Jianhong et al., 2005) Lethal concentration LC50 = 43.64 μg/L.

![Graph showing death rate vs. concentration](image1)

**Fig 3** Effect of different concentrations of ANT on the death rate of *B. plicatilis* individuals under the UV-A radiation at 24 h

Figure 3 shows the effect of different concentrations of anthracene on the survival rate of *B. plicatilis* under UV-A induction. In combination with UV-A and UV-B, the lethal rate of anthracene to rotifers was dose-dependent only under UV-A radiation. According to the linear regression analysis of anthracene concentration and mortality of rotifers for 24 hours under UV-A irradiation conditions (Chen Jianhong et al., 2005), the regression equation was: \( Y = 4.17X - 8.33 \), \( R^2 = 0.921 \) where \( P < 0.01 \), the half lethal concentration LC50 = 58.71 μg/L.

![Graph showing death rate vs. concentration](image2)

**Fig 4** Effect of different concentrations of ANT on the death rate of *B. plicatilis* individuals under the UV-B radiation at 24 h
Under the condition of UV-B radiation, the mortality of *Brachionus plicatilis* increased with the increase of anthracene concentration (Fig. 4). Linear regression analysis was performed on 24 hours of death and mortality in rotifers under UV-B exposure (Chen et al., 2005). The regression equation was: $Y = 2.81X - 6.27$, $R^2 = 0.98$ where $P < 0.01$, the median lethal concentration LC50 = 170.42 μg/L.

### 3.1. Effects of anthracene on the morphological structure of *Brachionus plicatilis* induced by UV radiation

![Effect of ANT with different concentrations on B. plicatilis morphology under inverted microscopy at 24h. A: Blank control; B: treatment with 10 μg /L ANT under UV irradiance; C: treatment with 10 μg /L ANT.](image)

As shown in Figure 5, the morphological structure of the rotifers in the 10μg/L anthracene-treated group and the 10μg/L anthracene-treated group, which was induced by UV radiation in the control group, was observed under the inverted microscope for 24 hours. The results showed that in the control group, the rotifers were full and spherical, and the inside of the body cavity was enriched, and the internal tissues were clearly discernible. Compared with the normal individuals, anthracene stress had little effect on the microstructure of rotifers. Under the induction of UV radiation, the individual morphology of rotifers changed significantly under anthracnogenic stress, which showed that the rotifers were thin and trendy. And the internal blur, the color becomes darker; the organ tissue shrinks to the middle of its body, and the opacity increases.

### 3.2. Effects of UV radiation and anthracene on the total protein content (TSP) of *B. plicatilis*

![Effect of ANT on TSP content in *B. plicatilis* with or without UV radiation exposure. Note: * represents statistically significant differences between control and treatments at the $P < 0.05$ level.](image)
The effect of anthracene stress on TSP content in *B. plicatilis* was shown in Fig. 6. Compared with the control group, the total protein content of *B. plicatilis* decreased significantly at 24 h in the 4 μg/L anthracene treatment group, which was only 88.59% of the control group, and the difference was significant (P <0.05) and then rose to the control group level at day 48. When the UV radiation and 0.4μg/L anthracene stress, the content of TSP in *B. plicatilis* was induced at the 24th hour, which was about 1.11 times (P <0.05), and the TSP (P <0.05); In the UV radiation and high concentration anthracene compound treatment group, the content of TSP in *B. plicatilis* increased to the highest level in the initial stage, and reached the initial value of the control group (P <0.05) (P <0.05), and then decreased rapidly at 48 hours, only 74.56% of the control group (P <0.05). The results showed that the content of TSP in Cynodon dactylon was increased first and then decreased under the condition of UV radiation. The change of TSP content was related to anthracene concentration.

3.2.1. *Lipid Peroxidation of B. plicatilis by UV radiation and anthracene*

![Diagram](image)

**Fig 7** Effect of different concentrations on MDA levels affected in *B. plicatilis* with or without UV radiation exposure. Note: *represent statistically significant differences between control and treatments at the P<0.05 level.

According to Figure 7, it is not difficult to find that the changes of MDA level and the change of ROS content in *B. plicatilis* have similar trends. In the 4 μg/L anthracene treatment group, the MDA in the *B. plicatilis* increased significantly (P <0.05) at 24 h and then decreased to the control group at the 48th hour. When the UV radiation was applied, the content of MDA in the *B. plicatilis* increased gradually, which was about 2.29 times and 2.39 times higher than that of the control group at the initial stage of stress, 0.4 μg/L and 4 μg/L anthracene treatment. The content of MDA in the *B. plicatilis* increased (P <0.05). The results showed that UV radiation and anthracene complex induced more MDA in the *B. plicatilis*. Therefore, the anthrax stress under UV radiation increased the lipid peroxidation of *B. plicatilis* and caused more severe oxidative damage.
3.2.2. Effects of UV radiation and anthracene on SOD activity in *B. plicatilis*

The effect of anthracene stress on SOD activity in *Brachionus plicatilis* was shown in Figure 8. After 24 hours, the activity of SOD in *B. plicatilis* was significantly increased by 4 μg/L anthracene stress, which was 1.23 times of that of the control group, which was significantly different from that of the control group (P < 0.05), and then inhibited at 48th hour Higher than the control group (P <0.05). When the UV radiation was applied, the activities of SOD in the *B. plicatil* were significantly increased with the increase of the stress time, and then the inhibitory trend was significantly inhibited. The SOD activity of *B. plicatilis* in the anthracene-treated group was higher than that in the non-UV-irradiated anthracene-treated group, and the activity of SOD was inhibited at 48 hours, which was much higher than that in the non-UV-irradiated 4 μg/L anthracene Group. The results showed that the SOD activity induced by UV radiation decreased the SOD activity of Brachionus plicatili significantly more strongly.

3.2.3. Effects of UV radiation and anthracene on POD activity of *B. plicatilis*

The effect of anthracene stress on POD activity in *Brachionus plicatilis* was shown in Figure 9. After 24 hours, the activity of POD in *B. plicatilis* was significantly increased by 4 μg/L anthracene stress, which was 1.23 times of that of the control group, which was significantly different from that of the control group (P < 0.05), and then inhibited at 48th hour Higher than the control group (P <0.05). When the UV radiation was applied, the activities of POD in the *B. plicatil* were significantly increased with the increase of the stress time, and then the inhibitory trend was significantly inhibited. The POD activity of *B. plicatilis* in the anthracene-treated group was higher than that in the non-UV-irradiated anthracene-treated group, and the activity of POD was inhibited at 48 hours, which was much higher than that in the non-UV-irradiated 4 μg/L anthracene Group. The results showed that the POD activity induced by UV radiation decreased the POD activity of Brachionus plicatili significantly more strongly.
The effect of anthracene stress on POD activity in *B. plicatil* was shown in Figure 9. Compared with the control group, the POD of *C. dactylon* in 4 μg/L anthracene stress increased first and then decreased to the control group (P>0.05). The activity of POD in *B. plicatilis* was strongly induced at 24 hours in the two anthracene-treated groups induced by UV radiation, which were 1.24,1.33 for the initial values of the control group (P<0.05), and it was strongly inhibited at 48th day, only 74.56% and 62.69% of the control group (P<0.05). At 24 o, the POD activity of *B. plicatilis* in the 4 μg/L anthracene group induced by UV radiation was higher than that in the 4 μg/L anthracene group without UV, while the 48% inhibition of POD activity was much greater No UV radiation in the 4 μg/L anthracene group. The results showed that anthracene stress had stronger effect on POD activity under UV radiation.

3.2.4. Effects of UV radiation and anthracene on CAT activity of *B. plicatilis*

![Fig 10 Effect of different concentrations of ANT on CAT activities in *B. plicatilis* with or without UV radiation exposure. Note: * represent statistically significant differences between control and treatments at the *P*<0.05 level.](image)

Effects of UV and anthracene on CAT activity in *B. plicatil* were shown in Figure 10. The CAT activity of *B. plicatilis* did not change significantly with time in the 4 μg/L anthracene treatment group (P> 0.05). The activity of CAT in the *B. plicatilis* did not change significantly at 24 hours compared with the control group (P> 0.05), and the difference between the control group and the control group was not significant (P> 0.05). At the 48th hour, the activity was strongly inhibited, only 81.89% of the control group (P <0.05). In the UV radiation and 4 μg/L anthracene complex stress group, the CAT content of *B. plicatilis* was always strongly inhibited (P <0.05). The results showed that the activity of CAT was lower than that of anthracene in the anthracene-treated group, and the effect of anthracene on CAT activity was related to anthracene concentration.

4. Discussion

Pterodactylus is a zooplankton that lives in water for life. Exogenous poisons are introduced into the *B. plicatilis* by direct absorption. Therefore, exposure to acute toxicity test is an important aspect of the acute toxicity test of *B. plicatilis*. Through preliminary understanding of the characteristics and intensity of the action of exogenous pollutants on *B. plicatilis*, the target organ of the action can be judged, and its toxicity and potential risk are evaluated. In this study, it was found that anthracene had a significant effect on the survival of *B. plicatilis* in the case of UV radiation, and its effective
concentration range was far lower than the effective concentration range without UV radiation. It was concluded that anthracene had acute light-induced toxicity to B. plicatilis under UV conditions. The intensity of UV-A and UV-B radiation in this experiment is basically set according to the ratio of sunlight. Compared with UV-B radiation, UV-A radiation triggers anthracene to produce more intense phototoxicity due to UV-A is more likely to cause the photo-sensitized material to produce reactive oxygen species, which in turn causes damage to the organism [13, 14]. Under normal physiological conditions, the production and clearance of ROS in the living body maintain a dynamic equilibrium state. But in the external environment, may lead to metabolic disorders and suddenly produce a large number of ROS. When the ROS exceeds the antioxidant defense system clean-up capability, it will attack the polyunsaturated fatty acids of the B. rotundus biofilm, the formation of lipid peroxides, causing cell damage, which is an important aspect of the negative impact of ROS. Malondialdehyde (MDA) is one of the most common products, is an important indicator of the level of biological peroxidation. The degree of lipid peroxidation can be detected by measuring the content of MDA in the organism, and the degree of oxidative damage of the organism cells is reflected from the side. In this study, the accumulation of anthracene in rotifers can increase the content of MDA and absorb UV light after the anthracene can induce more ROS production, which led to a more significant increase in MDA levels, which means that more serious oxidative damage occurred. Therefore, the increase of ROS-induced membrane lipid peroxidation may be the main mechanism of the anthracene on the photoxicity of B. plicatilis [15].

Antioxidant defense system is a line of defense against ROS peroxidation in vivo, mainly including antioxidant enzymes and antioxidant small molecules. Therefore, it is helpful to study the process and mechanism of the activity of anthracene on the photoinduced toxicity of the anthracene to the B. plicatilis by the change of the enzyme activity in the anti-oxidative defense system after the UV radiation and no UV radiation. The elimination of superoxide anion radicals (O$_2^·$-) produced by the main catalyzed biodegradation of SOD is a kind of oxidoreductase formed by the combination of protein and metal ions. CAT is erythropoietin, which exists in biological cells that can breathe. It can be used with POD to remove SOD catalyzed O$_2^·$ product H$_2$O$_2$, and H$_2$O decomposition of water and oxygen, thus eliminating its oxidative toxicity to organisms. In this experiment, the activity of SOD in B. plicatilis in the single anthracene treatment group was inhibited before induction and the degree of induction and inhibition was much higher than that of UV radiation, which indicated that during the process, SOD In the anthracene induced by light induced ROS play an important role. CAT activity was not sensitive to the response of single anthracene stress compared with SOD, but the activity of CAT was significantly inhibited by UV radiation and anthracene complex. It is generally believed that low-toxic pollutants have an induced effect on SOD and CAT, which is the adaptive response of organisms to pollutants, mainly to enhance the ability of the body to eliminate reactive oxygen free radicals, to avoid damage to the body; high toxic pollutants against oxidation Inhibition of enzyme activity is the role of pollutants on the body has been more than the body's ability to adapt, the resulting reduction in enzyme activity is a precursor to the poisoning reaction.

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