Streptomycin Pharmaceutical Wastewater affects the T-AOC and CAT activity of Zebrafish

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Abstract. Streptomycin is increasingly being used to treat human diseases. Recently, its toxic effects on aquatic organisms have attracted global attention. The aim of this study was to investigate the toxic effects of streptomycin on zebrafish by measuring T-AOC and CAT activity. Zebrafish were exposed to four different volume concentrations of streptomycin wastewater and their T-AOC and CAT activities were measured at 3, 6, 9, 12 and 15 days. T-AOC activity shows an inverted "N" trend, and T-AOC activity reached the maximum (1.90U·mgprot\textsuperscript{-1}) on the 12th days. Furthermore, the CAT activity shows "M" tendency, and concentrations of streptomycin can affect CAT activity. Streptomycin wastewater induced oxidative stress in Zebrafish muscle tissue.

1. Introduction

With the development of industry, industrial wastewater has a serious threat to aquatic ecosystems and human health, especially antibiotic pharmaceutical wastewater\textsuperscript{[1]}. According to Wise, more than 200,000 tons of antibiotics are used worldwide each year\textsuperscript{[2]}. Therefore, large amount of antibiotic wastewater is discharged into the environment. The main way for antibiotics to enter the water environment is the discharge of antibiotic pharmaceutical wastewater. So antibiotic pharmaceutical wastewater is getting more and more attention.

Streptomycin is extracted from the culture solution of gram streptomycin bacteria and is an aminoglycoside basic compound. Streptomycin is part of the earliest antibiotics used in humans and is mainly used for anti-tuberculosis treatment\textsuperscript{[3]}. Streptomycin wastewater has a high COD concentration, complex composition, low carbon-nitrogen ratio, poor biodegradability, and contains many biochemical inhibitors such as formaldehyde and oxalic acid\textsuperscript{[4~6]}. Studies have shown that streptomycin wastewater has certain biological toxicity to koi\textsuperscript{[7]}, but there is no reaction toxicity mechanism from biomarkers. Therefore, the aquatic toxicity of streptomycin wastewater needs further research.

Fish is at a critical position in the aquatic life food chain. After oxidative damage, the fish body will produce reactive oxygen-free radicals to resist damage. The zebrafish is a small tropical fish that is sensitive to environmental changes\textsuperscript{[8~10]}. At present, the determination of wastewater toxicity mainly includes chemical methods and biological methods. The toxicity of chemical methods for detecting wastewater is complicated. The application of biological monitoring is fast, simple, and low in cost, and can fully reflect the ecological effects of pollutants in antibiotic wastewater. Therefore, biological monitoring is indispensable in determining the biological toxicity of wastewater\textsuperscript{[11]}. In this study,
Zebrafish was utilized to examine the acute toxicity of Streptomycin wastewater, and determine T-AOC activities and CAT activities by Zebrafish muscle tissue. The aims are to indicate the ecological risks of Streptomycin wastewater in the aquatic ecosystem.

2. Materials and Methods

2.1 Experimental Materials

2.1.1 Experimental Equipments and Instruments. Microplate reader (SpectraMax190) were purchased from Molecular Devices (Silicon Valley, USA). UV-visible light spectrophotometer (UV-2550) were purchased from SHIMADZU (Japan). Desktop high speed refrigerated centrifuge (2-16PK); High-purity water distiller (SYZ-A); Low temperature refrigerator (BBC-226STV); Constant temperature water bath (DK-S26); Analytical balance (EL204); Dissolved oxygen analyzer (JPBJ-608); water hardness meter (YD300); Vortex mixer (XW-80A); Acidity meter (FE20); Pipette (Pipet-Lite; TopPette Pipettor); Glass homogenizer; Filter; Bucket; Aeration pump. Other are laboratory basic equipment. The kit of T-AOC and CAT were purchased from Nanjing Jiancheng Bioengineering Institute.

2.1.2 Test Organisms. Zebrafish is one of the five fish experimental animals recognized by the International Organization for Standardization. Zebrafish were purchased from Hebei Medical University in Shijiazhuang. Before the experiment, it is necessary to strictly select the zebrafish of appropriate body length and weight. The length is controlled at 2.50±0.03 cm. The weight of the fish is controlled at 0.30±0.03 g. The zebrafish culture conditions are a temperature of 26 ± 1 °C, a 12 / 12 h – light / dark cycle, an oxygen saturation of more than 70%, and a pH range of 7.4 - 8.1.

2.1.3 Experimental wastewater. Streptomycin wastewater comes from the secondary sedimentation tank effluent of a pharmaceutical company. The Streptomycin wastewater sample is stored in a freezer at 0-4 ℃. Before the experiment, regular projects of Streptomycin wastewater were determined, and the data is shown on table 1.

| Item    | Data     | Methods                          |
|---------|----------|----------------------------------|
| COD     | 58 mg/L  | Microwave sealed digestion COD rapid detector |
| NH₃-N   | 5.66 mg/L| Nessler reagent - spectrophotometry |
| TOC     | 101.33 mg/L| Shimadzu TOC-Vcpn analyzer       |
| TN      | 35.46 mg/L| Shimadzu TOC-Vcpn analyzer       |
| pH      | 7.06     | Laboratory pH meter              |

2.2 Experimental Methods

2.2.1 Subacute toxicity test methods. The subacute toxicity test of streptomycin wastewater on zebrafish was carried out by using the equal ratio series method to set the concentration interval. Four exposure groups and one blank group were set in the experiment (0, 7.78%, 12.96%, 21.61% and 36.01%, respectively), and each group was set up with three parallels. The period is 15 d. The experiment was carried out by static experiment. The raw water of streptomycin wastewater was diluted with fully dechlorinated tap water to prepare experimental liquids with different volume concentrations. 4 L of the corresponding concentration of experimental liquid was added to the 5 L glass aquarium, and the zebrafish which were domesticated for more than two weeks in the laboratory were placed in each concentration group. T-AOC activities and CAT activities in muscle tissue were respectively determined at 3 d, 6 d, 9 d, 12 d and 15 d after exposure.
2.3 Assay Methods

2.3.1 Determination of total antioxidant capacity (T-AOC) activity. The measurement was carried out by taking 0.15 mL of the prepared 10% tissue sample, and the measurement method was carried out according to the instructions provided in the kit. The final solution was measured at 550 nm, and the T-AOC in the sample was calculated according to the formula (2-1).

\[
T-AOC \quad (mgprot \cdot mL^{-1}) = \frac{OD_2 - OD_1}{0.01} \times 30 \times \frac{L_2}{L_1} \div c
\]  
(2-1)

OD_2 is determination of OD value; OD_1 is control OD value; L_2 is total volume of reaction solution; L_1 is sample volume; c is sample protein concentration

2.3.2 Determination of catalase (CAT) activity. The measurement method was carried out according to the instructions provided in the kit, and the final solution was measured for OD value at 412 nm. The activity of CAT in the sample was calculated according to formula (2-2).

\[
CAT \quad (mgprot \cdot ml^{-1}) = (OD_1 - OD_2) \times 271 \times \frac{1}{60 \times L} \div c
\]  
(2-2)

OD_2 is determination of OD value; OD_1 is control OD value; L is sample volume; c is sample protein concentration

2.4 Statistical Analysis

The data obtained in the experiment were the average of 3 parallel groups, and the standard deviation was calculated. The statistical results were expressed as mean ± standard deviation (Mean ± SD). One-way Anova was performed on the data with SPSS 22.0 software. The least significant difference (LSD) method was used to analyze the significant difference between the groups in the same day. 0.01 < P < 0.05, indicating significant difference; P < 0.01, indicating that the difference is extremely significant.

All the images were drawn using the software Origin 8.5, and the effects of oxytetracycline wastewater on the indexes of T-AOC and CAT in zebrafish muscle tissue were obtained.

3. Results and Discussions

3.1 Effect of T-AOC activity in zebrafish muscle tissue

Figure 1. T-AOC activities in Zebrafish muscle tissue after 15 d exposure to streptomycin pharmaceutical wastewater. Values are presented as means ± SD. *p < 0.05 and **p < 0.01. As shown in Figure 1. There was no significant change in the control group during the experiment (0.398–0.951 U·mgprot⁻¹). On the 3th day, 7.78% and 36.01% showed significant differences (0.01 < P < 0.05). This may be caused by the stress of streptomycin wastewater, which causes the body to generate oxygen free radicals in the early stages of exposure. The body consumes its own substances (CAT, SOD, GSH, etc.) in order to remove oxygen free radicals, and the T-AOC activity decreases. In the experimental study of benzo(a)pyrene, it was found that the total antioxidant capacity of the liver increased with increasing concentration, but the difference was not significant[12]. On the 6th day, there...
was a significant difference of 7.78% (0.01 < P < 0.05), and 12.96% showed a significant difference (P < 0.01) compared with the control group. This may be because the body needs more antioxidant enzymes to prevent the induction of reactive oxygen species after a period of exposure. Streptomycin wastewater may also cause direct damage to the body. On the 9th day, the exposed group reaches a steady state. On the 12th day, it may be that the body is exposed to the streptomycin environment for a long time. A large amount of active oxygen free radicals are produced in the body, and the body increases the amount of antioxidant enzymes in order to eliminate the foreign products. The 21.61% and 36.01% exposure groups were significantly induced (P < 0.01), and the T-AOC activity reached the maximum during the experimental period (1.47 U·mgprot⁻¹, 1.90 U·mgprot⁻¹). On the 15th day, due to prolonged exposure, streptomycin causes certain oxidative damage to the body, preventing the body from producing normal antioxidant enzymes to resist the external environment.

### 3.2 Effect of CAT activity in zebrafish muscle tissue

![Figure 2. CAT activities in Zebrafish muscle tissue after 15d exposure to streptomycin pharmaceutical wastewater. Values are presented as means ± SD. *p < 0.05 and **p < 0.01.](image)

Figure 2 shows that there has been a “M” trend during exposure. In addition, the CAT activity is significantly changed with the increase of the volume concentration, and the higher the concentration, the more obvious the stress. On the 3th day, 12.96% and 21.61% showed significant difference (0.01 < P < 0.05), 36.01% showed extremely significant difference (P < 0.01), which may be the entry of foreign substances (streptomycin wastewater), and the body produced oxidative stress. By consuming CAT, H₂O₂ can be decomposed into oxygen (O₂) and water (H₂O), thereby scavenging oxygen free radicals in the body and protecting the body from peroxygen free radicals. Studies have shown that it is known as the first line of defense against external stress with SOD. On the 6th day, CAT can resist the external environment and return to normal level. CAT catalyzes the reaction of H₂O₂ in dynamic equilibrium. On the 9th day, Significant inhibition was observed in the 36.01% exposed group (P < 0.01). The CAT activity was the minimum value during the experiment (1.04 U·mgprot⁻¹), and the inhibition rate was 62.7%. This may be because the streptomycin wastewater has a greater impact on the muscle tissue of the fish. The body produces antioxidant enzymes such as CAT and SOD to eliminate active oxygen free radicals, resulting in decreased CAT activity. On the 12th day, The low-exposure group (7.78%) was significantly induced (0.01 < P < 0.05), reaching the maximum during the experimental period (3.681.04 U·mgprot⁻¹). On the 12th day, The CAT activity of each exposed group was lower than that of the control group, and the significant exposure was observed in 36.01% of the exposed group (P < 0.01). It indicated that a large number of cells in the body were damaged, and the accumulation of active oxygen exceeded the exposure time. The limits of this have caused damage to its biofilm and enzyme system, resulting in a decrease in CAT activity.

### 4. Conclusion

In this study, streptomycin wastewater was used for subacute toxicity test. In different streptomycin wastewater volume concentrations, T-AOC activity in four exposed groups showed a trend of "inhibition-induction-inhibition" over time. On the 15th day, T-AOC enzyme activity was significantly
inhibited (P < 0.01). CAT activity is "M" type change during the experiment. As the volume concentration of streptomycin wastewater increases, the stress of CAT activity is more obvious. Therefore, streptomycin wastewater has certain toxic effects on zebrafish.

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