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# Ecological risk study on subacute toxicology experiment of streptomycin wastewater for Zebrafish

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**Abstract.** An exposure experiment was conducted to study the effect of different volume fraction of effluent streptomycin wastewater on the activity of the peroxidase (POD) activity and the malondialdehyde (MDA) content in muscles of Zebrafish for 20 days. The results show that POD activity is significantly induced on the eighth day. POD activities in the muscles of Zebrafish exposed to the streptomycin wastewater of 20% exposure group were significantly different ( $0.01 < P < 0.05$ ), and others were very significantly different ( $P < 0.01$ ). The POD activity of the other was almost equal to that of the control group. The MDA content was the effect of induction-inhibition-induction. The results show that as the value of volume fraction of the streptomycin wastewater increase, the Integrated Biomarker Responses (IBR) increase at first and then decrease. The IBR value of Zebrafish that exposed streptomycin wastewater ranged from 10.42 to 54.26, and the wastewater of 10% exposure group induced the maximum value on the twelve day. The study indicates that low concentration streptomycin wastewater has impacts on the antioxidant defense system and antioxidant ability of Zebrafish.

## 1. Introduction

Streptomycin is an antibiotic extracted from the culture medium of streptomycin, which is an aminoglycoside basic compound. It is one of the earliest antibiotics used by humans, and it has been the main drug in anti-tuberculosis treatment[1,2]. It is estimated that the worldwide antibiotic consumption varies from 100,000 and 200,000 t per year[3]. Streptomycin production process is divided into fermentation, extraction and purification process, which produced a complex wastewater, with a large number of organic matter, high sulfate concentration[4,5]. Some of these are very difficult removed by the wastewater treatment plants and cannot be completely removed, resulting in the sustained discharge of toxins to the aquatic environment[6].

Fish have been extensively used as toxicity test organisms[7-10]. Zebrafish is a small tropical aquarium fish with short generation cycle and high reproductivity, and it is inexpensive and easy to care for in laboratory[7-10]. Zebrafish has been widely utilized in environmental toxicology, pathological toxicology and pharmaceutical toxicology research, and so on. So it is a small ideal fish model that has been widely used to test the toxicity of wastewaters and some chemical[7-10].



The aim of this work was to study the relationship between peroxidase (POD) and malondialdehyde (MDA) content in muscles of Zebrafish in the presence of effluent streptomycin wastewater. Getting the degree of damage to the fish was obtained by subacute toxicology experiment of streptomycin wastewater, better assessing what happens in ecological risk.

## 2. Materials and methods

### 2.1. Experimental Materials

*2.1.1. Instruments and reagents.* UV-visible light spectrophotometer (UV-2550); high speed centrifuge (TG16-WS); hypothermia refrigerator (BBC-226STV); High purity water distiller (SYZ-A). Protein kits, MDA and POD were purchased from a biological engineering research institute.

*2.1.2. Test animals.* Zebrafish were obtained from aquafarm in Shijiazhuang. In this experiment, mean length of fish used was  $34.0 \pm 0.3$  mm. Mean weight of fish used in this experiment was  $0.24 \pm 0.03$  g. Before entering the laboratory, disinfection treatment employ 5% salt water, and then domesticate a week in 48h of aeration tap water. Feeding was halted 7d prior to testing. Healthy individuals of fish with similar size and weight were employed for test.

### 2.2. Experimental Methods

*2.2.1. Preliminary experiments.* Health Zebrafish were taken in 100% streptomycin wastewater (water volume concentration percentage), and no death within 96 h. Subacute experimental set up six experimental groups (0, 10%, 20%, 30%, 40%, and 50%, respectively). Experiments measure effect of different volume fraction effluent streptomycin wastewater on the oxidation resistance in Zebrafish muscle tissue in different periods.

*2.2.2. Exposure experiment.* The subacute toxicity experiments lasted 20d. Each group set up two parallel, each group randomly put in 15 fish. The experiment uses the static displacement method and change the water every day. Besides, experiment assay on 4, 8, 12, 16, and 20d. Experiments measure effect of different volume fraction streptomycin wastewater on the MDA contents and POD activities in Zebrafish muscle tissue in different periods.

### 2.3. Assay Methods

Anatomy, taking 0.2g muscle tissue rinse in physiological saline, blot moisture, weighing. According to the volume ratio of 1:9 join physiological saline, grinding 6-8min in the ice water bath, fully grinding. Take 10% of the organization, under the condition of 3500 r per min centrifuge for 10 min. The supernatant was collected for the experiment. Activities of Peroxidase (POD) and Malondialdehyde (MDA) content in samples were estimated by the method of POD and MDA kit.

### 2.4. Statistical Analysis

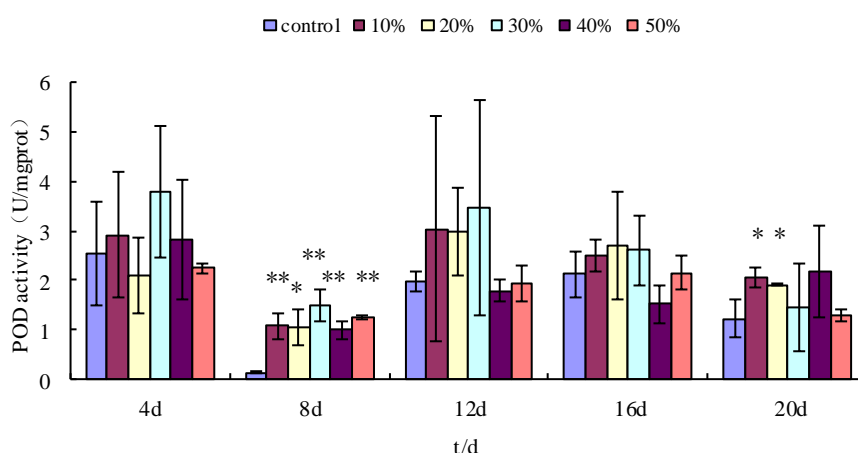
All data were deal with using Excel. Significant difference analysis uses the method of least significant difference (LSD). It analyzes the data in the group on the same day. P value of  $<0.05$  was considered statistically different,  $0.01 < P < 0.05$  statistically significantly different, and  $p < 0.01$  statistically very significantly different.

## 3. Results and Discussions

### 3.1. The effect of POD activities and MDA content in muscles of Zebrafish

As shown in Fig.1, the POD activity in the groups exposed to 10%, 30% and 40% was slightly different from that of the control group, when Zebrafish were exposed to streptomycin wastewater for 4 days. When the experiment was carried out to eighth days, the POD activity was higher than the

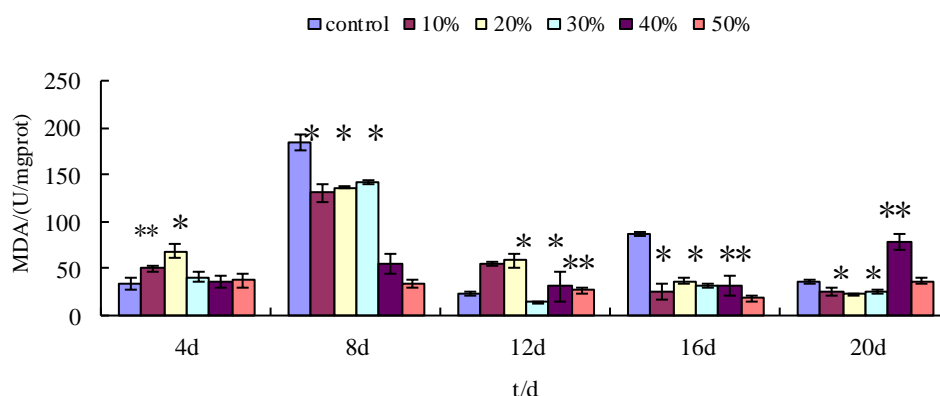
control group, and all groups of the enzyme activity. The POD activity of 10%, 30%, 40%, and 50% exposure group was very significantly different ( $P < 0.01$ ). The POD activity of 20% exposure group was significantly different ( $0.01 < P < 0.05$ ). On the twelfth day, the POD activity in the 10%, 20% and 30% exposure groups was higher than that in the control group. While the POD activity in the 40% and 50% exposure group was not significantly different from that in the control group. On the Sixteenth day, the activities of POD in the group of 10%, 20%, 30% and 50% were slightly induced, which was higher than that of the control group, and the POD activity of the 40% groups was slightly inhibited, which was slightly lower than that of the control group. On the twentieth day, the activity of enzyme in the exposed group was higher than that in the control group, and the activity of POD was significantly increased in the 10% and the 20% groups ( $0.01 < P < 0.05$ ). The POD activity in muscles of Zebrafish was any less sensitive, and it was in consistent with the research of Li [11]



**Figure 1.** POD activity in muscular tissue of Zebrafish exposed to streptomycin wastewater

As shown in Fig.2, the MDA content in the groups exposed to 10% was slightly different from that of the control group, when Zebrafish were exposed to streptomycin wastewater for 4 days. The MDA content of 10% exposure group was significantly different ( $0.01 < P < 0.05$ ), and the MDA content was very significantly different in the 20% groups ( $P < 0.01$ ). On the eighth day, the MDA content of each group was very significantly inhibited ( $P < 0.01$ ), and the content of enzyme was lower than that of the control group. On the twelfth day, the MDA of 10%, 20%, 40% and 50% exposure group was higher than the control group, and the MDA content of 10% and 20% exposure groups was very significantly different ( $P < 0.01$ ). The MDA content of 30% exposure group was very significantly different ( $P < 0.01$ ). On the sixteenth day, the MDA content of each group was very significantly different ( $P < 0.01$ ). On the twenty day, the MDA content in the 10%, 20% and 30% exposure groups was lower than that in the control group. And the MDA of 10% exposure group was significantly different ( $0.01 < P < 0.05$ ). The MDA content of 20% and 30% exposure groups was very significantly different ( $P < 0.01$ ). The MDA of 40% and 50% exposure groups was higher than the control group, of which 40% exposure group were very significantly different ( $P < 0.01$ ). The MDA content showed an obvious relationship between dose and response, and it was in consistent with the research of Shen [12]

In general, no significant relationship between dose and response was found in POD. But MDA content shows a significant relationship with dose and dose-time. So the MDA content can be applied as potential biomarkers for evaluating toxicological effect of streptomycin wastewater on Zebrafish. In another study, Liang[13] et al. showed that the MDA content was the more sensitive measured parameters and maybe used as potential biomarker for the antibiotic NFLX (norfloxacin) to swordtail fish (*Xiphophorus helleri*).



**Figure 2.** MDA contents in muscular tissue of Zebrafish exposed to streptomycin wastewater

### 3.2. Integrated Biomarker Responses (IBR) value

According to the IBR method, the integrated biomarker index of Zebrafish exposed to streptomycin wastewater was calculated in table 2. The results show that as the value of volume fraction of the streptomycin wastewater increase, the Integrated Biomarker Responses (IBR) increase at first and then decrease. The greater the value IBR indicates that the greater impact of pollutants on organisms, the greater environmental risks of its existence. It is indicating that the eighth day effect of streptomycin wastewater effluent on Zebrafish is larger at a high level, and its influence has also been in the process of changing. Some environmental pollutants can enter the organism. The metabolic activity in the body can metabolize and degrade these pollutants. And the IBR value of 10% exposure group is bigger than other exposure group on the transverse comparison. It shows that this phenomenon is more remarkable of low exposure group, which maybe related to the biological markers selected in the experiment. And the relative sensitivity of biomarkers to pollutants can affect the IBR value.

Table 1 the Integrated Biomarker Responses of Zebrafish exposure to streptomycin wastewater

	10%	20%	30%	40%	50%
4d	31.95	17.18	10.42	10.53	20.09
8d	41.34	34.42	33.22	34.72	37.68
12d	54.26	23.34	15.90	13.90	17.99
16d	23.28	12.76	14.09	19.00	21.72
20d	20.78	13.43	10.96	13.83	15.42

## 4. Summary

1) This paper exposes Zebrafish in streptomycin wastewater with 0, 10%, 20%, 30%, 40% and 50% volume percent for 20 days. The POD activities in muscles of Zebrafish exposed to the Streptomycin wastewater of 20% exposure group were significantly induced, and others were very significantly different. The POD activity is significantly induced on the eighth day, and the other day is equal to the control group. The MDA content in the muscles of Zebrafish exposed in the streptomycin wastewater was significantly induced. The MDA content was the effect of induction-inhibition-induction. The MDA content shows a significant relationship with dose and dose-time. So the MDA content can be used as a biomarker of Streptomycin wastewater stress.

2) In general, no significant relationship between dose and response was found in POD. But MDA content shows a significant relationship with dose and dose-time. So the MDA content can be applied as potential biomarkers for evaluating toxicological effect of streptomycin wastewater on Zebrafish. The IBR value of Zebrafish that exposed streptomycin wastewater ranged from 10.42 to 54.26, and the

wastewater of 10% concentration induced the maximum value on the twelve day. Streptomycin wastewater discharged into the environment has an ecological risk impact on Zebrafish.

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