OXIDATIVE DAMAGE AND ANTIOXIDANT FUNCTION OF SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) AND FLOWER CARP (*HYPOPHTHALMICHTHYS NOBILIS*) EXPOSED TO MANGANESE

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Abstract. Accurate analysis of the oxidative damage and antioxidant function of heavy metals on aquatic organisms has become increasingly important. To analyze the impact of Mn exposure on the antioxidant capacity and oxidative loss of silver carp and flower carp, the study measured and assessed the oxidative damage, antioxidant effects, and toxic effects of manganese on silver carp (*Hypophthalmichthys molitrix*) and flower carp (*Hypophthalmichthys nobilis*) from the aspects of total antioxidant capacity, catalase activity, acetylcholinesterase activity, malondialdehyde content, tissue protein content. It was found that the average mortality rate of silver carp and flower carp increased rapidly with the rise of Mn concentration in the water, until the average mortality rate reached 100% at 600 mg/L and 750 mg/L concentrations, respectively. The activities of catalase and acetylcholinesterase in the liver, gills, digestive tract, and kidneys of silver carp and flower carp were inversely proportional to the concentration of manganese in the water. The content of malondialdehyde in the liver, gills, digestive tract, and kidneys of silver carp and flower carp increased with the rise of Mn concentration. The protein content in the liver and digestive tract of silver carp and flower carp decreased with increasing Mn concentration. This result indicated that Mn exposure had a strong negative impact on the oxidative loss and antioxidant function of silver carp and flower carp, which can provide theoretical basis for research in aquaculture and other fields.

Keywords: *manganese toxicity, oxidative stress, catalase, acetylcholinesterase, malondialdehyde*

Introduction

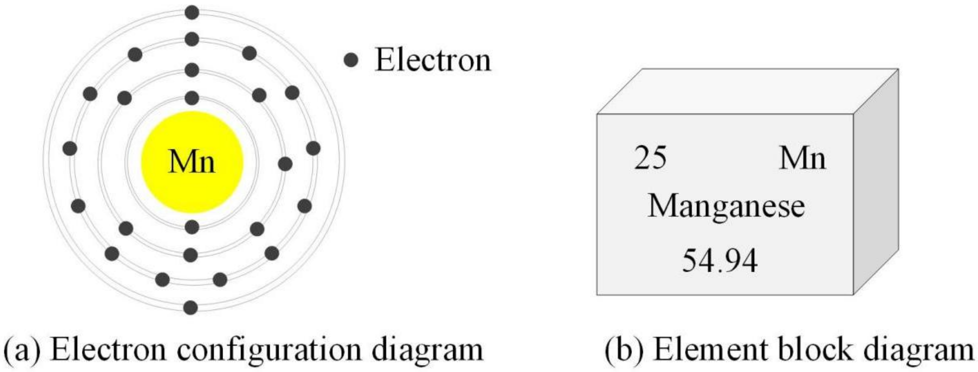
With the swift advancement of industrial manufacturing, the uncontrolled discharge of a large amounts of industrial wastewater has led to increasingly serious water pollution (Wu et al., 2022). Mn (*manganese*) in industrial wastewater is an essential trace element for living organisms (Li et al., 2023). Within a certain concentration range, Mn can become a harmful pollutant (Uddin et al., 2022). Fish are one of the most common and ubiquitous aquatic organisms. Mn is difficult to decompose in fish, accumulates in their tissues and organs over a long time. The accumulation and transmission in its ecosystem will have a negative impact on the entire ecosystem, including reducing water quality and affecting the survival and reproduction of aquatic organisms (Shaheen et al., 2025). Therefore, studying the effects of Mn on fish has become one of the key areas of focus in ecological pathology (Xu et al., 2022). Understanding the toxic effects of Mn exposure (Mn-EXP) on fish is of great significance for protecting the ecological environment and aquaculture. Many scholars have conducted relevant research, such as Ehiemere et al. (2022), to evaluate the pollution level of heavy metals (HMs) such as Cd, Cr, Cu, Mn, Pb, and Zn on fish ponds and the related human health exposure risks of these HMs through consumption of fish, the samples were digested with aqua regia, and the metal concentration was measured using an air acetylene flame atomic absorption spectrophotometer. The analysis outcomes indicated that the pollution level of HMs in water to ponds was very high, but the hazard index to human health was less than 1. For example, in response to the highly overlooked toxic effects and mechanisms of excessive Mn on vertebrates, Zhao et al. (2022) added Mn to the feed and analyzed its effects on yellow catfish through various experimental methods. The outcomes indicated that excessive Mn not only induced mitochondrial dysfunction in yellow catfish, but also induced hepatic lipotoxicity. For example, Abdel-Halim et al. (2022) studied the accumulation patterns of HMs in tilapia and catfish collected from four branches of the Nile River. In the study, inductively coupled plasma optical emission spectrometer was employed to detect seven metals including zinc, cadmium, lead, iron, nickel, copper, and Mn. The outcomes indicated that tilapia had a low Mn bioaccumulation factor, but cadmium had the highest. These metal accumulations may have adverse effects on the physiological status of fish and increase their risk of infection, which may have adverse effects on consumer health. Abbas et al. (2022) evaluated the level of HM pollution in two fish ponds in Egypt and its potential impact on farmed fish and human health. By detecting the concentration of HMs in water, sediment, and fish tissues, it was found that fish tissues in severely polluted ponds had higher levels of HMs, and consuming fish muscle from these ponds may pose a cadmium carcinogenic risk to children. Mehnaz et al. (2022) evaluated the potential impact of industrial wastewater on fish and human health by detecting the HM content in wastewater with different concentration ratios and their effects on the health of climbing perch and humans. The results indicated that as the concentration of wastewater rose, water quality parameters decreased, and the HM pollution index showed that chromium, Mn, and iron content led to higher levels of water pollution. The concentration and exposure time of wastewater had a significant impact on fish mortality, color changes, mucus secretion, and HM accumulation, and posed a higher risk of cancer in children.

The above relevant research results indicate that there are several key knowledge gaps in the current research. Firstly, although existing studies have focused on the effects of manganese exposure on fish, the understanding of how it specifically affects the oxidative damage and antioxidant function mechanisms of fish is still insufficient. Secondly, the differences in the responses of different fish species to manganese have not been fully studied, which limits our comprehensive understanding of the ecological impact of manganese pollution. In addition, the long-term ecological impacts of manganese pollution, including its cumulative effects in ecosystems and potential ecotoxicological effects, also require more in-depth exploration. Finally, research on the biological detection and bioreremediation potential of manganese pollution is also relatively scarce, which is crucial for the development of effective pollution management and remediation strategies. Therefore, in response to the above knowledge gap, the research adopts static exposure methods. To analyze the effects of manganese on oxidative damage and antioxidant function of silver carp (*Hypophthalmichthys molitrix*) and flower carp (*Hypophthalmichthys nobilis*). silver carp and flower carp are ideal models for studying the impact of manganese pollution because they are the most widely used in freshwater fish aquaculture and have a high sensitivity to environmental changes. By evaluating the effects of different concentrations of manganese on the physiological indicators of fish, this study will provide new insights into the impact of manganese pollution on aquatic organisms and offer a theoretical basis for the biological detection and remediation of manganese pollution in water bodies. The innovation of this study lies in the use of static exposure methods to determine the toxic effects of manganese on these two types of fish, which will provide a scientific basis for the assessment and management of manganese pollution.

Materials and methods

Characteristics and toxicity mechanism of Mn

Mn is a chemical element with the element symbol Mn. Its elemental form is a gray white, hard, brittle, and glossy transition metal, with a content greater than 99% in nature (Chao et al., 2023). The atomic structure and elemental block diagram of Mn are shown in *Figure 1* (Mao et al., 2023; Ahmadpour et al., 2023).



(a) Electron configuration diagram (b) Element block diagram

Figure 1. *Block diagram of Mn atomic structure and elements*

From *Figure 1(a)*, Mn is composed of 25 electrons and one atomic nucleus, with electrons distributed on different orbitals according to their energy. *Figure 1(b)* provides the information of Mn in the periodic table. Its atomic number is 25, element symbol is Mn, element name is Mn, and relative atomic mass is 54.94. The physical properties of Mn include a density of 21g/cm³ at 20 °C, a melting point of up to 1244 °C, a boiling point of 2095 °C, and a resistivity of 185×10^{-8} Ω at 25 °C▪m (Kącki et al., 2022). The chemical properties of Mn are that it is a relatively active element that is easily combustible in oxygen, easily oxidized in air, and prone to chemical reactions with many other elements and compounds (Qian et al., 2022). Mn exists in various oxidation states such as+2,+3,+4,+6, and+7 in nature, among which+2 and+4 titanium oxides are the most common (Chai et al., 2022). Due to these unique chemical and physical properties, Mn is widely used in industrial fields, chemical formulations, and metallurgy (Kwan et al., 2023). In addition, there are extremely trace amounts of Mn in the bones, liver, kidneys, pancreas, and other tissues of living organisms, which are considered an essential nutrient. Some global health organizations recommend daily intake of appropriate amounts of Mn to ensure good health (Wenaas, 2025). As in the "Guidelines for Drinking Water Quality (4th Edition, 2017)" of the World Health Organization, the provisional guideline value of manganese is 0.4 mg L-1. Long-term intake of drinking water above this level may have neurological effects. Excessive intake of Mncan cause damage to the body of organisms. The impact of Mn on fish and humans is shown in *Figure 2* (Qiu et al., 2022; Guo, 2025).

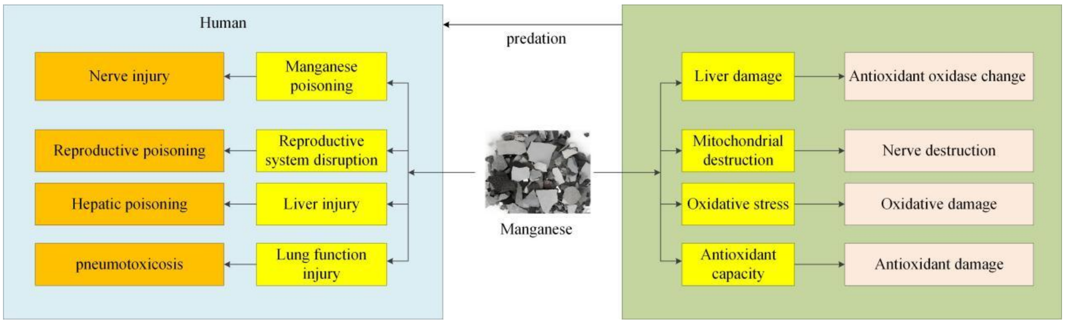


Figure 2. *Effects of Mn on fish and humans*

From *Figure 2*, on the one hand, Mn has negative effects on the liver, mitochondria, oxidative stress, and antioxidant capacity of fish. On the other hand, Mn causes damage to the human nervous system, liver, lung function, and reproductive system. With the improper discharge of industrial wastewater, a large amount of Mn is ingested by fish and accumulates in their bodies. After humans prey on these fish, Mn may cause damage to the body, thereby damaging human health. Therefore, Mn-EXP is crucial for protecting the environment and public health by mitigating oxidative damage and antioxidant effects in fish. In view of this, the study uses malondialdehyde assay, total antioxidant capacity assay, and catalase assay to analyze the effects of Mn-EXP on oxidative damage and antioxidant activity in fish. Acetylcholinesterase assay and tissue protein content assay methods were employed to analyze the toxic effects of Mn on fish and the changes in protein in fish tissues. The calculation expression for the determination method of malondialdehyde is shown in equation (1) (Panwar et al., 2022).

$$MDA = ((OD - AD)/(CD - DD)) \times A / B \tag{Eq.1}$$

In *Equation (1)*, MDA represents the content of malondialdehyde in the tissue in nmol/mg protein (NMP), OD represents the measured optical density value, AD represents the absorbance of the control sample without it, CD and DD represents the absorbance of the standard sample at a known concentration under the same conditions and the absorbance of the blank control of the standard sample. A represents the concentration of the standard sample in 10 nmol/mL, and B represents the protein concentration of the sample to be measured in mg protein/mL. The calculation expression for total antioxidant capacity is shown in *Equation (2)* (Hasnul Hadi et al., 2024).

$$TAOC = ((E - F)/0.01)/30 \times D / C \times G / H \tag{Eq.2}$$

In *Equation (2)*, $TAOC$ is the total antioxidant capacity in U/mg protein, E is the absorbance value of the reaction tube containing the test sample at a specific wavelength, F is the absorbance value of the control tube without the test sample measured under the same conditions, D is the total volume of the reaction solution in mL, C is the sampling amount in mL, G is the dilution factor of the sample before testing, H is the protein content in mg protein/mL. The expression for calculating the activity in catalase activity testing is shown in *Equation (3)* (Esfahani et al., 2023).

$$CAT = (AD - OD) \times 271 \times (1/(60 \times C)) / H \tag{Eq.3}$$

The expression for calculating the protein concentration in the tissue sample waiting for measurement is shown in *Equation (4)* (Pan et al., 2023).

$$B = ((OD - DD)/(CD - DD))) \times K \qquad \text{(Eq.4)}$$

In *Equation (4)*, K is the concentration of the standard substance, with a value of 0.563mg/mL.

Materials and methods

Experimental materials

After analyzing the characteristics of Mn and its effects on fish, the study used the following experimental materials to analyze in more detail the effects of Mn on oxidative loss and antioxidant function in fish, as shown in *Table 1.*

Table 1. *Experimental materials and details*

Items	Detailed information
Experimental object	Silver carp and flower carp larvae
Source	Hunan Liushahe flower pig ecological animal husbandry Co., LTD. Shu Long farm
Domestication condition	The water used for the experiment was aerated tap water with a hardness of 105.1 milligrams per liter of calcium carbonate equivalent, the water temperature was kept constant at 26.5°C, allowing a fluctuation range of 0.5 degrees Celsius, and the dissolved oxygen content of the water was more than 6 milligrams per liter. The ratio of light and dark time is 13 hours of light to 11 hours of darkness.
Change the water	Change half the water every day at 17:00
Feed	During the domestication period, food was fed twice a day, and feces and food residues were cleaned up after they were full. The domestication lasted for 30 days
Experimental reagent	Mn chloride (analytical purity >99.0%, Chengdu Jinshan Chemical Reagent Co., LTD.)
Assay kit source	Thermo Fisher Scientific

From *Table 1*, the experimental materials included flower carp and white carp fry from Shulong Farm of Liushahe River Flower Pig Ecological Breeding Co., Ltd. in Hunan Province. The experimental water used was aerated tap water with a hardness of 105.1 mg/L, a water temperature of 26.5 ℃, a dissolved oxygen content greater than 6 mg/L, and a light cycle of 13 hours with light and 11 hours of darkness. Half of the water was changed every afternoon at 5 o'clock. In terms of feeding, during the domestication period, it was fed twice a day for 30 days. The experimental reagent was Mn chloride with an analytical purity greater than 99.0% provided by Chengdu Jinshan Chemical Reagent Co., Ltd. The determination kit was provided by Thermo Fisher Scientific.

Experimental methods

After the experimental materials were prepared, the study simulated the situation of fish exposed to Mn and Mn, and observed and analyzed the effects of Mn-EXP on fish through simulation experiments. The experimental process is illustrated in *Figure 3*.

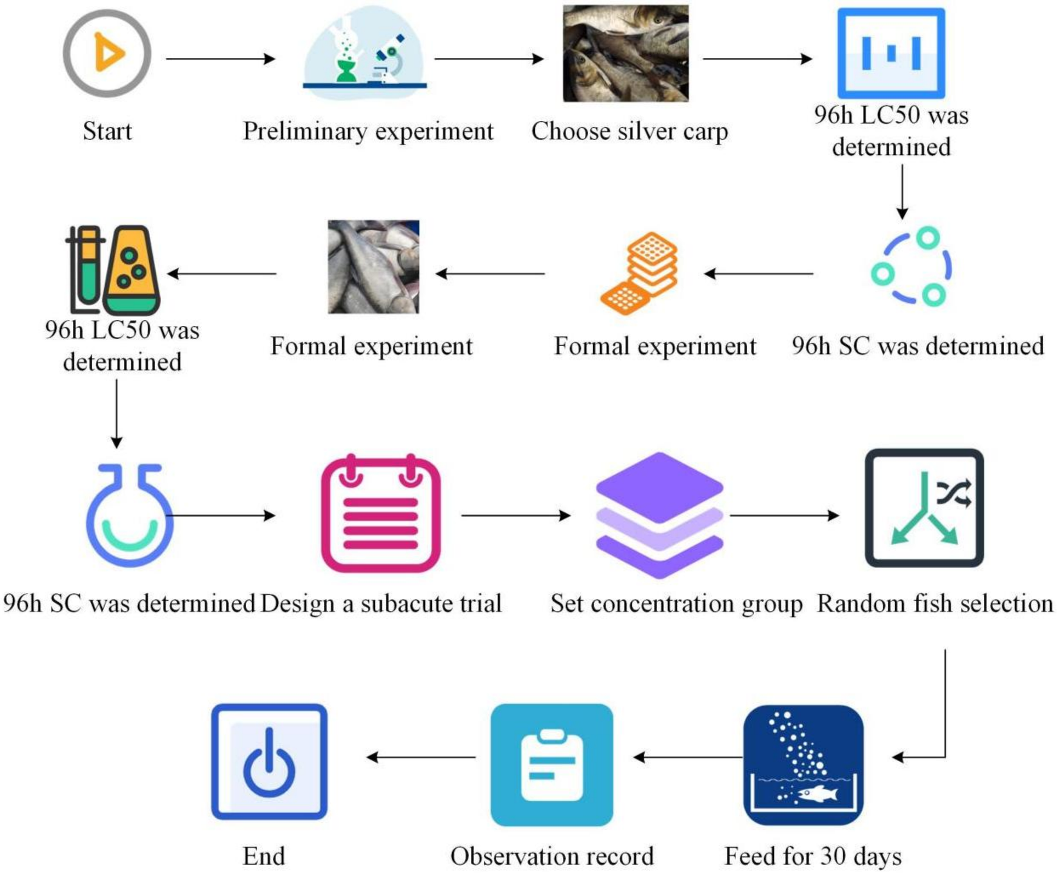


***Figure 3.** Experimental process*

In *Figure 3*, LC50 is the semi lethal concentration and SC is the safe concentration. The experiment first conducted a pre experiment on silver carp, selecting silver carp with a body weight of 21.5 ± 0.5 grams and a body length of 18.5 ± 0.5 centimeters. The lethal concentration range of the chemical substance was determined through a 96-hour exposure period, which was 270 to 800 mg/L. And the lethal concentration was determined to be 270 mg/L to 800 mg/L through a 30-day chronic exposure experiment. Based on this, the LC50 and SC50 of silver carp were calculated to be 539.98 and 53.98 mg/L. Subsequently, based on these data, a formal experiment was designed, in which different concentrations of chemicals ranging from 300 mg/L to 800 mg/L were used, and three experimental groups were divided, each containing 10 silver carp fish. Next, a similar pre experiment was conducted on flower carp, selecting flower carp with a body weight of 21.5 ± 0.5 grams and a body length of 17.5 ± 0.5 centimeters. The lethal concentration range of the chemical substance was determined through a 96-hour exposure period, from 330 mg/L to 750 mg/L. The LC50 and SC50 of flower carp were calculated to be 555.21 and 57.73 mg/L. Based on these data, a formal experiment was designed, in which different concentrations of chemicals such as 30, 60, 120, and 240 mg/L were used, with 10 flower carp in each group and a control group (CG). In the formal experiment, 10 fish were chosen at random from each group and a parallel group was set up to ensure the reproducibility and reliability of the experiment. Moreover, in the formal experiment, the control group was not exposed to manganese, while the experimental group was exposed to different concentrations of manganese. Each group contains 10 fish and three repeated experiments are conducted to ensure the reliability and repeatability of the data. During the experiment, environmental factors such as water temperature, pH value and dissolved oxygen were controlled to simulate natural conditions and ensure the consistency of the experiment. Data analysis was conducted using analysis of variance and Tukey's HSD multiple comparison tests to determine the significant differences among different concentration treatment groups. The significance level was set at $p<0.05$. During the experiment, the fish are fed twice a day, the experimental setup is cleaned regularly, the cleanliness of the experimental environment is maintained, and a 30-day exposure experiment is conducted. Finally, the experiment is completed and data analysis is carried out to evaluate the acute toxicity effects of different concentrations of chemicals on fish. Each experiment adopts an independent water circulation system to ensure that there is no water crossing between water tanks and avoid systematic errors. During the experiment, the water and fish bodies in each tank were not mixed, which met the "true repetition" standard. Furthermore, in the acute experiment, three parallel groups of silver carp and flower carp were set up each, with 10 fish in each group. A total of 6 concentrations + 1 control group = 7 groups × 3 groups = 21 parallel groups. In the subacute experiment, 4 concentrations of silver carp and 4 concentrations of flower carp were set up respectively, plus 1 control group = 5 groups. Each group consists of 10 fish, and each group is further divided into one parallel group. This experimental plan has been approved by the local Laboratory Animal ethics committee and is strictly implemented in accordance with the "Regulations on the Administration of Laboratory Animals in China" and the "Guidelines for the Welfare and Ethics Review of Laboratory Animals".

Sample extraction and processing

After the experimental method design is completed, samples need to be collected and processed. The process from sample collection to sample processing is shown in *Figure 4*.

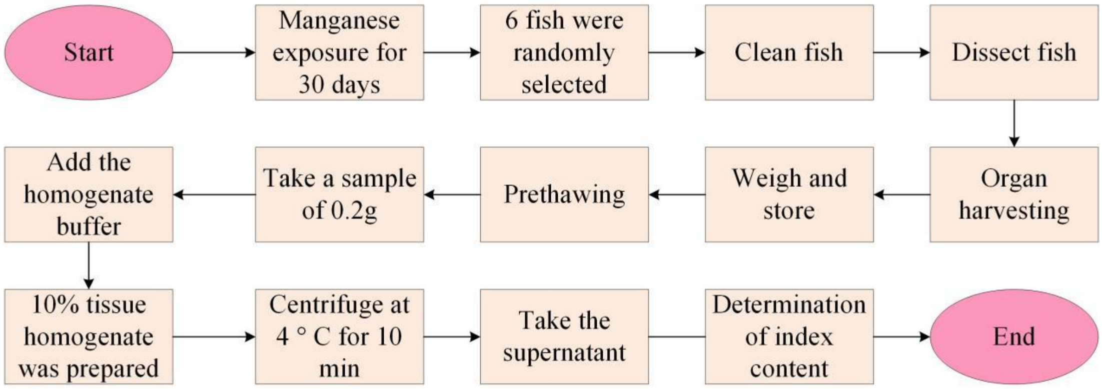


Figure 4. *Process from sample collection to sample processing*

From *Figure 4*, during the subacute toxicity test, the silver carp and flower carp were first allowed to live in an environment with Mn concentration for 30 days. Afterwards, 6 fish were chosen at random from each concentration group, and their surface water and mucus were washed away with deionized water. The fish were then placed on ice for dissection to quickly remove internal organs. Subsequently, weigh the organs and place them in a -60 °C freezer for later use. And thaw the frozen organs. After thawing, take

0.2 grams of tissue and place it in a new centrifuge tube. Then, at a weight to volume ratio of 1:9, physiological saline cooled with ice cubes was added, and the sample was homogenized into a 10% concentration tissue homogenate on ice using a tissue grinder. Finally, centrifuge the homogenate for 12 minutes and collect the clear liquid layer. Finally, the total antioxidant capacity, catalase activity, acetylcholinesterase activity, malondialdehyde content, and tissue protein content were measured using a clear liquid layer, and the experiment was completed and data analysis was conducted. Catalase is an important antioxidant enzyme in fish, mainly responsible for decomposing hydrogen peroxide into water and oxygen, thereby reducing the accumulation of hydrogen peroxide. Hydrogen peroxide is a reactive oxygen species. When it accumulates in excess within cells, it can cause oxidative damage. The increase in catalase activity indicates that fish are enhancing their antioxidant defense mechanisms to cope with oxidative stress caused by manganese exposure. This increase in activity helps to reduce oxidative damage and protect cells from harm. Malondialdehyde is a product of lipid peroxidation, and an increase in its level reflects the degree of oxidative damage to the lipids in the cell membrane. Lipid peroxidation is a form of cell membrane damage that can lead to cell dysfunction. The increase in malondialdehyde levels indicates that fish are experiencing oxidative stress, as lipid peroxidation is an important marker of oxidative damage. This oxidative damage may lead to the destruction of cell structure and function, affecting the health and survival of fish. Acetylcholinesterase activity is used as a biomarker for assessing the health status of fish and environmental stress. Its changes can provide important information about the health status of the fish's nervous system. By monitoring acetylcholinesterase activity, the potential toxic effects of manganese exposure on the nervous system of fish can be evaluated, which is crucial for understanding the ecological risks of manganese pollution.Justify the chosen concentration ranges and exposure durations for toxicity tests, and describe the design of control and treatment groups in detail.

Data processing and analysis tools

In this study, the experimental data were initially organized in Excel 2013 first to facilitate the subsequent statistical analysis. Secondly, one-way analysis of variance was conducted using SPSS 26.0 to compare the means of multiple independent sample groups. Then, after one-way analysis of variance showed significant differences between the groups, a post hoc multiple comparison test of minimum significant differences was conducted to determine which specific groups had significant differences. Immediately after, the significance level was set at $p<0.0.05$, which means that if the p value is less than 0.0.05, the difference between groups is considered statistically significant. Before conducting one-way analysis of variance, hypothesis tests for normality, homogeneity of variance and independence were also carried out. Normality was evaluated by the Shapiro-Wilk test, homogeneity of variance was checked by Levene's test, and the independence hypothesis was verified by observing the randomness of the data. Finally, the analysis results are presented in the form of mean ± standard error. When the p value is less than 0.5, it is considered that the differences between groups are significant (Pedraza, 2023).

Results

Analysis of the toxic effects of Mn-EXP on silver carp and flower carp

After completing the experiment, the study measured and analyzed the oxidative damage, antioxidant effects, and toxic effects of Mn on silver carp using indicators such as total antioxidant capacity, catalase activity, acetylcholinesterase activity, malondialdehyde content, tissue protein content, and toxicity effects. Firstly, under different concentrations of divalent Mn (Mn^{+2}) water environment, the experiment was divided into six different Mn^{+2}concentration groups, namely 300, 400, 500, 600, 700, and 800 mg/L. Each concentration group underwent three repeated experiments (labeled A, B, C), with 10 silver carp used in each experiment. The death rates and average mortality rates in the same water environment with different concentrations of Mn+2 are shown in *Table 2*.

Table 2. *Number of deaths and mean mortality results*

A total (tail)	B total (tail)	C total (tail)	A Total Deaths (tail)	A Total Deaths (tail)	A Total Deaths (tail)	Mn^{+2}concentration (mg/L)	Average mortality rate (%)
10	10	10	1	1	1	300	10.00%
10	10	10	2	3	4	400	30.00%
10	10	10	4	4	5	500	43.33%
10	10	10	5	5	7	600	56.67%
10	10	10	8	8	8	700	80.00%
10	10	10	10	10	10	800	100.00%

From *Table 2*, at a Mn^{+2}concentration of 300 mg/L, one silver carp died in each of the three replicate groups, for a total of three, with an average mortality rate of 10.00%. As the Mn^{+2}concentrationrose to 400 mg/L, the number of deaths rose to 6, and the average mortality rate rose to 20.00%. At a concentration of 500 mg/L, a total of 13 silver carp died, with an average mortality rate of 43.33%. When the Mn^{+2}concentration further rose to 600 mg/L, the number of deaths reached 17, with an average mortality rate of 56.67%. At a concentration of 700 mg/L, a total of 24 silver carp died, with an average mortality rate of 80.00%. At the highest concentration of 800 mg/L, all silver carp died, with an average mortality rate of 100.00%. The above results indicated that the toxicity of Mn^{+2} to silver carp rose with concentration and had lethal toxicity at higher concentrations. The death rates and average mortality rates in the same water environment with different concentrations of Mn+2 are shown in *Table 3*.

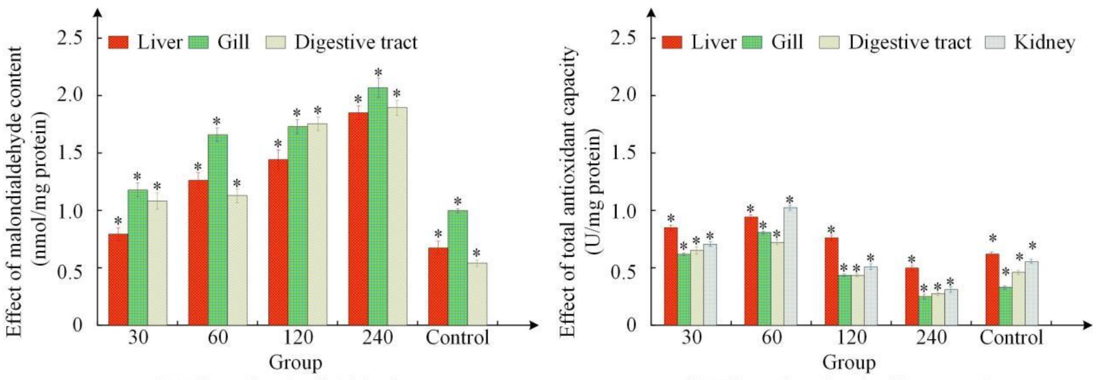
Table 3. *Number of deaths and mean mortality results*

D total (tail)	E total (tail)	F total (tail)	D Total Deaths (tail)	E Total Deaths (tail)	F Total Deaths (tail)	Mn^{+2}concentration (mg/L)	Average mortality rate (%)
10	10	10	1	1	1	350	10.00%
10	10	10	2	2	3	450	23.33%
10	10	10	4	4	4	550	40.00%
10	10	10	5	7	2	650	46.67%
10	10	10	10	10	10	750	100.00%

From *Table 3*, as the Mn concentration in the water rose from 350, 450, 550, 650, and 750 mg/L, the number of deaths of flower carp rose from 1 to 10, and the mortality rate rose from 10% to 100%. This result indicated that as the concentration of Mn in the water rose, the mortality rate of flower carp gradually rose until all of them die. Mn had a strong toxic effect on flower carp.

Analysis of the effects of Mn-EXP on the content of malondialdehyde and total antioxidant capacity in white silver carp and flower carp

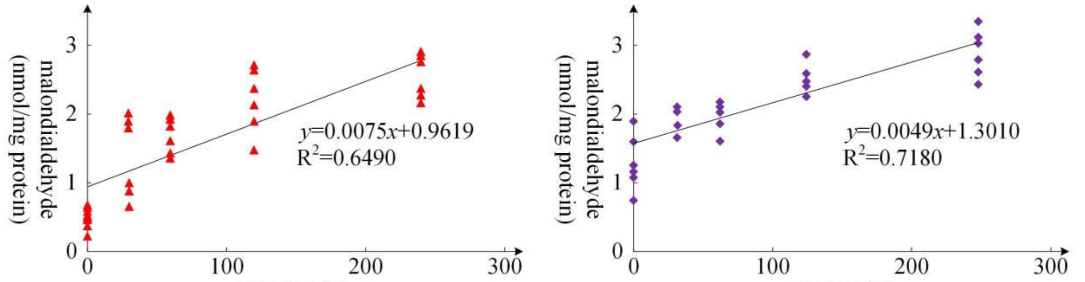
After completing the toxicity analysis of Mn-EXP on silver carp and flower carp, the study examined the impact of Mn-EXP on the content of malondialdehyde and total antioxidant capacity in the liver, gills, and digestive tract of silver carp and flower carp. The effects of different concentrations of Mn^{+2} in water environment on the content of malondialdehyde and total antioxidant capacity in the liver, gills, and digestive tract of silver carp are shown in *Figure 5*.



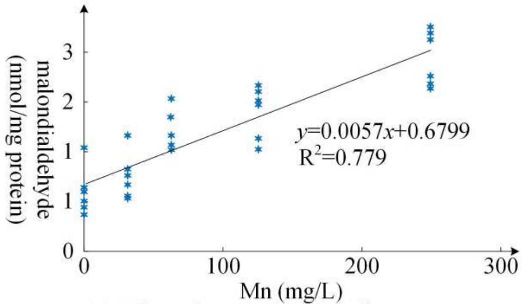
(a) Effect of malondialdehyde content
(b) Effect of total antioxidant capacity

Figure 5. *Effects of malondialdehyde content and total antioxidant capacity*

In *Figure 5*, "*" represents $p<0.05$, indicating a statistically significant difference (SSD) of 5% between the results and the CG. From *Figure 5(a)*, when the Mn concentrations were 30, 60, 120, and 240 mg/L, the malondialdehyde content in the liver was 1.29 ± 0.25, 1.59 ± 0.11, 2.41 ± 0.20, and 2.60 ± 0.14 NMP. The malondialdehyde content in the gills was 1.61 ± 0.13, 1.72 ± 0.05, 2.02 ± 0.08, and 2.39 ± 0.10 NMP. The malondialdehyde content in the digestive tract was 0.69 ± 0.08, 1.21 ± 0.09, 1.39 ± 0.12, and 2.0 ± 0.09 NMP. The levels of malondialdehyde in the liver, gills, and digestive tract of the CG were 0.39 ± 0.05, 0.99 ± 0.16, and 0.61 ± 0.09 NMP. From the above results, the content of malondialdehyde in the liver, gills, and digestive tract of silver carp in water with added Mn was substantially elevated than that in the CG without added Mn. With the rise of Mn concentration, the content of malondialdehyde in the liver, gills, and digestive tract of silver carp also rose. As shown in *Figure 5(b)*, the antioxidant capacity of the liver, gills, digestive tract, and kidneys of silver carp decreases with increasing Mn concentration. The antioxidant capacity of the CG without Mn addition was 0.64 ± 0.06, 0.45 ± 0.04, 0.55 ± 0.04, and 0.60 ± 0.02 NMP, which was substantially elevated than that of the liver, gills, digestive tract, and kidneys of silver carp with 240 mg/L Mn concentration. The above results indicated that, from the perspective of malondialdehyde and antioxidant capacity, with the rise of Mn concentration, the malondialdehyde in the liver, gills, digestive tract, and kidneys of silver carp rose, while the antioxidant capacity decreased. To further verify the effects of Mn on the liver, gills, and digestive tract of silver carp, regression analysis was conducted, and the regression outcomes are presented in *Figure 6*.



(a) Effect of manganese on liver malondialdehyde (b) Effect of manganese on gill malondialdehyde

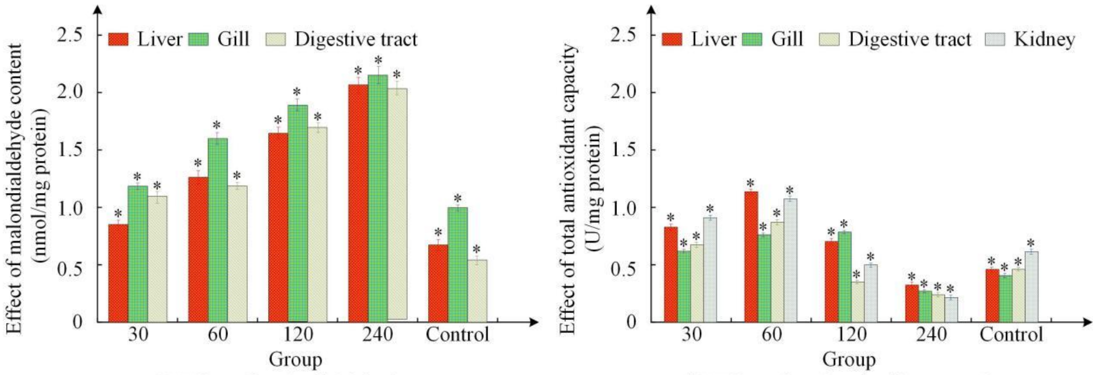


(c) Effect of manganese on Alimentary tract malondialdehyde

Figure 6. *Regression results*

From *Figure 6(a)*, with the rise of Mn concentration, the content of malondialdehyde in the liver of silver carp showed a significant upward trend. The linear regression equation (LRE)was y=0.0075x+0.9619, and the coefficient of determination R^2=0.6490 indicated that Mn concentration could explain about 64.90% of the changes in liver malondialdehyde content. From *Figure 6(b)*, the rise in Mn concentration also led to a rise in the content of malondialdehyde in the gills of silver carp. The LRE was y=0.0049x+1.3010, and R^2=0.7180 indicated that the explanatory power of Mn concentration on the content of malondialdehyde in the gills of silver carp reached 71.80%. From *Figure 6(c)*, the content of malondialdehyde in the digestive tract of silver carp also rose with the rise of Mn concentration. The LRE was y=0.0057x+0.6799, and the coefficient of determination R^2=0.779 showed that Mn concentration had the highest explanatory power on the changes in malondialdehyde content in the digestive tract, reaching 77.90%. These results indicated that different tissues had varying degrees of Mn induced oxidative stress response, with the digestive tract being the most sensitive. The effects of different concentrations of Mn^{+2} in water environment on the content of malondialdehyde and total antioxidant capacity in the liver, gills, and digestive tract of flower carp are shown in *Figure 7*.

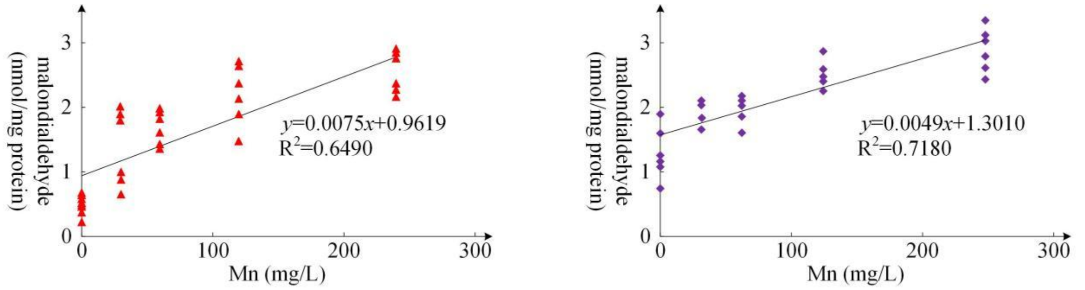
In *Figure 7*, "*" represents $p<0.05$, indicating an SSD of 5% between the results and the CG. From *Figure 7(a)*, when the Mn concentrationwas 30 mg/L, the malondialdehyde content in the liver, gills, and digestive tract of flower carp was 0.80 ± 0.04 nmol/mg protein, 1.31 ± 0.06 nmol/mg protein, and 1.20 ± 0.09 nmol/mg protein, respectively. When the Mn concentration reached 240 mg/L, the malondialdehyde content in the liver, gills, and digestive tract of flower carp was 1.99 ± 0.07 nmol/mgprotein, 2.19 ± 0.14 nmol/mg protein, and 1.89 ± 0.10 nmol/mg protein, respectively, showing an increasing state. substantially elevated than the CG's 0.70 ± 0.04 nmol/mg protein, 0.99 ± 0.01 nmol/mg protein, and 0.55 ± 0.04 mol/mgprotein. This result indicated that a rise in Mn concentration in water could lead to a rise in malondialdehyde content in the liver, gills, and digestive tract of flower carp. In *Figure 7(b)*, the total antioxidant capacity of the liver, gills, and digestive tract of flower carp decreased with increasing Mn concentration. When the Mn concentration was 60 mg/L, its total antioxidant capacity was the highest, which was 1.01 ± 0.09 U/mg protein, 0.81 ± 0.06 U/mg protein, 0.73 ± 0.04 U/mg protein, 0.99 ± 0.07 U/mg protein, and substantially elevated than the CG's 0.66 ± 0.04 U/mg protein, 0.45 ± 0.01 U/mg protein, 0.49 ± 0.06 U/mg protein, and 0.60 ± 0.02 U/mg protein. When the Mn concentration reached 240 mg/L, the total antioxidant capacity of the liver, gills, and digestive tract reached its lowest level, substantially reduced than other groups. The above results indicated that, overall, with the rise of Mn concentration, the total antioxidant capacity of the liver, gills, and digestive tract of flower carp decreased, while the content of malondialdehyde rose. To further verify the effects of Mn on the liver, gills, and digestive tract of silver carp, regression analysis was conducted, and the regression outcomes are presented in *Figure 8*.



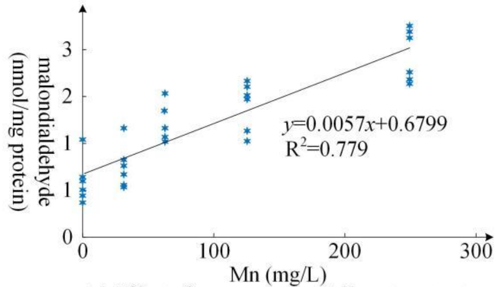
(a) Effect of malondialdehyde content
(b) Effect of total antioxidant capacity

***Figure 7.** Effects of malondialdehyde content and total antioxidant capacity*

From *Figure 8(a)*, with the rise of Mn concentration, the content of malondialdehyde in the liver of silver carp showed an upward trend. The LRE was y=0.0075x+0.9619, with a coefficient of determination R2=0.6490, indicating that Mn concentration could explain approximately 64.90% of the changes in liver malondialdehyde content. From *Figure 8(b)*, the rise in Mn concentration also led to a rise in the content of malondialdehyde in the gills of silver carp. The LRE was y=0.0049x+1.3010, and R^2=0.7180, indicating that the explanatory power of Mn concentration on the content of malondialdehyde in the gills reached 71.80%. From *Figure 8(c)*, the content of malondialdehyde in the digestive tract of silver carp also rose with the rise of Mn concentration. The LREwas y=0.0057x+0.6799, and the coefficient of determination R^2=0.779, indicating that Mn concentration had the highest explanatory power on the changes in malondialdehyde content in the digestive tract, reaching 77.90%. These results indicated that different tissues had varying degrees of Mn induced oxidative stress response, with the digestive tract being the most sensitive.



(a) Effect of manganese on liver malondialdehyde (b) Effect of manganese on gill malondialdehyde

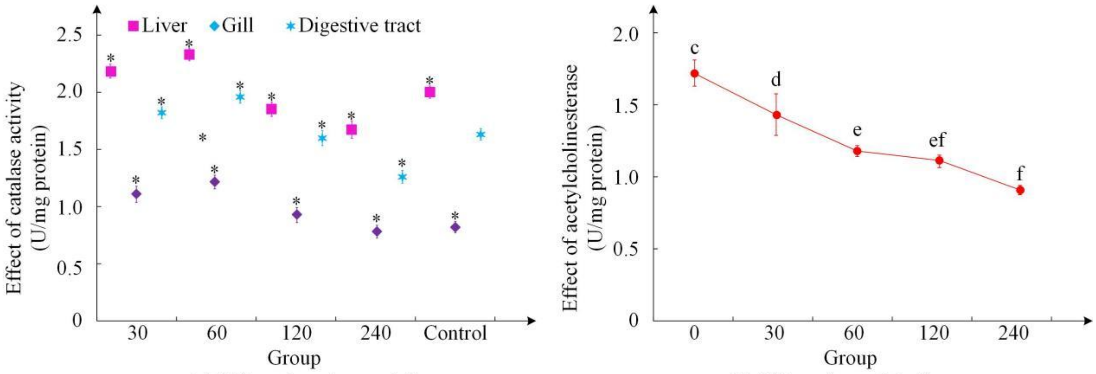


(c) Effect of manganese on Alimentary tract malondialdehyde

Figure 8. *Regression results*

Analysis of the effect of Mn-EXP ontissue catalase activity in silver carp and flower carp

After analyzing the malondialdehyde content and total antioxidant capacity of silver carp and flower carp, the effects of Mn-EXP on catalase and acetylcholinesterase in the tissues of silver carp and flower carp were studied and analyzed. The effects of different concentrations of Mn-EXP on catalase activity and acetylcholinesterase in the liver, gills, and digestive tract of silver carp are shown in *Figure 9*.



(a) Effect of catalase activity (b) Effect of acetylcholinesterase

Figure 9. *Effects on hydrooxidase activity and brain acetylcholinesterase*

In *Figure 9*, if the letters in the table are not the same, it indicates $p<0.05$, which means that statistically speaking, the results have an SSD of 5% compared to the CG. Otherwise, there is no significant difference. From *Figure 9(a)*, as the Mn concentration in the water

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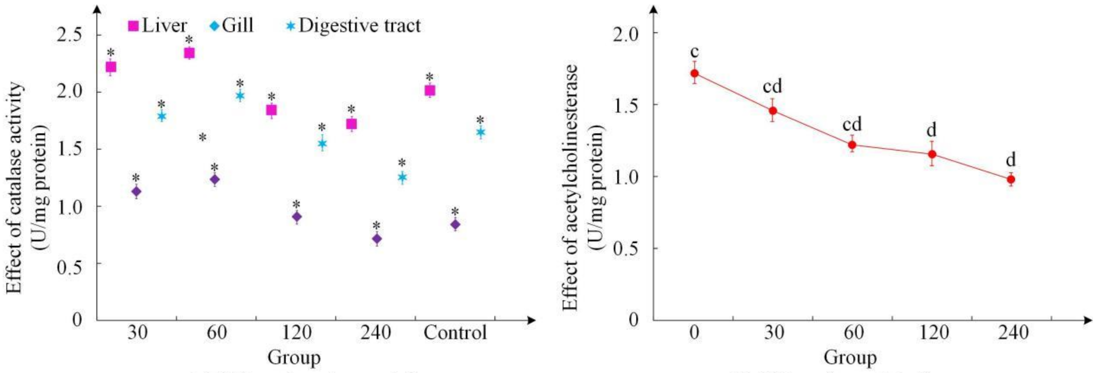
rose, the catalase activity in the liver, gills, and digestive tract of silver carp decreased. When the Mn concentration reached 240 mg/L, the catalase activity in the liver, gills, and digestive tract of silver carp was 1.91 ± 0.05 U/mg protein, 1.01 ± 0.99 U/mg protein, and 1.59 ± 0.10 U/mg protein, respectively, which were substantially reduced than the 2.10 ± 0.12 U/mg protein, 0.81 ± 0.09 U/mg protein, and 1.73 ± 0.09 U/mg protein of the CG. As shown in *Figure 9(b)*, with the rise of Mn concentration in the water, the acetylcholinesterase activity in the brain of silver carp decreased. When the Mn concentration reached 240 mg/L, the acetylcholinesterase activity in the brain was 0.89 ± 0.03 U/mg protein, which was substantially reduced than that when the Mn concentration in the water was 0. The above results indicated that a rise in Mn concentration in water could reduce the catalase activity in the liver, gills, and digestive tract of silver carp, as well as the acetylcholinesterase activity in the brain of silver carp. The effects of different Mn concentrations on proteins in the liver, digestive tract, kidneys, and brain of silver carp in water are presented in *Table 4*.

Table 4. *Influence results*

Tissue	**Sample size (tail)**	**Protein content (mg/g)**				
		30 mg/L	**60 mg/L**	**120 mg/L**	**240 mg/L**	**CG**
Liver	6	37.91 ± 0.09^{d}	38.49 ± 0.12^{d}	38.79 ± 0.16^{d}	24.59 ± 0.10^{e}	37.13 ± 0.08^{d}
Gill	6	17.31 ± 0.09^{de}	18.69 ± 0.03^{def}	19.09 ± 0.05^{def}	19.92 ± 0.04^{ef}	16.91 ± 0.09^{d}
Alimentary tract	6	28.41 ± 0.13^{d}	26.61 ± 0.05^{de}	25.99 ± 0.09^{df}	25.29 ± 0.10^{d}	27.99 ± 0.13^{de}
Kidney	6	24.71 ± 0.07^{de}	26.19 ± 0.07^{d}	29.39 ± 0.15^{f}	22.29 ± 0.07^{d}	23.29 ± 0.07^{de}
Brain	6	44.09 ± 0.07^{d}	30.51 ± 0.07^{f}	31.91 ± 0.04^{f}	31.69 ± 0.04^{f}	24.01 ± 0.07^{d}

Note: If the letters in the table are not the same, it indicates$p<0.05$, which means that there is an SSD of 5% between the results and the CG in statistics. Otherwise, there is no significant difference

According to *Table 4*, the protein content of the liver rose to 38.49 ± 0.12 mg/g, 38.79 ± 0.16 mg/g, and 38.79 ± 0.16 mg/g at concentrations of 30, 60, and 120 mg/L Mn, respectively, which were substantially elevated than the CG's 37.13 ± 0.08 mg/g. At a Mn concentration of 240 mg/L, the protein content was 24.59 ± 0.10 mg/g, substantially reduced than the CG's 37.13 ± 0.08 mg/g. The protein content of gill tissue rose with the rise of Mn concentration. When the Mn concentration reached 240 mg/L, its protein content was 19.92 ± 0.04 mg/g, substantially elevated than the CG's 16.91 ± 0.09 mg/g. The protein content in the digestive tract decreased with increasing Mn concentration. When the Mn concentration reached 240 mg/L, the protein content was 25.29 ± 0.10 mg/g, substantially reduced than the CG's 27.99 ± 0.13 mg/g. The protein content in the kidneys rose from 30, 60, and 120 mg/L Mn concentrations to 24.71 ± 0.07 mg/g, 26.19 ± 0.07 mg/g, and 29.39 ± 0.15 mg/g, respectively, substantially elevated than the CG's 23.29 ± 0.07 mg/g. When the Mn concentration reached 240 mg/L, the protein content was 22.29 ± 0.07 mg/g, substantially reduced than the CG's 23.29 ± 0.07 mg/g. The protein content in the brain decreased with the rise of Mn concentration, until the Mn concentration reached 240 mg/L, and its protein content was 31.69 ± 0.04 mg/g, substantially elevated than the CG's 24.01 ± 0.07 mg/g. The effects of different concentrations of Mn-EXP on catalase activity and acetylcholinesterase in the liver, gills, and digestive tract of silver carp are shown in *Figure 10*.



(a) Effect of catalase activity

(b) Effect of acetylcholinesterase

Figure 10. *Effects on hydrooxidase activity and brain acetylcholinesterase*

In *Figure 10*, if the letters in the figure are different, it indicates $p<0.05$, which means that statistically speaking, the result has an SSD of 5% compared to the CG. Otherwise, there is no significant difference. From *Figure 10(a)*, as the Mn concentration in the water rose, the catalase activity in the liver, gills, and digestive tract of silver carp generally decreased. When the Mn concentration reached 240 mg/L, the catalase activity in the liver, gills, and digestive tract of silver carp was 1.60 ± 0.07 U/mg protein, 0.69 ± 0.99 U/mg protein, and 1.39 ± 0.05 U/mg protein, respectively, which was substantially reduced than that of the CG and had an SSD. As shown in *Figure 10(b)*, with the rise of Mn concentration in the water, the acetylcholinesterase activity in the brain of silver carp decreased. When the Mn concentration reached 240 mg/L, the acetylcholinesterase activity in the brain was 1.19 ± 0.08 U/mg protein, which was substantially reduced than that when the Mn concentration in the water was 0. The above results indicated that a rise in Mn concentration in water could reduce the catalase activity in the liver, gills, and digestive tract of silver carp, as well as the acetylcholinesterase activity in the brain of silver carp. The effects of different Mn concentrations on proteins in the liver, digestive tract, kidneys, and brain of flower carp in water are shown in *Table 5*.

Table 5. *Influence results*

Tissue	**Sample size (tail)**	**Protein content (mg/g)**				
		30 mg/L	**60 mg/L**	**120 mg/L**	**240 mg/L**	**CG**
Liver	6	27.91±0.04^d	28.09±0.09^d	33.31±0.12^d	40.49±0.13^f	26.71±0.04^d
Gill	6	16.29±0.04^d	16.01±0.02^d	16.19±0.05^d	19.81±0.03^e	16.29±0.04^d
Alimentary tract	6	36.59±0.09^d	34.85±0.15^d	40.39±0.09^d	40.39±0.09^f	36.59±0.09^d
Kidney	6	25.91±0.02^d	25.69±0.6^d	43.29.40±0.17^f	49.49±0.09^f	25.69±0.13^d
Brain	6	25.49±0.11^d	24.29±0.16^d	25.19±0.15^f	25.221±0.11^d	25.91±0.07^d

Note: If the letters in the table are not the same, it indicates$p<0.05$, which means that there is an SSD of 5% between the results and the CG in statistics. Otherwise, there is no significant difference

According to *Table 5*, at a Mn concentration of 240 mg/L, the protein content in the liver significantly rose to 40.49 ± 0.13 mg/g, the protein content in the digestive tract reached 40.39 ± 0.09 mg/g, and the protein content in the kidneys rose to

49.49 ± 0.09 mg/g, all substantially elevated than the CG's 26.71 ± 0.04 mg/g, 36.59 ± 0.09 mg/g, and 25.69 ± 0.13 mg/g. In addition, the protein content of brain tissue significantly rose to 25.22 ± 0.1 mg/g at a concentration of 240 mg/L Mn, compared to 25.91 ± 0.07 mg/g in the CG. In contrast, the protein content of gill tissue did not show significant changes at different Mn concentrations. The 240 mg/L treatment group had a protein content of 19.81 ± 0.03 mg/g, which was not significantly different from the CG's 16.29 ± 0.04 mg/g. These results revealed the specific effects of Mn on the protein content of different tissues in flower carp, suggesting its potential physiological and ecological impacts, with the liver, digestive tract, and kidneys being particularly sensitive to Mn-EXP.

Discussion

To investigate the effects of Mn-EXP on the antioxidant capacity and oxidative loss of silver carp and flower carp, the study measured and analyzed the oxidative damage, antioxidant effects, and toxic effects of Mn on silver carp and flower carp from the aspects of total antioxidant capacity, catalase activity, acetylcholinesterase activity, malondialdehyde content, tissue protein content, and toxic effects. In the toxicity impact analysis results, it was found that when the Mn concentration in the water was300 mg/L and 350 mg/L, the average mortality rate of silver carp and flower carp in the three groups was 10%. With the rise of Mn concentration, the average mortality rate reached 100% when the Mn concentration in the water was 600 mg/L and 750 mg/L, respectively. This result indicated that at higher concentrations, the toxic effect of Mn on silver carp and flower carpwas significantly enhanced, causing oxidative damage to their functions and disrupting their antioxidant defense system, ultimately leading to death. Chen et al. (2023) research indicates that in liver injury models, excessive Reactive Oxygen Species can disrupt the antioxidant system and trigger cell death. Prussian blue nanozyme with multivalent Mn significantly alleviates liver injury by scavenging Reactive Oxygen Species. This result is consistent with the conclusion drawn from the research that excessive Mn content in organisms can be fata. The total antioxidant capacity outcomes indicated that the antioxidant capacity of the liver, gills, digestive tract, and kidneys of silver carp and flower carp decreased with the rise of Mn concentration in the water. When the Mn concentration reached 240 NMP, their antioxidant capacity was substantially reduced than that of the CG. This result indicated that under high concentrations of Mn-EXP, the antioxidant defense system of fish was severely disrupted, unable to effectively eliminate free radicals produced in the body, leading to a rise in oxidative stress levels and making cells and tissues more susceptible to oxidative damage. The research (Azimzadeh and Jelodar, 2022) indicates that an increase in Mn concentration in the body significantly aggravates oxidative damage. This study also found that high environmental Mn eroded the total antioxidant capacity of the liver, gills, intestines and kidneys of silver carp and bighead carp, suggesting that exogenous pro-oxidative stress can disrupt the trace element dependent antioxidant network in fish, thereby inducing systemic oxidative stress. According to the results of catalase activity and acetylcholinesterase activity, with the rise of Mn concentration in water, the catalase activity and acetylcholinesterase activity in the liver, gills, and digestive tract of silver carp and flower carp generally decreased. Meanwhile, when the Mn concentration reached 240 mg/L, the catalase activity and acetylcholinesterase activity in the liver, gills, digestive tract, and brain of flower carp were 1.60 ± 0.07 U/mg protein, 0.69 ± 0.99 U/mg protein, 1.39 ± 0.05 U/mg protein, and 1.19 ± 0.08 U/mg protein, respectively. The activities of catalase and acetylcholinesterase in the liver, gills, digestive tract, and brain of silver carp were 1.91 ± 0.05 U/mg protein, 1.01 ± 0.99 U/mg protein, 1.59 ± 0.10 U/mg protein, and 0.89 ± 0.03 U/mg protein, respectively, which were substantially reduced than those in the CG. This result indicated that Mn-EXP had a significant inhibitory effect on the activities of catalase and acetylcholinesterase in silver carp and flower carp, and this inhibitory effect was more pronounced under high concentration Mn-EXP. Mn posed a potential toxic threat to the oxidative defense system and nervous system of fish. Hao et al. (2023) demonstrated that MnO_2 nanostructures can deplete intracellular glutathione and inhibit antioxidant enzymes, leading to ROS accumulation and neurotoxicity. Correspondingly, our data indicated that 240 mg/L Mn^{2+} significantly reduced CAT and AChE activities in the livers, gills, intestines and brains of the two types of fish, confirming that excessive Mn - whether as a soluble ion or engineered nanomaterium - would damage the same antioxidant system and cholinergic system. This poses a dual oxidation-neurotoxicity threat to fish. According to the results of malondialdehyde content, as the Mn concentration in the water rose until the Mn concentration reached240 mg/L, the malondialdehyde content in the liver, gills, and digestive tract of flower carp was 1.99 ± 0.07 nmol/mg protein, 2.19 ± 0.14 nmol/mg protein, and 1.89 ± 0.10 nmol/mg protein, respectively. With the rise of Mn concentration, the content of malondialdehyde in the liver of silver carp showed a significant upward trend, with anLRE of $y=0.0075x+0.9619$ and a determination coefficient $R^2=0.6490$. The content of malondialdehyde in the gills rose, and the LRE was $y=0.0049x+1.3010$, with $R^2=0.7180$. The LRE for the digestive tract was $y=0.0057x+0.6799$, with a coefficient of determination R2=0.779, indicating that Mn concentration had the highest explanatory power for changes in malondialdehyde content in the digestive tract. Meanwhile, with the rise of Mn concentration in the water, the content of malondialdehyde in the liver of flower carp showed an upward trend. The LREwas $y=0.0075x+0.9619$, with a coefficient of determination R2=0.6490. The content of malondialdehyde in the gills rose, and the LRE was $y=0.0049x+1.3010$, with $R^2=0.7180$. The content of malondialdehyde in the digestive tract also rose with the rise of Mn concentration, and the LRE was $y=0.0057x+0.6799$, with a coefficient of determination R2=0.779. This result indicated that Mn-EXP caused oxidative damage to the cell membrane structure and function of silver carp and flower carp, and this damage intensified with increasing Mn concentration. Different tissues had different sensitivities to Mn toxicity. Feng et al. (2024) found that environmental strontium exposure could increase MDA levels in the human liver, suggesting ROS-induced lipid peroxidation and membrane damage. This study also showed that the MDA in the liver, gills and digestive tract of the two types of silver carp significantly increased with the increase of Mn dose, among which the slope of the digestive tract was the largest ($R^2 = 0.779$). This consistency indicates that, similar to strontium, excessive Mn can also accelerate lipid peroxidation, further supporting MN-driven oxidative stress in destroying membrane integrity in a tissue-specific manner. The results of tissue protein content showed that with the rise of Mn concentration in the water, the protein content in the liver and digestive tract of silver carp decreased, while the protein content in the gills rose, and the protein content in the kidneys rose to decrease. The protein content of the liver, gills, digestive tract, and kidneys of flower carp rose with the rise of Mn concentration, and was substantially elevated than that of the CG. However, the protein content of the flower carp alarm clock decreased with the rise of Mn concentration, until the Mn concentration reached 240 mg/L, and its protein content was

25.221 ± 0.11 mg/g, substantially reduced than the CG. This result indicated that Mn affected protein synthesis and metabolism by interfering with intracellular metal ion balance, inhibiting enzyme activity, and increasing oxidative stress. Smith and Strupp (2023) observed that chronic Mn exposure disrupts intracellular metal-ion homeostasis, suppresses key metabolic enzymes, and elevates ROS, collectively impairing protein synthesis in rodent brain regions. Consistently, we found that ≥240 mg/L Mn reduces hepatic and digestive-tract protein levels in silver carp, while flower carp exhibit tissue-specific increases or marked depletion (e.g., “alarm clock” protein 25.22 mg/g vs. CG). These opposing yet significant changes mirror the Mn-induced imbalance between oxidative damage and compensatory synthesis reported by Smith and Strupp (2023), confirming that Mn interferes with protein metabolism through metal-ion dyshomeostasis, enzyme inhibition, and ROS overproduction. The above results indicated that Mn-EXP could have negative effects on the digestive tract, liver, gills, and kidneys of silver carp and flower carp.

Conclusion

The impact of increasing Mn concentration in water on oxidative damage and antioxidant capacity of silver carp and flower carp was analyzed from the aspects oftotal antioxidant capacity, catalase activity, acetylcholinesterase activity, malondialdehyde content, tissue protein content, and toxic effects. The experimental analysis outcomes indicated that the concentration of Mn was directly proportional to the mortality rate of silver carp and flower carp. When the concentration reached 600 mg/L and 750 mg/L, the average mortality rate reached 100%. The catalase activity and acetylcholinesterase activity of silver carp and flower carpdecreased with increasing Mn concentration. The content of malondialdehyde in the liver, gills, and digestive tract of silver carp and flower carp rose with the rise of Mn concentration. The protein content in the liver and digestive tract of silver carpdecreased, while the protein content in the gills rose, and the protein content in the kidneys first rose and then decreased. The protein content of the liver, gills, digestive tract, and kidneys of flower carp rose with the rise of Mn concentration, and was substantially elevated than that of the CG. The protein content of the flower carp alarm clock decreased with the rise of Mn concentration, until the Mn concentration reached 240 mg/L, and its protein content was 25.221 ± 0.11 mg/g, substantially reduced than the CG. The above results indicated that Mn had a negative impact on the oxidative damage and antioxidant capacity of both silver carp and flower carp. The limitation of this study lies in its main focus on the impact of manganese on two fish species, silver crucian carp and flower crucian carp, without examining the influence of manganese pollution on broader ecosystems, including food webs, biodiversity, and ecosystem services. Future research can explore the impact of manganese pollution on these ecosystem components to gain a more comprehensive understanding of their ecological risks. Although this study only focused on the impact of manganese, the results have practical significance for aquaculture and environmental monitoring. For instance, this discovery can serve as a basis for improving water quality standards, especially for setting safe manganese level guidelines in fish farming systems. In addition, these findings can also be used to develop early warning biomarkers to monitor changes in manganese pollution levels in water bodies. Through these practical applications, aquatic ecosystems can be better protected and the sustainability of aquaculture can be enhanced.

Conflict of interest statement. The author declares that there's no conflict of interest.

Data availability. The data associated with the paper are not publicly available but are available from the author on reasonable request.

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