Effects of cadmium on LC 50 , histological characteristics and liver DNA damage of juvenile largemouth bass (Micropterus salmoides)

Xinhong Zhou
Southwest University of Science and Technology
Qiuhong Wu
Southwest University of Science and Technology
Jin Li
Southwest University of Science and Technology
Songsong Bao
Southwest University of Science and Technology
Yachao Wang
Southwest University of Science and Technology
Li Jiang (1305462380@qq.com)
Southwest University of Science and Technology

Research Article

Keywords: Cadmium, Largemouth bass, Semi-lethal concentration, Pathological sections, DNA damage

Posted Date: June 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1752596/v1

Abstract

This experiment aimed to investigate the effects of cadmium (Cd) on Semi-lethal concentration (LC_{50}), histological characteristics and liver DNA damage of juvenile largemouth bass. Healthy juvenile largemouth bass was randomly divided into 6 treatment groups (0 mg/L, 85 mg/L, 90 mg/L, 95 mg/L, 100 mg/L and 105 mg/L,) with 3 replicates per treatment (9 fish/replicate), and was subjected to 96h toxicity test. The experimental results show that: The LC_{50} for 48h, 72h and 96h were 88.90mg/L, 86.74mg/L and 86.65mg/L, respectively, and the safe concentration (SC) was 8.67mg/L. The liver and spleen of largemouth bass were collected to make pathological sections, and the results showed that Cd had an obvious toxic injury to the liver and spleen, manifested by vacuolar degeneration of liver cells, and disorganized arrangement of spleen corpuscles and increased macrophages. The results of single-cell gel electrophoresis (SCGE) showed that when the exposure concentration reached 90 mg/L, the tail distance, tail DNA content, tail length and Olive tail distance of liver DNA of juvenile largemouth bass were significantly higher than those of the control group ($P < 0.05$); with the increase of Cd concentration, the tail length, tail moment, tail DNA and olive tail moment of DNA also increased.

Introduction

Largemouth bass (Micropterus salmoides), a carnivorous warm-water fish from California, U.S.A., was introduced to China for its adaptability, fast growth rate and tender flesh[16; 41]. Largemouth bass has high requirements for the water environment in their lives[1]. The water environment is an important part of where human society lives and develops, and it is also the most seriously disturbed and damaged by human beings, and heavy metal pollution is one of the most important factors of water pollution[11; 26].

Cadmium (Cd) is an unnecessary element in living organisms and an important foreign element that cannot be purified in the body[17]. Cd is widely used in electroplating, pigments, batteries, electronic devices and other fields due to its good stability and corrosion resistance[12; 33]. With the rapid development of industry, more and more cadmium into the water environment through a variety of ways, leading to the increasingly serious situation of cadmium pollution in aquatic bodies[24; 2]. Aquatic Cd pollution mainly comes from the direct discharge of Cd wastewater from industrial production, as well as metal mining and smelting, which leads to the accumulation of Cd in the environmental media, and enters the aquatic environment after rainwater leaching[6]. Cd toxicity has a significant impact on the organism, producing toxic effects in physiology and biochemistry, leading to DNA damage and changes in cell membrane structure and function[4; 7]. It was found that Cd exposure induces oxidative stress, activates DNA damage response pathways, leads to DNA damage and induces apoptosis of mud crabs[3]. Cd^{2+} exposure caused intestinal Cd accumulation and histopathological damage, inhibited intestinal digestive enzyme activity and related gene expression, suppressed intestinal antioxidant activity, and decreased the expression of intestinal immune response genes of yellow catfish[35]. Cd exposure induced oxidative stress, excessive autophagy and mitochondrial dysfunction in Procypris merus liver, decreased enzymatic activity and gene expression of SOD, CAT and GST[5]. In addition, Cd can also affect the energy metabolism of fish, change motor behavior, interfere with the endocrine

system, threaten the survival and growth of fish, and endanger human health through the food chain[28; 18; 8]. However, there is a lack of studies on the toxicity of Cd to largemouth bass.

The purpose of this study is to investigate the effects of Cd on the LC_{50}, behavior, histopathology and DNA damage in the liver of largemouth bass. It makes people aware of the importance of healthy and safe production and protection of the environment, and also has important theoretical and practical significance for the in-depth understanding of the resistance of largemouth bass to Cd and the understanding of the toxicity of Cd.

Material And Methods

Experimental method

Healthy juvenile largemouth bass (average weight of about 5 g) were obtained from Meishan City, Sichuan Province, China. Upon arrival, all fish were tamed in a test environment for 2 weeks. At beginning of the experiment, 180 fish of the same size were randomly placed in 18 tanks with 6 L of water, and 9 fish were placed in each tank. Then, the prepared CdCl2 liquid was added to the tanks (control group was not added) and exposed to Cd^{2+} concentrations of 0 mg/L (control group), 85 mg/L, 90 mg/L, 95 mg/L, 100 mg/L and 105 mg/L, respectively. Three parallel tanks were set up for each concentration. During the experiment, the commercial feed was fed twice a day (10:00 and 16:00). To ensure water quality, 1/2 volume of water in the tank was changed daily and supplemented with a quantitative CdCl2 liquid to achieve the set exposure mass concentration of Cd^{2+} in the treatment group, pH 7.0±0.2, oxygen content greater than or equal to 8 mg/L, water temperature 24±0.5°C, and the whole study duration of Cd exposure treatment was 96h. The mortality rate of each group was counted promptly during the experiment to calculate the LC_{50}.

Sample collections

At the end of the experiment, the livers and spleens of the experimental fish were fixed with 4% paraformaldehyde at each concentration for making pathological sections. Fresh liver cells were rinsed 2~3 times with phosphate buffer and cut up with scissors into a mortar with 2 ml of phosphate buffer and crushed into a slurry, then filtered into 5 ml centrifuge tubes at 7500 r/min for 5 min, the supernatant was discarded and resuspended with 2 ml of PBS and stored at 4°C for use.

Sample measurement

Histopathology section preparation

The liver and spleen were fixed with 4% paraformaldehyde, then dehydrated, transparent, embedded, and 5-μm sectioned, then dewaxed, rehydrated, subjected to hematoxylin-eosin (HE) staining, dehydrated, transparent, and sealed using neutral gum (specific steps are shown in Table 1).

Table 1

Detailed steps of paraffin sectioning

Steps	Reagents	Times
Dehydration	75% ethanol solution	3h
	85% ethanol solution	2h
	95% ethanol solution	2h
	100% ethanol solution Ⅰ	1h
	100% ethanol solution Ⅱ	1h
Transparent	Xylene Ⅰ	0.5–1h
	Xylene Ⅱ	10–30min
Dewaxing	Xylene Ⅰ	5–10min
	Xylene Ⅱ	5–10min
Rehydration	100% ethanol solution	2–5min
	100% ethanol solution	2–5min
	95% ethanol solution	2–5min
	85% ethanol solution	2–5min
	75% ethanol solution	2–5min
Dyeing	Sumac Extract	5–10min
	Hydrochloric acid alcohol	Seconds
	Eosin Dye Solution	5min
Dehydration	75% ethanol solution	1min
	85% ethanol solution	1min
	95% ethanol solution	1min
	100% ethanol solution Ⅰ	1–2min
	100% ethanol solution Ⅱ	3–4min
Transparent	Xylene Ⅰ	2–5min
	Xylene Ⅱ	2–5min

Single-cell gel electrophoresis test (comet experiment)

Absorb 100μl of ordinary melting point agarose drops onto the middle of the slides washed and dried with anhydrous ethanol beforehand, take a coverslip to cover it, and put it into the refrigerator at 4℃ for

30 minutes to make the first layer of gel. Mix 100μl of cell suspension with 200μl of low melting point agarose gel, absorb 75μl of mixed sample drops on the first layer of gel-covered with a coverslip and cure at 4℃ for 10 minutes. The cured slides were lysed in cold basic lysis solution at low temperature and protected from light for 1h. Remove the slide from the lysate, rinse it 3 times with distilled water, put the slide in a horizontal electrophoresis tank, add pre-cooled electrophoresis buffer and soak for 30 minutes, wait for the DNA double-stranded to unserialize, and gently rinse it 3 times in phosphate buffer after finishing. Electrophoresis at 4°C, 25V, 200mA constant current for 30 minutes. Rinse the slides 3 times with distilled water after the end of electrophoresis, neutralize them with prepared Tris-HCl neutralization solution for 15 minutes, rinse them 3 times with distilled water, store the slides in a moist cassette, add 25ul,20ug/L of ethidium bromide dropwise to each slide for observation, and cover the slides with a coverslip for observation. Ten cells per slide were randomly selected to measure tail length, tail length, tail moment, tail DNA and olive tail moment parameters.

Statistical Analysis

All data were expressed as mean ± SD. Significance levels were determined by IBM SPSS Statistics 23 one-way analysis of variance (ANOVA), and multiple comparisons were performed using the Tukey multiple range test. DNA damage pictures were measured using the comet analysis software CASP 1.2.3. Significant differences were indicated as *P < 0.05.

Results

Behavioral responses of juvenile largemouth bass after acute Cd poisoning

The appearance characteristics of juvenile largemouth bass under Cd exposure are shown in Fig. 1. After Cd exposure, largemouth bass at two low concentrations of 85mg/L and 90mg/L were able to maintain vitality and swim normally without obvious damage to their appearance in the early stage of exposure. After 24 h Cd exposure group the juvenile largemouth bass was relatively anxious and started to die. A layer of pinkish-white mucus was formed on the skin at three high exposures of 105mg/L, 100mg/L and 95mg/L. After a certain time of Cd exposure, the swimming direction of the fish becomes confused and cannot swim smoothly, they will rush to the water surface at a faster speed and stay or stick to the glass tank wall. With increasing exposure time, the fish reach the limit of their tolerance to Cd as they swim sluggishly or stay quietly in the tank and almost stop swimming. Largemouth bass juveniles die of Cd poisoning with a large mouth spread. The dead largemouth bass has a lot of mucus inside the throat and outside the body, the whole head is slightly red, and there is red blood around the inside of the muzzle, the outside of the eye and the gill cover. moreover, part of the tail will show a curved state. After dissection, the gills of the fish were found to be edematous, the inside of the gills contained a large amount of transparent mucus, the liver had deep red color, and after a certain time of death, a yellowish fluid would flow from the abdomen. At the later stage of the experiment, the white foam was floating on the water surface, a layer of the scale was left on the fish tank wall, the color of the water was slightly yellow and emitted a fishy smell.

LC_{50} of Cd in juvenile largemouth bass

The average mortality in each group of juvenile largemouth bass under acute Cd exposure is shown in Table 2. Information related to the LC_{50} of juvenile largemouth bass is shown in Table 3. The LC_{50} results for 48h, 72h and 96h under cadmium exposure were 88.90mg/L, 86.74mg/L and 86.65mg/L respectively which were in the upper and lower LC_{50} range and the safe concentration was 8.67mg/L.

Table 2

The average mortality of juvenile largemouth bass exposed to Cd for 24h, 48h, 72h and 96h

Exposure time (h)	85mg/L(tail)	90mg/L(tail)	95mg/L(tail)	100mg/L(tail)	105mg/L(tail)
24	0	1	2	4	9
48	0	3	6	8	9
72	2	5	7	8	9
96	2	6	8	9	9

Table 3

Linear regression calculation of LC_{50} results

Exposure time (h)	Regression equation	LC_{50} (mg/L)	LC_{50} lower limit (mg/L)	LC_{50} upper limit (mg/L)
48	y=22.282+10.556x	88.90	58.23	119.58
72	y=11.468+11.993x	86.74	64.30	109.18
96	y=11.018+11.054x	86.65	64.42	108.87

Pathological effects of Cd on the liver of juvenile largemouth bass

The liver pathology sections of juvenile largemouth bass after cadmium exposure are shown in Fig. 2. Compared with a and b in Figure 2, the hepatocytes in a are well-defined, but the hepatocytes in b are vacuolated (indicated by the arrow in b) and the nucleus is squeezed to the edge of the cell, each vacuole is well-defined, which can be observed even under 10x microscope (indicated by the arrow in c). The central vein shows vacuole-like degeneration and disorganized arrangement of hepatic corpuscles (indicated by the arrow in d). The liver tissue in e has a more obvious damage condition, the cells are

widely spaced, the central vein is dilated, some cells are detached (indicated by the arrow in e), and the hepatocytes are edematous (indicated by the arrow in f).

Pathological effects of cadmium on the spleen of juvenile largemouth bass

The spleen pathology sections of juvenile largemouth bass after Cd exposure are shown in Fig. 3. As seen in Figure 3, the boundaries of splenic white marrow and splenic red marrow can be distinguished from each other, and splenic cord cells are arranged in an orderly manner at the periphery of the splenic sinus. The lymphocytes in b are increased and the splenocyte spacing is increased (indicated by the arrow in b). The splenic cord cells in c are disorganized and difficult to distinguish (indicated by arrow 5), and macrophages are increased (indicated by arrow 4).

Effect of Cd exposure on DNA damage in the liver of largemouth bass

DNA damage to the liver of largemouth bass by Cd exposure is shown in Figure 4. After processing with the Comet Analysis software CASP, it can be visualized that the liver cells of the control group are a smooth red orb (shown in a). The cell trailing at 85 mg/L is short and dense (shown in b), the cells at 90 mg/L start to spread and become longer (shown in c), and the cells at 95 mg/L have long trailing edges and spread in an inverted triangular shape (shown in d). The tails of the liver cells at 100 mg/L and 105 mg/L were trailing increasingly longer and more severely fragmented. At the same time the cells in the head of the comet are breaking up and the edges are starting to become mutilated (shown in e, f). The tail length, tail moment, tail DNA and olive tail moment in Table 4 were obtained using SPSS23 analysis. When the exposure concentration reached 90 mg/L, tail length, tail moment, tail DNA and olive tail moment of juvenile largemouth bass were significantly higher than those of the control group ($P < 0.05$).

Table 4

Effect of Cd exposure on DNA damage of largemouth bass

Items (mg/L)	Tail length (um)	Tail moment (um)	Tail DNA (%)	Olive tail moment (um)
0	3.00±0.0	0.01±0.01	0.36±0.26	0.07±0.05
85	4.33±1.53	0.59±0.35	6.24±1.23*	1.53±0.10*
90	12.33±3.51*	5.33±3.24*	15.24±5.91*	3.63±0.60*
95	21.00±4.00*	13.76±2.04*	23.79±2.64*	8.83±2.36*
100	31.67±4.51*	18.99±2.41*	41.12±3.45*	15.52±1.96*
105	47.67±6.66*	27.23±4.90*	57.16±10.55*	20.75±2.65*

*Significant difference (P < 0.05).

Discussion

In the acute toxicity test, the concentration that kills half of the test animals is the LC_{50}, which is often used as an evaluation indicator for the contamination of aquatic organisms[39; 10]. The LC_{50} is an important parameter to measure the toxicity of toxic substances to aquatic animals in the water, and its lethal effect is related to the exposure time of the exposed animals to toxic substances[9; 34]. The toxicity of Cd to largemouth bass can be expressed by the LC_{50}, the smaller the LC_{50}, the greater the toxicity. We found that in the range of 24h to 96h, the LC_{50} was 88.90mg/L, 86.74mg/L and 86.65mg/L for 48h, 72h and 96h, respectively, and the Cd concentration and exposure time together determined the magnitude of the LC_{50}. It was found that LC_{50} of Cd was 49.5 mg/L for 96 hours of trichogaster fasciata[25]. the (24,48 and 96h) LC_{50} of Gambusia holbrooki were (37.29, 42.733 and 50.178) mg/l, respectively[36]. The 96h LC_{50} of Cd for carp and goldfish were 8.845 ml/L and 9.202 ml/L, respectively [37]. In agreement with previous studies, the LC_{50} for Cd in fish was low and highly toxic.

The liver is the largest digestive gland of fish and is also an important detoxification organ, which can remove pathogens, toxic substances and metabolic waste[38]. However, when the toxicity of exogenous toxins exceeds the detoxification capacity of the liver, it can lead to hepatic abnormalities. It has been found that Cd exposure can lead to the accumulation of Cd in the liver of fish, and then induce histological lesions, immune dysregulation and metabolic disorders[31; 32; 40]. In this study, we found that hepatocytes underwent steatosis, hepatocyte disarrangement, hepatic sinusoidal dilatation, cellular distention and focal cytoplasmic lysis with transparency after Cd exposure. Both acute and chronic liver disease can lead to cell and tissue deprivation of oxygen[14]. This explains the phenomenon of the largemouth black bass surfacing. The spleen is the largest immune organ in the body and contains a large number of macrophages and lymphocytes, whose main role is to remove senescent cells and other foreign substances from the body[13; 19]. Invasion of Cd into the spleen produces an immune response and an increase in the number of macrophages, which cannot continue to function normally if the concentration of Cd is excessive[20; 30]. In this study, we found that the splenic cords were disorganized and caused some damage to the spleen.

Single-cell gel electrophoresis (SCGE) is a technique that can detect DNA damage, and the damaged DNA shows a comet-like tail at the end of electrophoresis, which is why it is also called a comet experiment[21; 22; 23]. Under the influence of harmful substances, the organism will carry out the conversion process of biotransformation enzymes, resulting in the generation of various metabolites represented by reactive oxygen species, causing oxidative stress, and the intermediate metabolites can combine with DNA to produce free radicals to attack nucleic acids, directly or indirectly leading to DNA strand breakage, therefore DNA damage can directly represent the extent of the impact on the organism[15; 29; 27]. We found that within a certain range, the greater the concentration, the more serious the liver DNA damage, and if the concentration is set large enough beyond the cellular tolerance, the liver DNA fragmentation will

level off after a certain degree. From the data we measured, the damage of Cd to liver DNA is significant. Therefore, we should increase the treatment of Cd pollution, if there are substances to replace Cd or reduce the toxicity of Cd is also a good way to reduce Cd pollution.

In conclusion, our study shows that the LC_{50} of Cd for juvenile largemouth bass is in the range of 1 ~ 100mg/L. According to the fish toxicity classification standard, Cd is a highly toxic substance. Cd exposure caused certain lesions in the livers and spleens of largemouth bass and caused DNA damage to livers.

Declarations

Ethics statement

This trial was approved by the Southwest University of Science and Technology in China, Institutional Animal Care and Use Committee (Project A00655).

Funding

This research was financially supported by the Central Public-interest Scientific Institution Basal Research Fund, CAFS (2020TD59).

Consent of Publish

Agree.

Conflicts of Interest

All authors have declared no conflict of interest.

Data Availability Statement

Data is available on request due to restrictions on privacy. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to this paper is part of a series of studies, and disclosure of data may influence the publication of subsequent papers.

Author Contributions

XH. Z.: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. QH. W: Conceptualization, Methodology, Formal analysis. SS. B.: Investigation, Data curation. YC. W.: Investigation, Data curation L. J.: Funding acquisition, Investigation.

References

1. Bai, J., Li, S. (2018). Development of largemouth bass (Micropterus salmoides) culture. *Aquaculture in China: Success Stories and Modern Trends*, 421-429.http://doi.org/10.1002/9781119120759.ch4_5
2. Bai, J., Xiao, R., Zhang, K., Gao, H. (2012). Arsenic and heavy metal pollution in wetland soils from tidal freshwater and salt marshes before and after the flow-sediment regulation regime in the Yellow River Delta, China. *Journal of Hydrology*, *450-451*, 244-253.http://doi.org/10.1016/j.jhydrol.2012.05.006
3. Cheng, C.H., Ma, H.L., Deng, Y.Q., Feng, J., Jie, Y.K., Guo, Z.X. (2021). Oxidative stress, cell cycle arrest, DNA damage and apoptosis in the mud crab (Scylla paramamosain) induced by cadmium exposure. *Chemosphere*, *263*, 128277.http://doi.org/10.1016/j.chemosphere.2020.128277
4. Coogan, T.P., Bare, R.M., Waalkes, M.P. (1992). Cadmium-induced DNA strand damage in cultured liver cells: reduction in cadmium genotoxicity following zinc pretreatment. *Toxicol Appl Pharmacol*, *113*(2), 227-233.http://doi.org/10.1016/0041-008x(92)90118-c
5. Dai, Z., Cheng, J., Bao, L., Zhu, X., Li, H., Chen, X., Zhang, Y., Zhang, J., Chu, W., Pan, Y., Huang, H. (2020). Exposure to waterborne cadmium induce oxidative stress, autophagy and mitochondrial dysfunction in the liver of Procypris merus. *Ecotoxicol Environ Saf*, *204*, 111051.http://doi.org/10.1016/j.ecoenv.2020.111051
6. Das, S., Mukherjee, D. (2013). Effect of cadmium chloride on secretion of 17beta-estradiol by the ovarian follicles of common carp, Cyprinus carpio. *Gen Comp Endocrinol*, *181*, 107-114.http://doi.org/10.1016/j.ygcen.2012.10.010
7. Demchenkov, E.L., Nagdalian, A.A., Budkevich, R.O., Oboturova, N.P., Okolelova, A.I. (2021). Usage of atomic force microscopy for detection of the damaging effect of CdCl2 on red blood cells membrane. *Ecotoxicol Environ Saf*, *208*, 111683.http://doi.org/10.1016/j.ecoenv.2020.111683
8. Hameed, F. (2019). Hazardous effects of cadmium contaminated water on biological characteristics of fish; a review. *RHAZES: Green and Applied Chemistry*, *6*, 01-10.http://doi.org/10.48419/IMIST.PRSM/rhazes-v6.17566
9. Hashim, N.H., Mohamat-Yusuff, F., Joni, A.A., Mohd Kusin, F., Mohamed, K.N., Zulkeflee, Z., Asha'ari, Z.H., Zahmir Zulkifli, S. (2020). Determination of Median Lethal Concentration (LC50) and Nitrite Accumulation in the Blood and Tissue of Blood Cockle (Tegillarca granosa, Linnaeus 1758). *Water*, *12*(8), 2197.http://doi.org/10.3390/w12082197
10. Hojjati-Najafabadi, A., Mansoorianfar, M., Liang, T., Shahin, K., Karimi-Maleh, H. (2022). A review on magnetic sensors for monitoring of hazardous pollutants in water resources. *Sci Total Environ*, *824*, 153844.http://doi.org/10.1016/j.scitotenv.2022.153844
11. Inyinbor Adejumoke, A., Adebesin Babatunde, O., Oluyori Abimbola, P., Adelani Akande Tabitha, A., Dada Adewumi, O., Oreofe Toyin, A. (2018). Water pollution: effects, prevention, and climatic impact. *Water Challenges of an Urbanizing World*, *33*, 33-47.http://doi.org/10.5772/intechopen.72018
12. Jumbe. (2009). Heavy Metals Analysis and Sediment Quality Values in Urban Lakes. *American Journal of Environmental Sciences*, *5*(6), 678-687.http://doi.org/10.3844/ajessp.2009.678.687

13. Kaptaner, B., Unal, G., Dogan, E., Aykut, H. (2021). Histology of corpuscles of Stannius in Lake Van fish (Alburnus tarichi Guldenstadt, 1814) (Cyprinidae). *Anat Histol Embryol*, *50*(2), 404-410.http://doi.org/10.1111/ahe.12645
14. Konishi, T., Smith, J.L., Lin, K.M., Wan, Y.J. (2003). Influence of genetic admixture on polymorphisms of alcohol-metabolizing enzymes: analyses of mutations on the CYP2E1, ADH2, ADH3 and ALDH2 genes in a Mexican-American population living in the Los Angeles area. *Alcohol Alcohol*, *38*(1), 93-94.http://doi.org/10.1093/alcalc/agg021
15. Ladeira, M.S., Rodrigues, M.A., Freire-Maia, D.V., Salvadori, D.M. (2005). Use of Comet assay to assess DNA damage in patients infected by Helicobacter pylori: comparisons between visual and image analyses. *Mutat Res*, *586*(1), 76-86.http://doi.org/10.1016/j.mrgentox.2005.06.003
16. Li, X., Wei, X., Guo, X., Mi, S., Hua, X., Li, N., Yao, J. (2020). Enhanced growth performance, muscle quality and liver health of largemouth bass (Micropterus salmoides) were related to dietary small peptides supplementation. *Aquaculture Nutrition*, *26*(6), 2169-2177.http://doi.org/10.1111/anu.13155
17. Liu, D., Yan, B., Yang, J., Lei, W., Wang, L. (2011). Mitochondrial pathway of apoptosis in the hepatopancreas of the freshwater crab Sinopotamon yangtsekiense exposed to cadmium. *Aquat Toxicol*, *105*(3-4), 394-402.http://doi.org/10.1016/j.aquatox.2011.07.013
18. Liu, G.D., Sheng, Z., Wang, Y.F., Han, Y.L., Zhou, Y., Zhu, J.Q. (2016). Glutathione peroxidase 1 expression, malondialdehyde levels and histological alterations in the liver of Acrossocheilus fasciatus exposed to cadmium chloride. *Gene*, *578*(2), 210-218.http://doi.org/10.1016/j.gene.2015.12.034
19. Mallhi, T.H., Khan, Y.H., Adnan, A.S., Tanveer, N., Aftab, R.A. (2021). Dengue-Induced Lymphoreticular Complications. In *Expanded Dengue Syndrome*, Springer, pp. 115-123.
20. Mirkov, I., Popov Aleksandrov, A., Ninkov, M., Tucovic, D., Kulas, J., Zeljkovic, M., Popovic, D., Kataranovski, M. (2021). Immunotoxicology of cadmium: Cells of the immune system as targets and effectors of cadmium toxicity. *Food Chem Toxicol*, *149*, 112026.http://doi.org/10.1016/j.fct.2021.112026
21. Muniz, J.F., McCauley, L., Scherer, J., Lasarev, M., Koshy, M., Kow, Y.W., Nazar-Stewart, V., Kisby, G.E. (2008). Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. *Toxicol Appl Pharmacol*, *227*(1), 97-107.http://doi.org/10.1016/j.taap.2007.10.027
22. Nandhakumar, S., Parasuraman, S., Shanmugam, M.M., Rao, K.R., Chand, P., Bhat, B.V. (2011). Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay). *J Pharmacol Pharmacother*, *2*(2), 107-111.http://doi.org/10.4103/0976-500X.81903
23. Pacheco-Pantoja, E.L. (2022). Convolutional Neural Network for Segmentation of Single Cell Gel Electrophoresis Assay. In *Intelligent Computing Systems: 4th International Symposium, ISICS 2022, Santiago, Chile, March 23–25, 2022, Proceedings*, Springer, p. 57.
24. Rai, P.K. (2009). Heavy Metal Phytoremediation from Aquatic Ecosystems with Special Reference to Macrophytes. *Critical Reviews in Environmental Science and Technology*, *39*(9), 697-

753.http://doi.org/10.1080/10643380801910058

25. Roy, S., Karmakar, D., Pal, S. (2022). Acute Toxicity Bioassay and Determination of LC50 of Cadmium Chloride in Trichogaster (Colisa) fasciata. *Appl Biochem Biotechnol*, 1-11.http://doi.org/10.1007/s12010-022-03953-1
26. Singh, B.R., Steinnes, E. (2020). Soil and water contamination by heavy metals. In *Soil processes and water quality*, CRC Press, pp. 233-271.
27. Slobodskova, V.V., Zhuravel, E.V., Kukla, S.P., Chelomin, V.P. (2019). Evaluation of DNA Damage in the Marine Mussel Crenomytilus grayanus as a Genotoxic Biomarker of Pollution. *Journal of Ocean University of China*, *18*(1), 159-164.http://doi.org/10.1007/s11802-019-3573-7
28. Souid, G., Souayed, N., Yaktiti, F., Maaroufi, K. (2013). Effect of acute cadmium exposure on metal accumulation and oxidative stress biomarkers of Sparus aurata. *Ecotoxicol Environ Saf*, *89*, 1-7.http://doi.org/10.1016/j.ecoenv.2012.12.015
29. Tasneem, S., Yasmeen, R. (2018). Evaluation of genotoxicity by comet assay (single-cell gel electrophoresis) in tissues of the fish Cyprinus carpio during sub-lethal exposure to Karanjin. *The Journal of Basic and Applied Zoology*, *79*(1).http://doi.org/10.1186/s41936-018-0033-7
30. UĞUrlu, P., Satar, E.İ., ÇİÇEk, T. (2022). Histopathological and Cytopathological Determination of Alterations and Immune Response in Spleen Tissue of Oreochromis Niloticus Exposed to Sub-Lethal Concentration of Diazinon.http://doi.org/10.21203/rs.3.rs-1324492/v1
31. Van An, L., Hong, T.T., Ogle, B., Lindberg, J.E. (2005). Utilization of ensiled sweet potato (Ipomoea batatas (L.) Lam.) leaves as a protein supplement in diets for growing pigs. *Trop Anim Health Prod*, *37*(1), 77-88.http://doi.org/10.1023/b:trop.0000047937.41355.4d
32. van Dyk, J.C., Pieterse, G.M., van Vuren, J.H. (2007). Histological changes in the liver of Oreochromis mossambicus (Cichlidae) after exposure to cadmium and zinc. *Ecotoxicol Environ Saf*, *66*(3), 432-440.http://doi.org/10.1016/j.ecoenv.2005.10.012
33. Vena, M.P., Jobbagy, M., Bilmes, S.A. (2016). Microorganism mediated biosynthesis of metal chalcogenides; a powerful tool to transform toxic effluents into functional nanomaterials. *Sci Total Environ*, *565*, 804-810.http://doi.org/10.1016/j.scitotenv.2016.04.019
34. Wang, H.-y., Jia, Y., Jing, G.-f., Wu, X.-c. (2021). A novel toxicity prediction model for hydrazine compounds based on 1D–3D molecular descriptors. *Computational Toxicology*, *18*, 100169.http://doi.org/10.1016/j.comtox.2021.100169
35. Xie, D., Li, Y., Liu, Z., Chen, Q. (2019). Inhibitory effect of cadmium exposure on digestive activity, antioxidant capacity and immune defense in the intestine of yellow catfish (Pelteobagrus fulvidraco). *Comp Biochem Physiol C Toxicol Pharmacol*, *222*, 65-73.http://doi.org/10.1016/j.cbpc.2019.04.012
36. Yalsuyi, A.M., Hedayati, A., Vajargah, M.F., Mousavi-Sabet, H. (2017). Examining the toxicity of cadmium chloride in common carp (Cyprinus carpio) and goldfish (Carassius auratus). *Journal of Environmental Treatment Techniques*, *5*(2), 83-86

37. Yalsuyi, A.M., Hedayati, A., Vajargah, M.F., Mousavi-Sabet, H. (2017). Examining the toxicity of cadmium chloride in common carp (Cyprinus carpio) and goldfish (Carassius auratus). *Journal of Environmental Treatment Techniques*, *5*(2309-1185), 83-86
38. Zhang, Q.F., Li, Y.W., Liu, Z.H., Chen, Q.L. (2016). Exposure to mercuric chloride induces developmental damage, oxidative stress and immunotoxicity in zebrafish embryos-larvae. *Aquat Toxicol*, *181*, 76-85.http://doi.org/10.1016/j.aquatox.2016.10.029
39. Zhang, Y., Jiao, Y., Li, Z., Tao, Y., Yang, Y. (2021). Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. *Sci Total Environ*, *771*, 145418.http://doi.org/10.1016/j.scitotenv.2021.145418
40. Zheng, J.L., Yuan, S.S., Wu, C.W., Lv, Z.M. (2016). Acute exposure to waterborne cadmium induced oxidative stress and immunotoxicity in the brain, ovary and liver of zebrafish (Danio rerio). *Aquat Toxicol*, *180*, 36-44.http://doi.org/10.1016/j.aquatox.2016.09.012
41. Zhu, S., Gao, W., Wen, Z., Chi, S., Shi, Y., Hu, W., Tan, B. (2022). Partial substitution of fish meal by Clostridium autoethanogenum protein in the diets of juvenile largemouth bass (Micropterus salmoides). *Aquaculture Reports*, *22*.http://doi.org/10.1016/j.aqrep.2021.100938

Figures

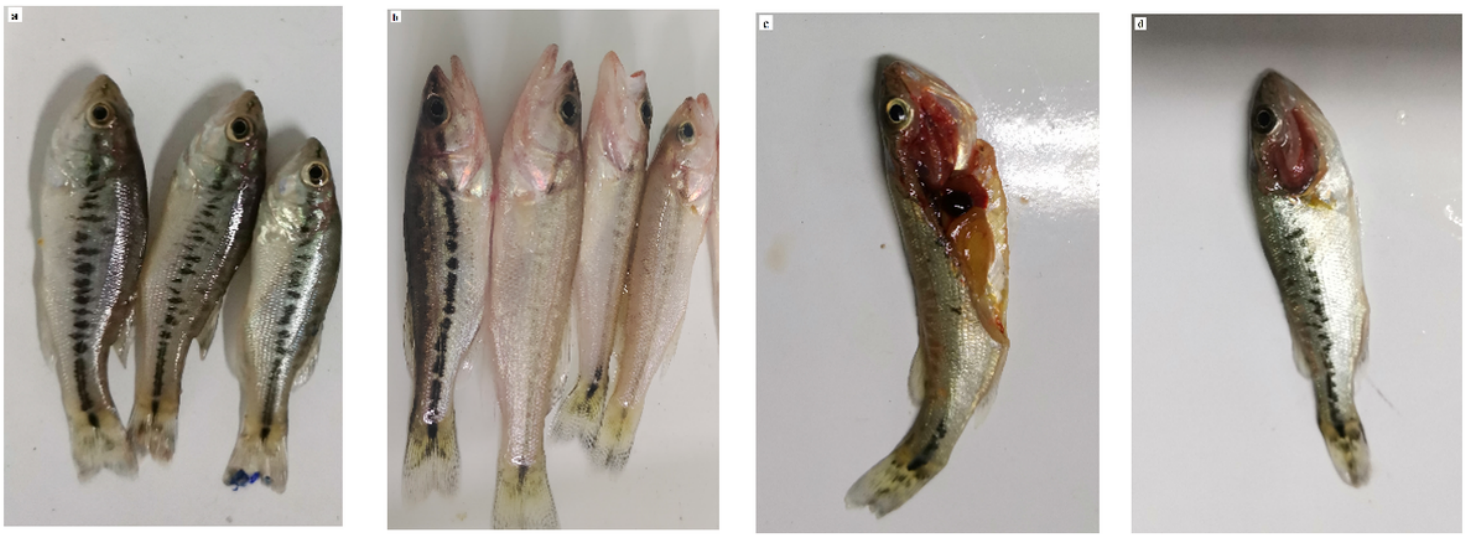

Figure 1

Appearance characteristics of juvenile largemouth bass under Cd exposure,

a: control mortality picture, b: experimental mortality picture, c dissection picture, d: gill cover dissection picture

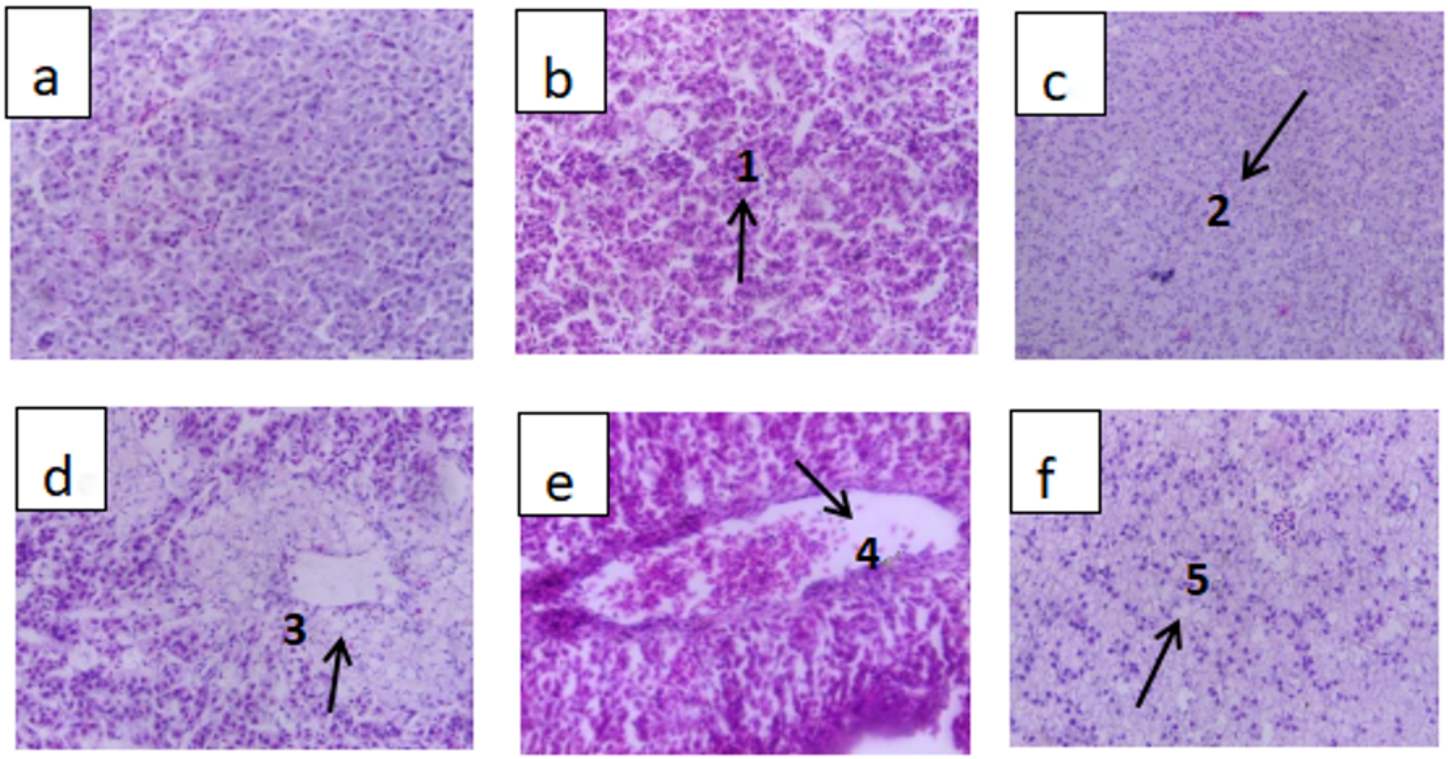

Figure 2

Pictures of Cd exposure on liver pathology sections of juvenile largemouth bass

Note: Liver pathology sections a, b, d, e, f is 40X objective, c is 10X objective, a: control, b: hepatocyte vacuole-like degeneration, c: hepatocyte vacuolation-like degeneration, d: central venous vacuolation-like degeneration, e: central vascular dilatation with cell detachment. f: hepatocellular edema, 1:40X cell vacuolation-like degeneration, 2:10X vacuolation, 3: central venous vacuolation, 4 central venous blood cell detachment, 5: cellular edema.

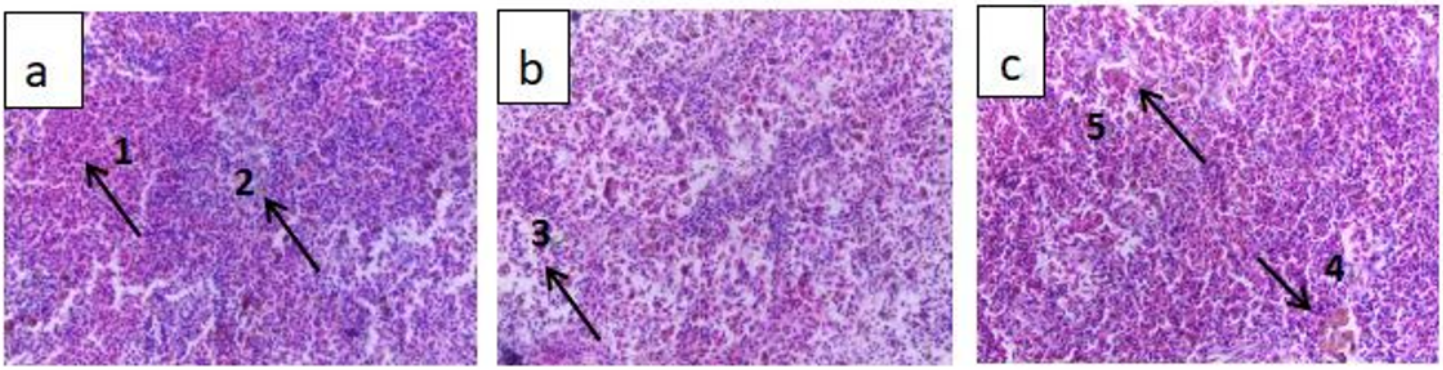

Figure 3

Pictures of Cd exposure on pathological sections in the spleen of largemouth black bass

Note: Spleen sections were obtained under 40X objective, a: control group, b, c: experimental group, 1: splenic red marrow, 2: splenic white marrow, 3: enlarged spleen cell gap, 4: macrophages, 5: disorganized splenic cord arrangement.

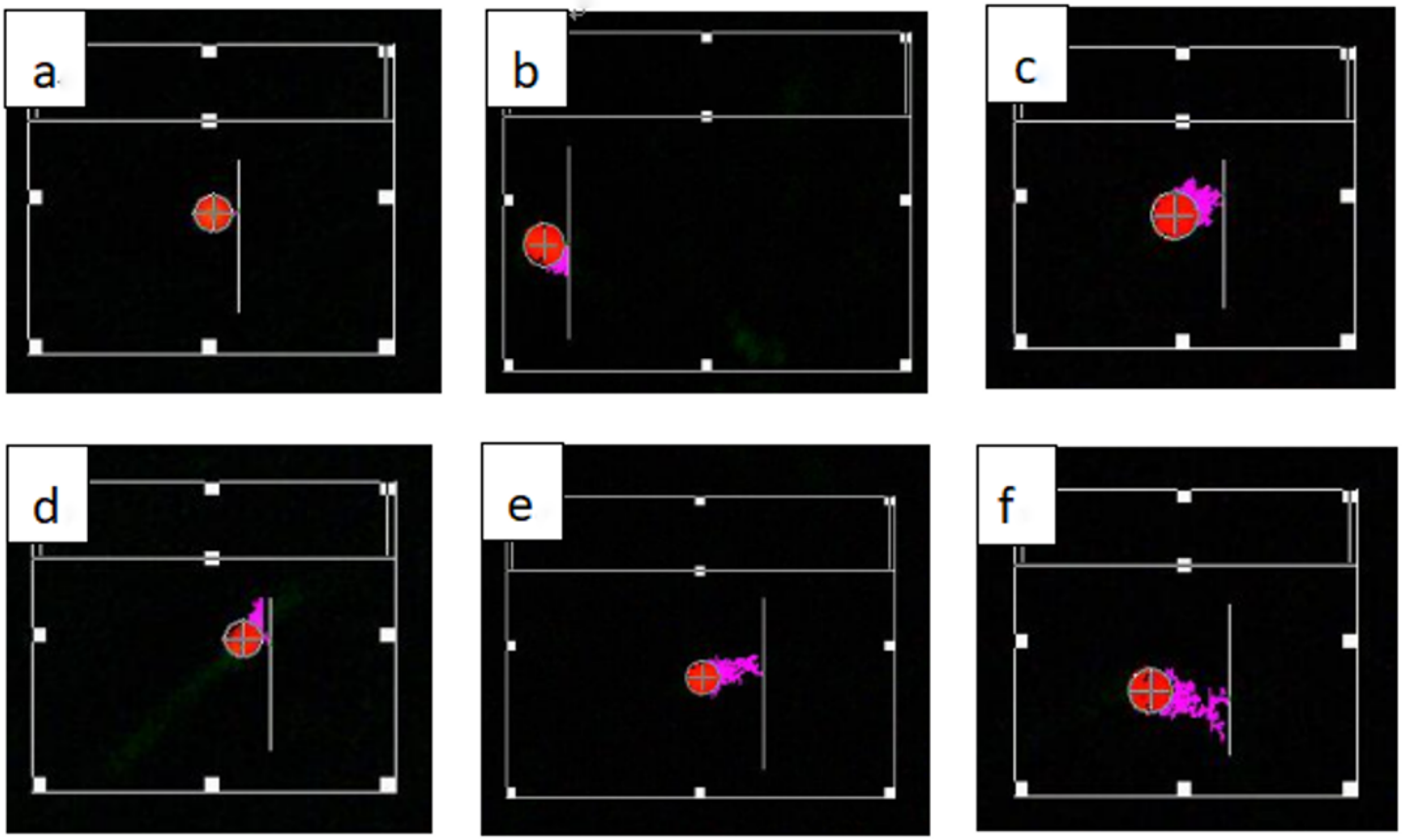

Figure 4

Pictures of DNA damage to the liver of largemouth bass by Cd

Note: all pictures were obtained under 200x objective, a:0mg/L, b:85mg/L, c:90mg/L, d:95mg/L, e:100mg/L, f:105mg/L