Lead Exposure Induces Structural Damage, Digestive Stress, Immune Response and Microbiota Dysbiosis in Intestine of Silver Carp (*Hypophthalmichthys Molitrix*)

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Research Article

Abstract

Lead (Pb) is one of the most common toxic heavy metals in water, and it can cause harm to aquatic animals and humans when released into the environment. In the present study, the effects of Pb exposure on the morphology, digestive enzyme activity, immune function and microbiota structure of silver carp (*Hypophthalmichthys molitrix*) intestines within 96 h were detected. Moreover, the correlation between them was analyzed. The results showed that Pb exposure could severely damage the intestinal morphology on the one hand, including significantly shortening the intestinal villi's length, increasing the goblet cells' number, causing the intestinal leukocyte infiltration, and thickening the intestinal wall abnormally, and on the other hand, increasing the activity of intestinal digestive enzyme (trypsin and lipase). In addition, the mRNA expressions of structure-related genes (*Claudin-7* and *villin-1*) were down-regulated, and the immune factors (*IL-8*, *IL-10* and *TNF-a*) were up-regulated after Pb exposure. Furthermore, data of the MiSeq sequencing showed that the abundance of membrane transport, immune system function and digestive system of silver carp intestinal microbiota was decreased, and the cellular antigens was increased. Finally, the canonical correlation analysis (CCA) found that there were correlations between silver carp's intestinal microbiota and intestinal morphology and immune factors. In conclusion, it is speculated that Pb may damage the intestinal barrier of silver carp, leading the microbiota dysbiosis, which further affects the intestinal immune and digestive function.

Introduction

In recent years, industrialization and related human activities have led to a significant increase in heavy metal content in freshwater environment (Sin et al. 2001; Yuan et al. 2011; Islam et al. 2015). A large number of studies have shown that metals pollutants in the water have negative effects on aquatic organisms, such as serious harm to freshwater and aquatic microorganisms (Kawser et al. 2016; Mohammad et al. 2018; Pradip et al. 2019). In addition, excessive accumulation in fish can even cause toxicity to humans through the food chain (Vargas et al. 2001; Odokuma and O. Ijeomah 2004; Tao et al. 2012). Lead (Pb), as one of the most common pollutants, is carcinogenic, teratogenic and to a certain extent mutagenic to animals like most other heavy metals (Eisler 1998; Bonacker et al. 2005).

Several studies have reported that Pb pollution may affect the structure and function of fish intestines, cause fish intestinal morphology disturbance, and affect the activity of intestinal enzymes activity and immune function (Crespo et al. 2006; Lei et al. 2020). In addition, it is reported that the stability of intestinal structure and function is closely related to the intestinal microbiota structure (Ni et al. 2009), and the studies have shown that water pollutants can destroy the structure of fish intestinal microbiota and cause harm to the physiological functions of fish intestines (Qiao et al. 2019a).

Intestinal microbiota is an important ecosystem in organism, which has a lot to do with metabolism and immunity, and affects the growth, survival and lipid synthesis mechanism of fish (O'Hara and Shanahan 2006; Carmody and Turnbaugh 2012; Daga et al. 2013; Bahrami et al. 2014; Wang et al. 2019). There are many studies on the influence of foreign substances on the structure of fish intestinal microbiota, such

as immunosuppression changed the intestinal microbiota structure of the silver carp, which in turn increased the susceptibility to pathogens (Qi et al. 2019). Although previous studies have suggested that heavy metal has a toxic effect on aquatic organisms, the relevant influence mechanism among the intestinal microbiota, intestinal structure, and intestinal function of fish under Pb exposure is still unclear.

Fish is one of the most important aquatic organisms in the aquatic environment and plays a key-role in human diet and provides an important source of high-quality protein (Milenkovic et al. 2019). Over the last twenty years, world consumption of fish has increased with increasing concerns about its therapeutic and nutritional benefits (Rajeshkumar and Li 2018). Among them, silver carp is one of the most common freshwater fish species in the environment, and is well-known as a food fish in many countries (Buchtova and Frantisek 2011; Islam et al. 2019). Meanwhile, silver carp as a filter-feeding fish, can reduce phytoplankton biomass, control algae bloom, and promote nutrient regeneration in water environment (Fu et al. 2020). Therefore, silver carp plays an important role in maintaining the balance of the water environment. In this study, we simulated the basic environment of the pond, and added Pb pollution. We found that Pb was accumulated in the intestine, and the intestinal structure, digestive enzyme activity, and the expression of immune factors was changed after Pb pollution. Then, the dynamics of microbiota in silver carp intestine were detected and analyzed.

Materials And Methods

Fish maintenance

Purchased silver carp from an aquaculture company in Qingyuan (Guangdong, China), transported to the experimental base in oxygenated polythene bag, and then cultured in an earthen pond The average body length of silver carp was 10.72 ± 0.35 cm and the average weight was 12.31 ± 0.54 g. Before sampling, fish were fed twice a day with a commercial fish food for two weeks as describe before (Liu et al. 2020). During domestication, silver carp has no clinical symptoms (normal swimming and active eating).

Exposure treatment and sample collection

According to the Pirsaheb's method, acute toxicity test and lethal concentration (LC_{50}) test at 96 h of $Pb(NO_3)_2$ were carried out (Pirsaheb et al. 2019). Referring to the method of EPA (version 1.5), design various concentrations of $Pb(NO_3)_2$ to determine the LC_{50} of the silver carp by a series of pre-experiments for acute toxicity tests (Protection Agency 2002). Then, confirmed the safe concentration (SC, SC = LC_{50} × 0.1) of the silver carp according to the previous method (Roopadevi and K Somashekar 2012).

Eighty silver carp were randomly selected from the pond and cultured in a four cubic meter of aquarium (to simulate fish original living environment, the culture water is taken directly from the local ponds where the fish originally lived). Before Pb exposure, twelve silver carp were randomly collected and sampled (L0 h group). Then, fish were exposed to a SC (3.84 mg/g) of $Pb(NO_3)_2$ until 96 h, and twelve silver carp were randomly collected at 6 h, 48 h and 96 h (L6 h, L48 h and L96 h group), respectively. The Pb content in

water was keep around 3.84 mg/L during the experiment. Before sampling, fish were anesthetized in 0.05% MS-222 (Aladdin, China) to euthanize, posterior intestines from each group were sampled to detect the activities of digestive enzyme, Pb content, and RNA expression of gene (n = 3). The samples were immediately stored frozen liquid nitrogen and stored at −80°C until analysis. For histological analyses, posterior intestines were collected from the fish euthanized and fixed in 4% paraformaldehyde solution (n = 3). For DNA Extraction and MiSeq sequencing, posterior intestines from the fish euthanized were collected and fixed in 95% alcohol (n = 3).

Pb accumulation

Posterior intestines from each group were sampled to detect Pb concentration. According to the method published by previous study, the Pb concentrations were measured (Liu et al. 2020). Briefly, the samples were collected and homogenized, using the 4 mL HNO_3 to digest for 1 h, and then put into microwave digestion apparatus (QiYao, China). Microwave digestion procedure: hold at 120°C for 10 min, hold at 150°C for 15 min, and then hold at 190°C for 25 min. After that, the Pb concentration of the sample was determined by ICP-MS (Liu et al. 2020).

Histological analysis

For histological analysis, using 4% paraformaldehyde solution (Biosharp, China) to fix the posterior intestine (1 cm segment before the anus) of all samples (each group, n = 3) for 48 h, and then processed according to the method described before (Liu et al. 2021). Using the light microscope (Nikon, Japan) to observe the number of goblet cells, intestinal villi, infiltration of leucocyte and wall thickness.

Digestive enzyme activities analysis

For digestive enzymes activities analysis, obtain intestinal homogenate: mix the intestine with saline solution in a ratio of 1 : 9, mechanical homogenate, and ice bath for 3-5 min to prepare tissue homogenate. Then, the intestinal homogenate was centrifuged at $4,000 \times g$ for 10 min at 4°C, and the supernatant was collected. Finally, use the total protein assay kit (Jiancheng, China), trypsin assay kit (Jiancheng) and lipase assay kit (Jiancheng) to determine the trypsin and lipase enzymes activities of silver carp intestine according to the manufacturer's instructions.

Quantitative real-time PCR for analysis intestinal structure and immune-related genes

Using Trizol reagent (Vazyme, China) to extract the total RNA of sample according to the method published by previous study. Briefly, tissues (100 mg / samples) were collected and ground under liquid nitrogen conditions, lysed with 1 mL Trizol, and RNA was extracted with chloroform and isopropanol. Dissolved RNA in DEPC-treated water. Dissolved the RNA in DEPC-treated water and then determined the RNA concentration and quality according to the previous method (Tan et al., 2018). Using SYBR® qPCR Master Mix (Vazyme, Nanjing, China) and the Bio-Rad CFX Connect PCR (Bio-Rad, USA) to performe the quantitative real-time PCR (qRT-PCR). The *β-actin* of the silver carp was used as a reference gene

(GenBank accession NO. JX274220.1). The primers of gene shown in Tab. 1. Detected the primer's efficiency and specificity before the qRT-PCR analysis and processed the qRT-PCR as the method of Fu et al (Fu et al. 2019).

Table 1 The primer sequences used in this study for qRT-PCR.

Primers	Nucleotide Sequence (5′→3′)
RT-*β-actin*-F	TCTGGTGAAGGCTGGTTTTGC
RT-*β-actin*-R	CTTTCTGACCCATACCGACCAT
RT-*IL-8*-F	TGTTGCTGTGGCATTTCTGACC
RT-*IL-8*-R	CAGTGAGGGCTGGGAGGGTA
RT-*TNF-α*-F	CAAAGTCAGGCGTATGGCGG
RT-*TNF-α*-R	TGGCAGCCCTGGAAGTGG
RT-*IL-10*-F	AATCCCTTTGAGTTTGCCACC
RT-*IL-10*-R	TGCTTTTCTCTCTTTGATGCCA
RT-*villin-1*-F	CTTGTGCCCTGTCCACCTAATA
RT-*villin-1*-R	GCCTTGCCCAGCCAATAAT
RT-*Claudin-7*-F	CAAGGTGTACGACTCCATCCTACA
RT-*Claudin-7*-R	CACTTCATGCCCATGCTGG

DNA Extraction

The posterior intestinal samples of all groups (each group, n = 3) were sent to Guangzhou JiRui Gene Technology Co. Ltd. (China) for extraction of DNA and PCR amplification by Illumina MiSeq Sequencing platform. PCR was performed from V3~V4 variable regions of 16S rRNA to taxonomically identify the bacteria (Xun et al. 2019).

16S rRNA gene amplification sequence analysis

Constructed and sequenced the high-throughput sequencing library according to previous methods (Abdolrasouli et al. 2015; Yang et al. 2015). The "CCTACGGRRBGCASCAGKVRVGAAT" sequence as the upstream primers and "GGACTACNVGGGTWTCTAATCC" sequence as the downstream primers were used to amplify the DNA samples, including V3 and V4 (two highly variable regions). In addition, the PCR product of 16S rDNA was subjected to NGS sequencing. Finally, read the sequence information of the sample and determine the library quality.

Data analysis

The results were analysed by SPSS 19.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) and R. Using image pro plus program to analyze intestinal structure indicators. One-way ANOVA was used to check for the significance of difference between the means of each group. Data were expressed as (Mean ± S.D.). Difference was considered significant at $0.01 < p < 0.05$(*). Extremely significantly difference was considered significant at $p < 0.01$(**).

Results

Pb accumulation in intestines

Determining the Pb concentration of silver carp intestine by ICP-MS. As shown in Fig. 1, the concentration of Pb in intestines significantly increased to the highest concentration (118.39 mg/kg) at 96 h after Pb exposure.

Histologic observations and analysis of intestines

Observing the morphology of silver carp intestines by a microscope (Nikon, Japan) (Fig. 2A). As shown in Fig. 2B, after Pb exposure for 48 h, the relative intestinal wall thickness was increased significantly and reached the highest level (up to 1.27-fold, $p < 0.01$). Besides, the relative depth of intestinal crypts decreased and reached the lowest level at 96 h (down to 0.41-fold, $p < 0.01$) (Fig. 2C), while the goblet cells' number in intestine increased and reached the highest level at 48 h (up to 3.17-fold, $p < 0.01$) (Fig. 2D).

The activity levels of trypsin and lipase in the intestines

Using the enzyme activity kit to determine the relative activity level of trypsin activity and lipase activity of silver carp intestine after Pb exposure. As shown in Fig. 3A, the relative activity of trypsin in the intestine increased significantly and reached the highest level after 6 h of Pb exposure (up to 6.38-fold, $p < 0.01$). Similarly, the relative activity level of lipase increased significantly and reached the highest level after 48 h (up to 3.55-fold, $p < 0.01$) (Fig. 3B).

The expression of immune and structure -related genes in the intestines

The mRNA expressions of immune-related genes (*IL-8*, *IL-10* and *TNF-α*) and structure-related genes (*Claudin-7* and *villin-1*) in intestine were measured by qRT-PCR. After Pb exposure, as shown in Fig. 4A and B, the expression of *TNF-α* and *IL-10* in intestine significantly increased to the highest level at 48 h (up to 2.36-fold, $p < 0.01$, and 2.28-fold, $p < 0.01$, respectively) after Pb exposure and gradually recovered at 96 h. In Fig. 4C, the expression of *IL-8* in intestine significantly increased to the highest level at 6 h (up to 2.76-fold, $p < 0.01$) and gradually recovered later. On the contrary, the expression of *Claudin-7* and *villin-1* in intestine decreased significantly and reached the lowest level at6 h after Pb exposure (down to 0.31-fold, $p < 0.01$, and 0.11-fold, $p < 0.01$, respectively) (Fig. 4D and E).

Characteristics of fish microbiota structures and diversity analysis

Quality and chimera filtration from effective sequences ranging 21,252 to 101,185 (each group, n = 3) per sample by Illumina MiSeq platform, a total of 779,971 quality-filtered sequences were obtained. A 97% similarity cutoff was applied to cluster the high-quality sequences of the microbiota in intestines which divided into 615 operational taxonomic units (OTUs) (excluding monad sequence). Among them, 324 OTUs were shared by all samples (Fig. S1).

Good's coverage of different samples was more than 99% (Tab. 2), and the statistical estimates of species richness and diversity indexes from each sample were presented in Tab. 2. ACE index ranged from 261.56 to 354.71, while chao1 index ranged from 261.07 to 362.55. The trends of ACE and chao1 after Pb exposure were shown in Fig. 5A and B. The shannon index ranged from 5.12 to 6.22, while the simpson index ranged from 0.90 to 0.97. In all samples, the shannon index and simpson index were the lowest at 48 h after Pb exposure (Fig. 5C and D). The corresponding rarefaction curves tended to reach the saturation plateau (Fig. S2).

Table 2 Diversity and Richness indexes as calculated by MOTHUR software (ver. 1.30.0). Operational taxonomic units (OTUs) are defined at 97% sequence similarity.

Samples	Total sequences passed quality check	Total OTUs	Diversity and Richness indexes[1]				Good's coverage
			Ace Index	Chao1 Index	Simpson Index	Shannon Index	
L0 h	221,047	884	261.56 ± 11.25	261.07 ± 8.99	0.96 ± 0.01	5.85 ± 0.24	0.999
L6 h	231,612	958	273.66 ± 47.05	273.07 ± 46.12	0.97 ± 0.03	6.22 ± 0.82	0.997
L48 h	122,601	905	265.86 ± 28.48	271.32 ± 29.39	0.90 ± 0.04	5.12 ± 0.79	0.998
L96 h	204,711	844	354.71 ± 103.11	362.55 ± 102.98	0.96 ± 0.02	6.02 ± 0.96	0.999

1: Values represent the average ± S.D (each mean value of 3 determinations).

Change in the bacterial community compositions

Main bacterium of silver carp at the phylum level was shown in Fig. 6, mainly including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*, and *Fusobacteria*. Before Pb exposure, the most abundant bacterium in intestine was *Proteobacteria* (29.47%). After Pb exposure, the most abundant bacterium in intestine was changed to *Firmicutes* (36.05%) at 6 h and then changed to *Bacteroidetes* at 48 h (39.50%) and *Proteobacteria* at 96 h (33.37%).

In Fig. 7A, 29 different family of microbiota were confirmed in silver carp's intestine and six of them were dominant. They were *Aeromonadaceae*, *Weeksellaceae*, *Burkholderiaceae*, *Flavobacteriacea*e, and

Erysipelotrichaceae. After Pb exposure for 6 h, the abundance of *Aeromonadaceae* in intestines were increased significantly and reached to the highest level (Fig. 7B). The relative abundance of *Weeksellaceae* and *Burkholderiaceae* significantly increased to the highest level at 48 h, and then recovered (Fig. 7C and D).

Canonical correlation analysis

CCA was carried out to analyze the relationship between intestinal microbiota, intestinal structure, immune factors, digestive factor, and Pb content. As shown in Fig. 8A, the goblet cell number was positively correlated with the intestinal microbial community in L48 h group, the intestinal crypt was correlation with intestinal microbial community in L0 h and L6 h groups, and the Pb content was positively correlated with the intestinal Pb content in L96 h group. In Fig. 8B, the expression of *TNF-α* and *IL-10* in silver carp were positively correlated with the intestinal microbial community in L48 h group. Meanwhile, the expression of *IL-8* correlated with intestinal microbial community in L6 h group. In Fig. 8C, the expressions of trypsin and lipase in intestine were correlated with intestinal microbial community in L6 h and L48 h groups, respectively.

Predictive functional profiling of microbial communities

PICRUST was used to predictive the functions of the microbiota in intestines. In Fig. 9A, 25 functions were identified by Cluster of Orthologous Groups of proteins (COG) analysis in this study. In COG analysis, except for general function prediction only (relative abundance from 11.48 to 11.72%), the highest represented category was transcription (7.04 to 8.07%) and amino acid transport and metabolism (7.87 to 8.20%) function categories. These COG function classification results indicated that the biological functions profiles of all groups were similar with each other.

Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, the functions of microbiota in carp intestinal were divided into six categories at level one, namely genetic information processing, cellular processes, human diseases, environmental information processing, metabolism and organismal systems, among which metabolism (47.54-49.26%) was the most abundant (Fig. 9B). At level two, membrane transport (10.94-12.94%) was the most abundant, while immune system and digestive system was the lower abundant but significant changes (Tab. S1). Similarly, cellular antigens had a significant change at level three (Tab. S2). The relative abundance change of membrane transport, immune system, digestive system and cellular antigens were shown in Fig. 9C, D, E and F, respectively. The relative abundance of membrane transport function of intestinal microbiota significantly decreased to the lowest level at 48 h, and the relative abundance of immune system function of microbiota also decreased at 6 h. In contrast, the relative abundance of digestive system and cellular antigens function of intestinal microbiota increased consistently and reached the highest level at 48 h.

Discussion

Nowadays, the water environment is generally polluted by heavy metals, which seriously affects the survival of fish in the water (Luczynska et al. 2018). Pb may cause damage to human nerves, hematopoietic system and kidneys (Sivaperumal et al. 2007; Hasschon et al. 2008). It has been reported that the accumulation of Pb in fish may adversely affect the structure and function of fish intestines. In this study, we found that Pb was enriched in the intestine of silver carp and the changes in the morphological structure, digestive enzyme's activity, and immune factors of the fish intestine. In addition, we also used high-throughput sequencing technology to detect changes of microbiota in silver carp's intestine after Pb exposure. CCA analysis showed that there is a significant correlation between the silver carp's intestinal microbial community and intestinal structural indicators, digestive enzyme activity levels and immune factors. The function prediction analysis further indicated that the changes of silver carp intestinal structure and function may be related to the intestinal microbiota. Therefore, Pb may destroy the structure and function of carp intestine by destroying the composition of silver carp intestinal microbiota.

Previous studies reported that heavy metals exposure could influence the intestinal function such as active transport, immune response, and so on (Iturri and Peña 1986; Wang and Chen 2015). In this study, the concentration of Pb in the silver carp intestine was significantly increased after Pb exposure (Fig. 1). Moreover, the intestinal morphology changed after Pb exposure, including infiltrated leukocytes, increased goblet cells' number, shallowed the depth of crypts, and increased intestinal wall thickness (Fig. 2). Crypts and intestinal wall thickness changes are related to inflammation, and the goblet cells' number increased and leukocyte infiltration will further promote inflammation (Lofgren et al. 2002; Qiao et al. 2019b; Liu et al. 2020). These results were consistent with the mRNA expression of structure-related genes (*Claudin-7* and *villin-1*) and immune-related genes (*TNF-a*, *IL-8* and *IL-10*) (Fig. 4). Meanwhile, the activity levels of intestinal digestive enzyme (trypsin and lipase) also increased significantly after Pb exposure (Fig. 3), which may be due to the compensation mechanism triggered by the environmental stress (Zare et al. 1996; Cao et al. 2010; Babaei et al. 2020). The data at the genetic, protein and structural levels indicated that Pb exposure can cause physiological damage to the intestinal barrier, which may be related to intestinal digestive function and immune response.

Fish intestinal microbiota plays an important role in intestinal morphology, digestion and immunity (Liu et al. 2020). After Pb exposure, the diversity of silver carp intestinal microbiota decreased (Fig. 5). The reduction of the diversity of fish intestinal microbiota will cause disorders of intestinal structure and immune function, destroy digestive power and function, and lead to the occurrence of diseases (Kuno et al. 2016; Passos and Moraes 2017).

After Pb exposure, *Firmicutes* and *Bacteroidetes* became the most abundant bacterium at the phylum level in intestine (Fig. 6). *Firmicutes*, a common phylum in the intestines of fish (Burgos et al. 2018; Meng et al. 2020). The increase of *Firmicutes* in relative abundance in this study may be due to its higher tolerance to heavy metals (Guo et al. 2019). *Bacteroidetes*, a Gram-negative bacterium, which closely relate to the occurrence of intestinal inflammation (Marchesi et al. 2016). At the family level, both *Aeromonadaceae*, *Burkholderiaceae* and *Weeksellaceae* in silver carp intestines were increased after Pb

exposure (Fig. 5). *Aeromonadaceae*, belonging to *Gammaproteobacteria*, is a common pathogenic bacterium in fishes, which destroys the intestinal lining, causes intestinal cell damage, promotes inflammation and alter intestinal morphology (Ring et al. 2004; Bhowmik et al. 2009; Dikow 2011; Mohamed et al. 2015). *Burkholderiaceae* is belonging to *Betaproteobacteria*, which can promote inflammation in various epithelial cells (Yang et al. 2006; Liu et al. 2020). This may be related to the up-regulation of intestinal *TNF-α* and *IL-8*. *Weeksellaceae* is a pathogen that can releases the proinflammatory cytokines and degrades intestinal glycoprotein (Ruseler-van et al. 1989; Nagy et al. 1998; Zhang et al. 2017). Glycoprotein is one of the main components of the intestinal epithelial cell membrane, which affects the structure of the epithelial membrane and the morphology of the intestinal villi (Quaroni et al. 1979; Gupta and Waheed 1992). So, the up-regulated of *Weeksellaceae* abundance may induce the intestinal morphology changes.

The CCA results showed the relationship between the intestinal microbiota and intestinal structure, immune factors, digestive enzyme, and Pb content in intestines, and the structure index in Fig. 8. As expected, CCA proved that increased expressions of immune factors (*IL-8 IL-10* and *TNF-α*) and increased level of digestive enzyme (trypsin and lipase) activity in the intestines closely related to the change of carp intestinal microbial community. Previous studies have also found that changes in intestinal microbial structure can affect the morphology of intestinal structure, digestive function and the expression of immune factors in fish (Geovanny and Jose 2010; Navarrete et al. 2013; Passos and Moraes 2017; Gao et al. 2017). As a filter-feeding fish, the structure and function of silver carp intestines maybe more closely related to intestinal microbiota (Dong and Li 1994). The results in our study indicated that changes in intestinal microbiota could affect the structure and immune function of the intestine.

Conclusion

In this study, the effects of Pb exposure on silver carp intestinal structure, digestive enzyme, and immune function within 96 h were detected. Through CCA analysis, we speculated that these effects may be related to the changes of intestinal microbial community. In addition, the function prediction results were also consistent with the correlation analysis. Overall, the results of this present study provide new information about the toxic effects of Pb on silver carp and gain a better understanding of the relationship between intestinal microbes and intestinal structure and function.

Declarations

Conflict of Interest

The authors declare no conflict of interests

Credit Author Statement

Haisu Liu: Writing - review & editing, Investigation, Visualization.

Hang Zhang: Investigation, Writing - review & editing:; Investigation.

Sanshan Zhang: Writing - review & editing, Resources.

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Data Availability Statements All data generated or analysed during this study are included in this published article (and its supplementary information files).

Compliance with ethical standards

Ethics statement All animal experimental procedures were carried out in accordance with the Regulations for Animal Experimentation of South China Normal University (SCNU-SLS-2020-001), and the animal facility was based on the National Institutes of Health guide for the care and use of Laboratory Animals (NIH Publications No. 8023).

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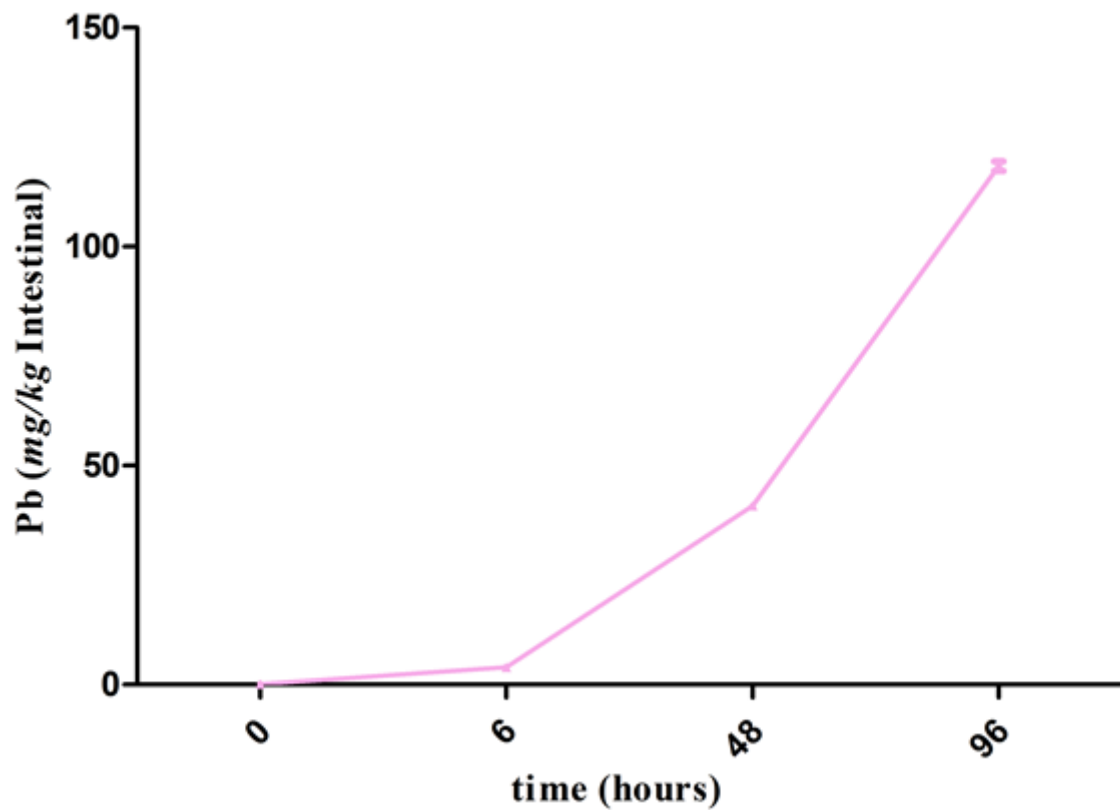
Figures

Figure 1

Accumulation of Pb in silver carp within 96 h.
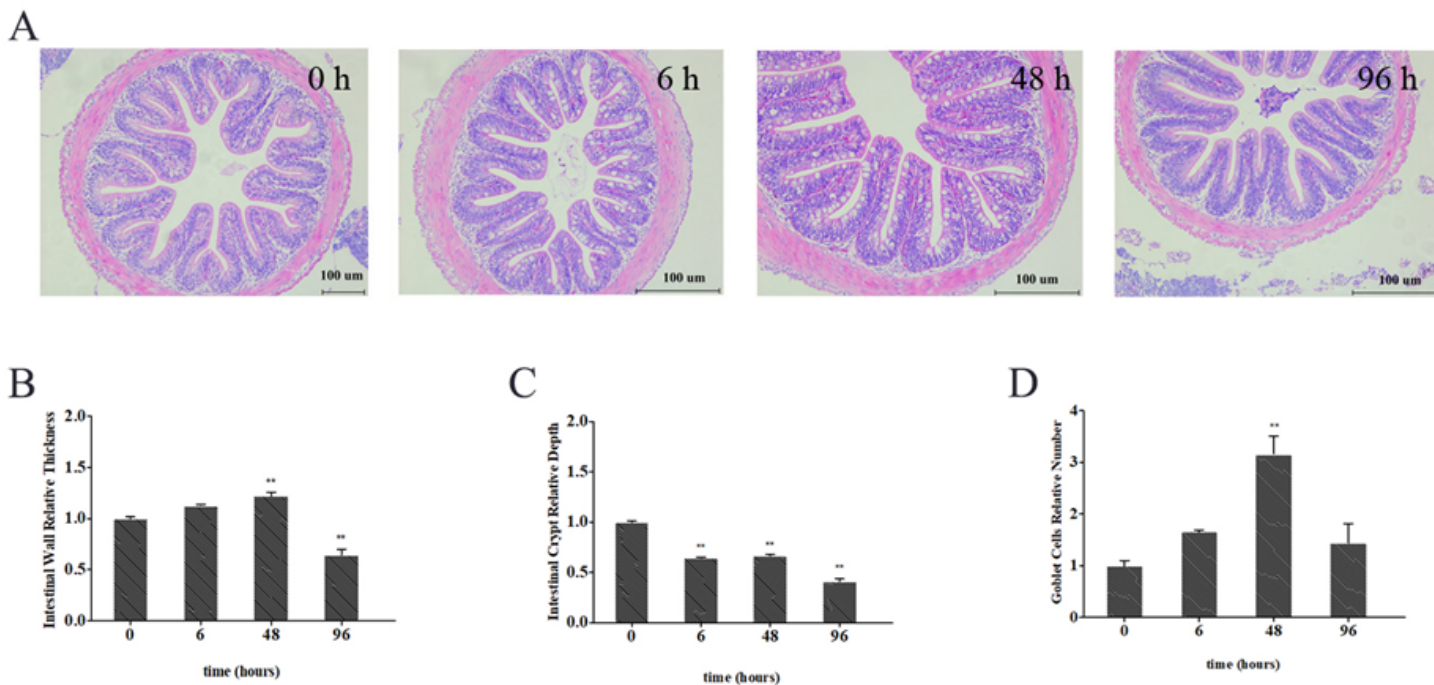

Figure 2

Representative micrographs of intestines from silver carp (A). intestinal wall relative thickness (B), intestine crypt relative depth (C), and goblet cells relative number (D) were analyzed. Leukocyte infiltration occurs in the area indicated by the arrow. The asterisk represents a statistically significant difference (*0.01 < p < 0.05 and **p < 0.01).
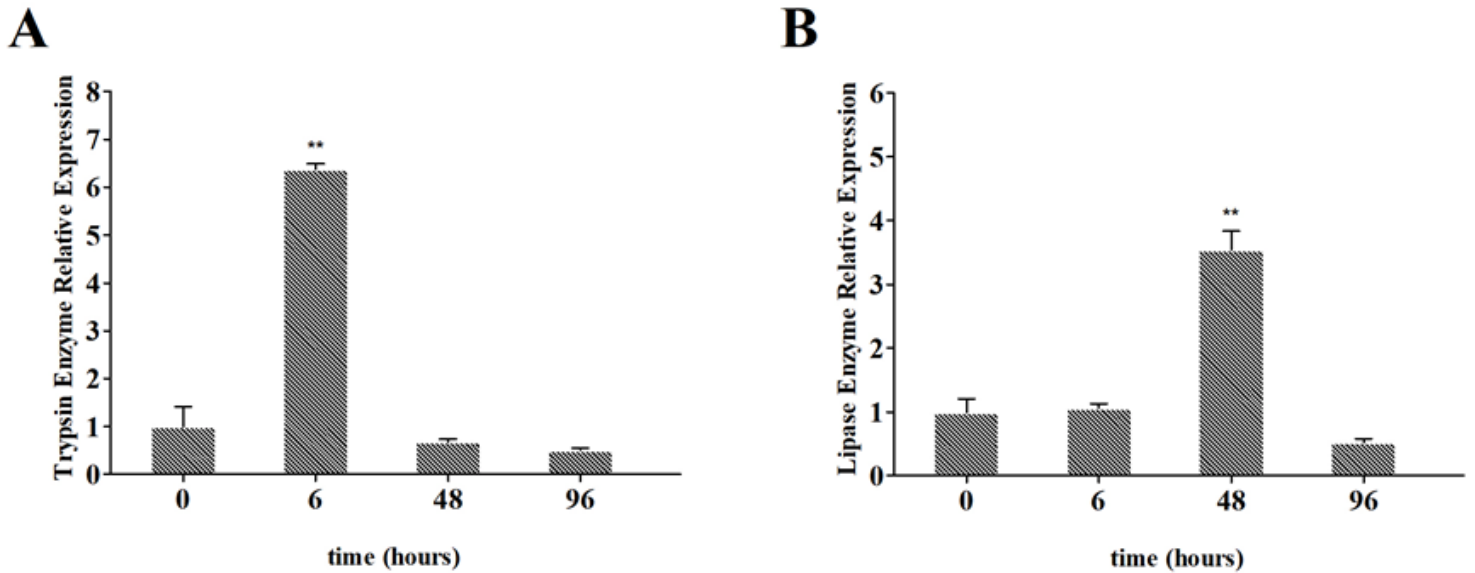

Figure 3

Relative enzyme activity of trypsin (A) and lipase (B) in silver carp intestines.
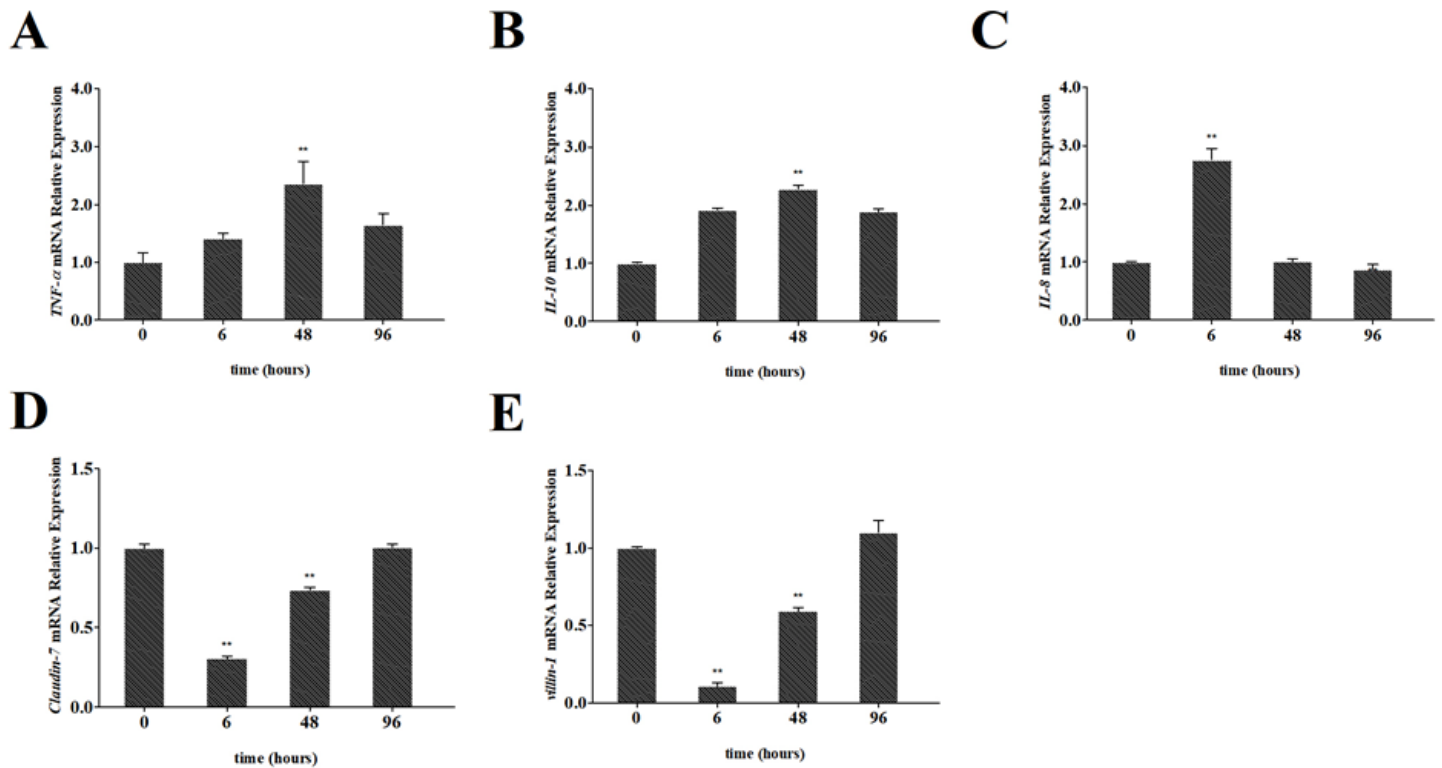
Figure 4

Relative expression of TNF-⍺ (A), IL-10 (B), IL-8 (C), Claudin-7 (D) and villin-1 (E) in the intestines of silver carp. The mRNA levels of each gene were normalized to that of β-actin. The asterisk represents a statistically significant difference (*0.01 < p < 0.05 and **p < 0.01).
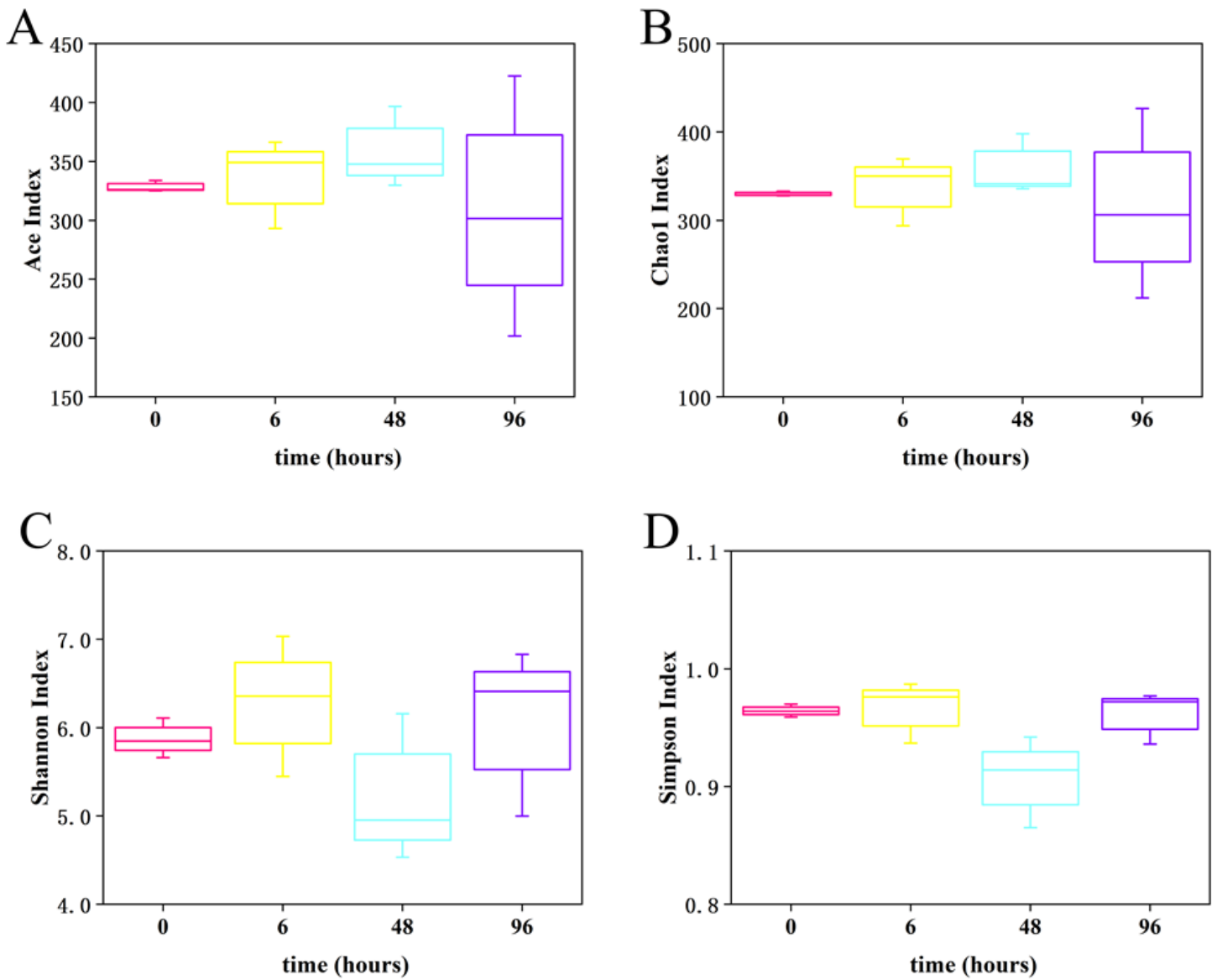

Figure 5

The effects of Pb on the richness and diversity of microbiota in the silver carp intestines in each sample. A. Ace index. B. Chao1 index. C. Shannon index. D. Simpson index.
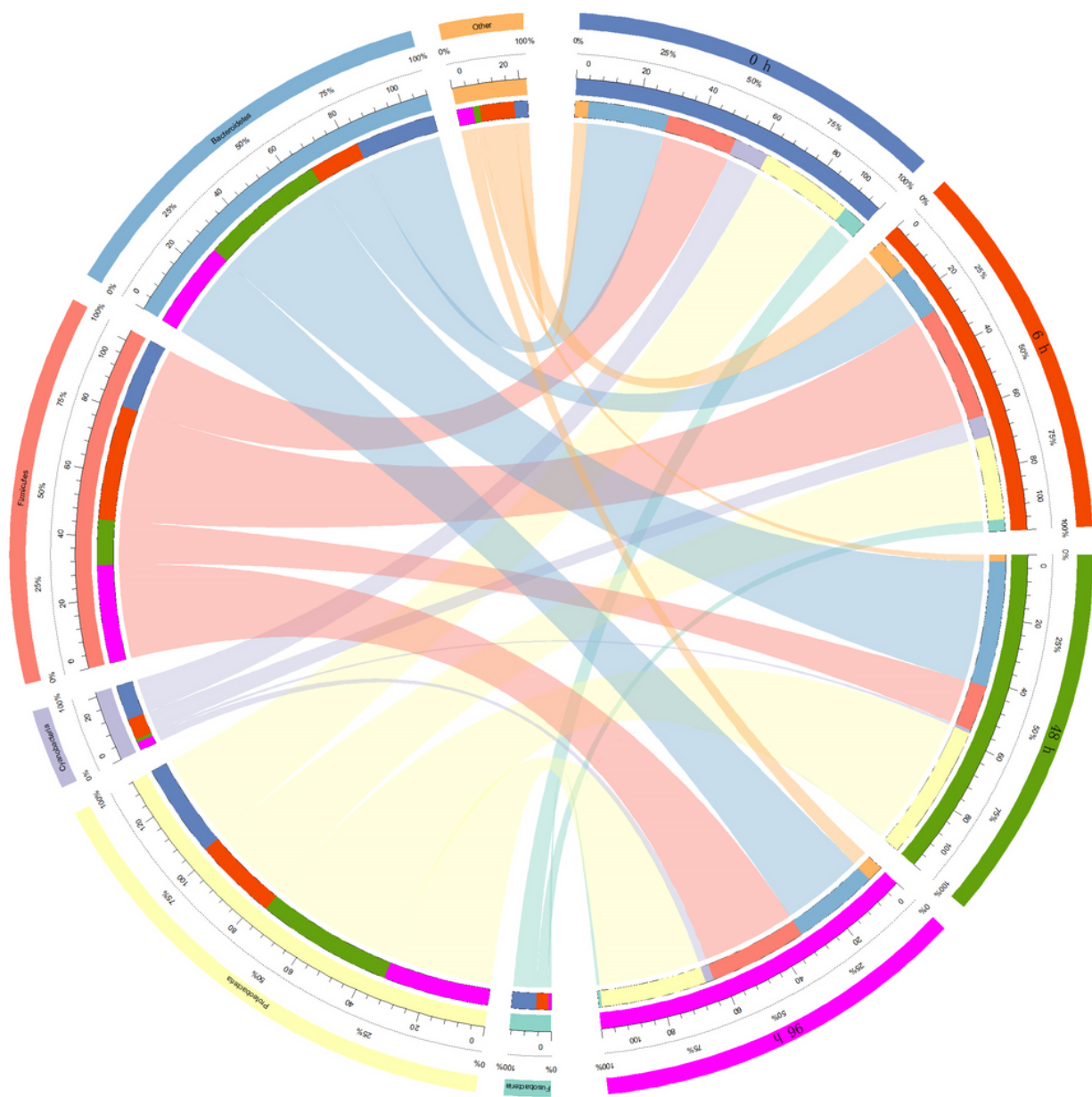
Figure 6

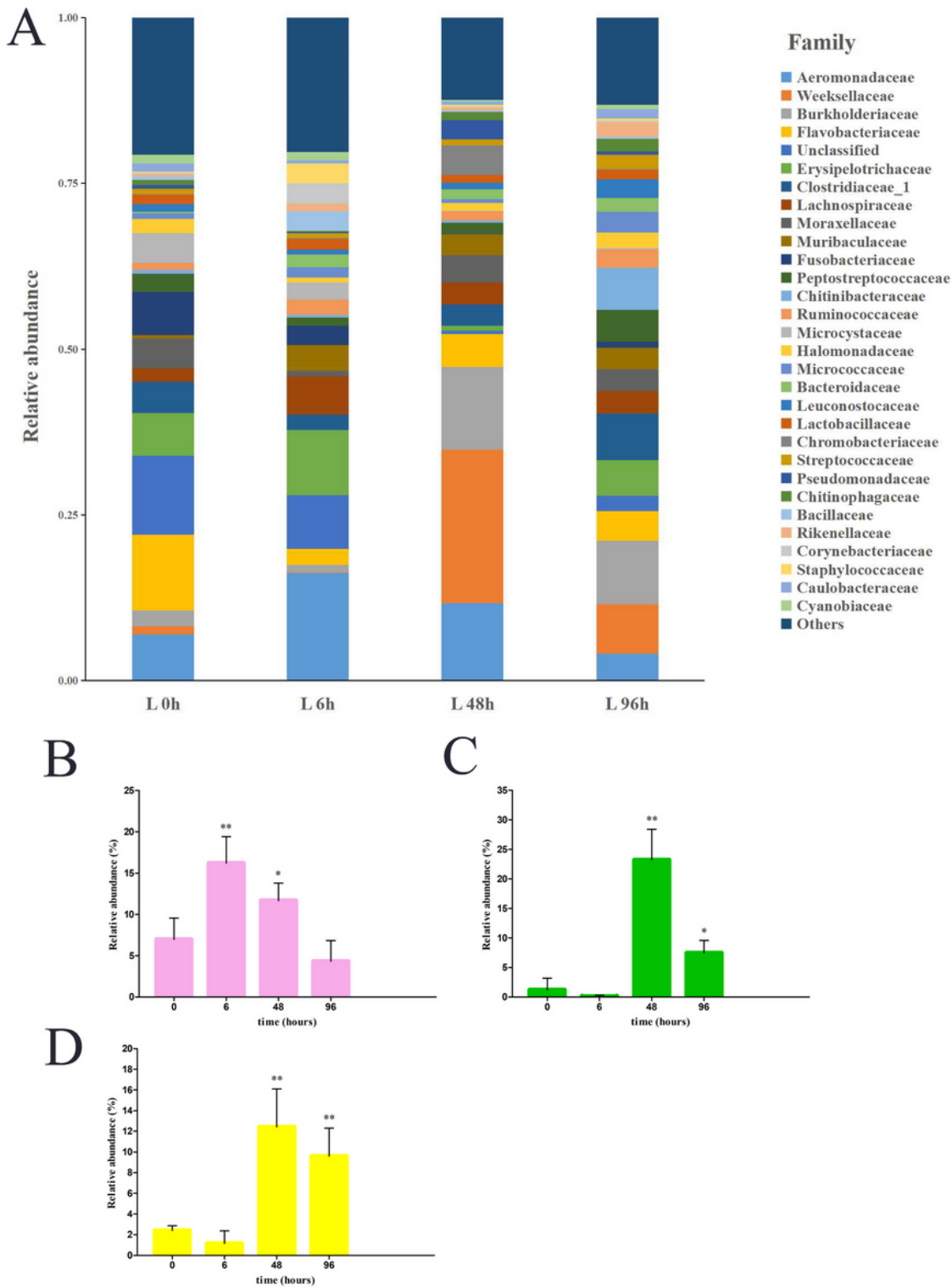The intestinal microbiota composition of the silver carp's intestines at phylum level.

Figure 7

The effects of Pb exposure on the composition and relative abundance of microbiota silver carp's intestines (A) at the family level. Relative abundance of Aeromonadaceae (B), Weeksellaceae (C) and Burkholderiaceae (D) were measured. The asterisk represents a statistically significant difference (*0.01 < p < 0.05 and **p < 0.01).
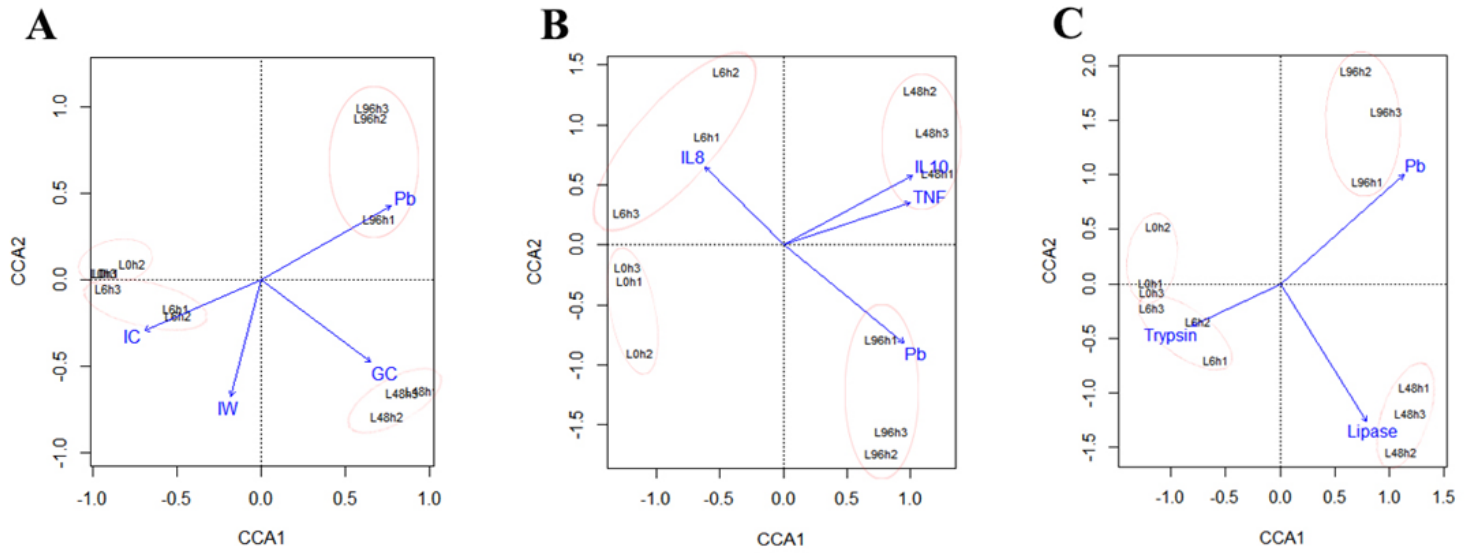
Figure 8

Canonical correlation analysis between intestinal microbial community structure of H. molitrix and intestinal structure (A), immune factors (B), digestive factor (C) and Pb content.
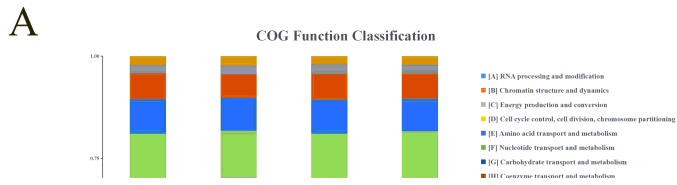
A

COG Function Classification

- [A] RNA processing and modification
- [B] Chromatin structure and dynamics
- [C] Energy production and conversion
- [D] Cell cycle control, cell division, chromosome partitioning
- [E] Amino acid transport and metabolism
- [F] Nucleotide transport and metabolism
- [G] Carbohydrate transport and metabolism
- [H] Coenzyme transport and metabolism

Figure 9

Functional prediction maps of all samples. A. he COG function classification of different groups. B. The KEGG function classification of the groups. C. Relative abundance changes of membrane transport in silver carp's intestines. D. Relative abundance changes of immune system in silver carp's intestines. E. Relative abundance changes of digestive system in silver carp's intestines. F. Relative abundance

changes of cellular antigens in silver carp's intestines. Asterisk represents a statistically significant difference (*0.01 < p < 0.05 and **p < 0.01).