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Lanthanum exerts acute toxicity and histopathological changes in gill and liver tissue of rare minnow (*Gobiocypris rarus*)

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Abstract We evaluated the acute toxicity effects of lanthanum (La(III)) on gill and liver of rare minnow (*Gobiocypris rarus*). The median lethal concentration of La (III) at 96 h was 1.92 mg L^{-1}. Rare minnow were reared in freshwater and exposed to 0.04, 0.08, 0.16, 0.32 and 0.80 mg L^{-1} La (III) for 21 d. Gill and liver samples were analyzed by light microscopy. The main histopathological changes induced by La (III) in gills were epithelial lifting, filamentary epithelial proliferation,edema, lamellar fusion, desquamation, and necrosis. Histopathological changes induced by La (III) in the liver included dilation of sinusoids, focal congestion, pyknotic nuclei, karyohexis and karyolysis, vacuolar degeneration, and numerous necrosis areas. Hypsometric analysis indicated significant changes in the measures of gill dimensions (average length, width, area), suggesting metabolic disturbance (gas exchange) upon La (III) exposure. The result showed that La (III) severely affects fish gill and liver.

Keywords Toxicity · *Gobiocypris rarus* · La(III) · Histopathological · Gill · Liver

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Introduction

"Rare earth elements" (REE) is a general designation for 17 chemical elements in the periodic table, including scandium, yttrium, and the lanthanum series. Because REE have specific effects on biological organisms, they have been widely used in agriculture, forestry, animal husbandry, aquaculture, and medicine (Qu and Xin 2001). For example, nutrient digestibility and meat quality were improved in broiler chickens whose diet was supplemented with REE-enriched yeast (Caia et al. 2015). In addition, in comparison with the fish fed with no REE-ehitosan chelate, crucian carp fed with 0.08% REE-ehitosan chelate had increased intestinal amylase, lipase activity, and protease activity (Liu et al. 2008).

REE have been approved by the Ministry of Agriculture China as feed additives. However, previous studies have revealed adverse effects of REE on plants and animals. In a rodent model, sub-acute exposure to indium during the period of sexual maturation affected male reproductive function during spermatogenesis, through an increase in oxidative stress and DNA damage of sperm chromatin (Lee et al. 2016). Cerium chloride heptahydrate ($CeCl_3 \cdot 7H_2O$) induced muscle paralysis in grasshoppers (*Melanoplus sanguinipes*) (Allison et al. 2015). REE also had toxic effects on human beings. Blood analyses showed that there are alterations in the values of many biochemical indices (WBC, PLT, and blood Ca) in people living in regions with high levels of REE (Zhang et al. 2000). These alterations are thought to be caused by prolonged intake of REE through food (Zhang et al. 2000).

The safety of aquatic ecosystems is in danger of contamination owing to waste water discharge in exploitation, smelting, and separation of REE, and also owing to surface runoff and leakage of REE used in agriculture. In recent

decades, large-scale exploitation of REE mines has led to increasingly serious ecological problems (Bai and Yang 2012). For example, research showed that in the natural waters of Taihu Lake the concentration of total dissolved REE was 46.86–112.5 ng/L (Zhu et al. 2011). But in the mining area (Jiangxi Province, CHN), the pH values (3.6–5.8) and total dissolved REE concentration (363–117520 μg/L) of surface waters exceed the national standard GB3838-2002, and regional background values (Liu et al. 2015).

In contrast to research performed on plants and terrestrial animals, research on the effects of REE on aquatic organisms has been extremely deficient, especially regarding their toxic effects and toxicological mechanisms. Therefore, the purpose of this study was to investigate toxic effects of lanthanum (La (III)) on rare minnow, and to explore its toxicological mechanisms.

Rare minnow (*Gobiocypris rarus*) is an endemic freshwater species in China. In nature, rare minnow is distributed mainly in shallow and static habitats, such as ditches, rice fields, and slow flow rivers. Rare minnows have considerable adaptability to environmental factors, such as temperature(0–35 °C), carbon dioxide concentration (up to 70 mg L^{-1}), and dissolved oxygen (as low as 0.5 mg L^{-1}). Rare minnows are a recommended test organism, according to the Guidelines for the Testing of Chemicals (Ministry of Environmental Protection of China).

Materials and methods

Fish

Fish of similar size (29.3 ± 3.0 mm, 0.51 ± 0.03 g; mean ± SD), were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Four hundred individuals were carefully transported to the laboratory in a sealed plastic bag with abundant oxygen. The plastic bag was placed inside a large water bucket for 20 min, until the inside and outside temperature were the same (approximately 21 °C). Fish were then removed from the plastic bag and placed into the plastic aquarium (300 L) equipped with an air compressor.

Fish were fed with red worms (Frozen Artificial Feed, Tianjin, China; crude protein 6.8%, crude fiber 0.5%, crude fat 0.8%, and moisture 89.8%) twice a day. Feed residuals and excrement were siphoned off. A third of the water was replaced daily to prevent excessive ammonia accumulation. The period of acclimatization was 7 d. The photoperiod was controlled, and consisted of a 12 h light/dark cycle. During the acclimatization period the activity of the experimental fish was normal (calm, active feeding,and cluster swimming), and mortality was below 5%. After acclimatization, fish were randomly divided into controls or experimental groups.

Water

Physical and chemical properties of the water (temperature, dissolved oxygen, pH, oxygen saturation) were measured daily, according to the guidelines of the chemical acute toxicity test in rare minnow (GB/T 29763-2013, China). Deionized water was aerated for 2 d before using for fish tests. An electromagnetic air compressor connected to stone aerators was set to provide dissolved oxygen in each experimental unit.

Test chemical and determination of median lethal concentration (LC50)

Lanthanum chloride heptahydrate ($LaCl_3{\cdot}7H_2O$, assay grade 99.999% purity) was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). The test substance was dissolved in test water to 10 mg L^{-1} (stock solution, La (III)), and then diluted to the different experimental concentrations. Fish were acclimated to glass aquaria containing 5 L of test water for 24–48 h before La (III) exposure. Fish were not fed during the final acclimatization period or the test period.

In a preliminary trial, 40 fish were exposed to eight different La (III) concentrations (0.0, 0.1, 0.5, 1.0, 5.0, 10.0 and 40.0 mg L^{-1}) for 96 h, and fish mortality was calculated. Based on the mortality rates observed in the preliminary trial, six concentrations were selected for the final experiment: 1.00, 1.58, 2.51, 3.98, 6.31, and 10.00 mg L^{-1}. These concentrations were selected because there was 100% mortality in the highest concentration, and 0% mortality in the lowest concentration (American Public Health Association, APHA 1980). There were three replicates of each experimental group. The final experimental groups were: La (III) at 1.00, 1.58, 2.51, 3.98, 6.31 and 10.00 mg L^{-1}, and a negative control with no La (III). Each experimental group had three replicates and each replicate consisted of eight individuals randomly selected. Fish were placed in aerated glass aquaria for 96 h. The test water was renewed every other day. Cumulative mortality was recorded at 24, 48, 72, and 96 h of exposure.

Histopathology

According to the calculated LC_{50} value at 96 h, five test La (III) concentrations were selected:0.04 mg L^{-1}, 0.08 mg L^{-1}, 0.16 mg L^{-1}, 0.32 mg L^{-1}, and 0.80 mg L^{-1}. Fish were exposed to the different La (III) concentrations for 21 d, or left untreated (control), in a semi-static system. Water was renewed on alternate days to maintain the concentration of

the toxicant. There were three replicates for each group, and each group consisted of 24 fish. Fish were fed to satiation twice a day. Feed residuals and excrement were siphoned off.

The liver and gills are organs often used for the analysis of the effects of pollutants in fish, through histological examination (Jabeen and Chaudhry 2013; Galat et al. 2010; Figueiredo-Fernandes et al. 2007; International Council for the Exploration of the Sea 1997). Thus, at the end of the exposure period, the liver and gills of two individuals were collected randomly from each group. The specimens were immediately fixed in 10% neutral-buffered formalin for 48 h, then dehydrated, cleared in xylene, and embedded in paraffin. Tissue sections of 4 mm were sliced, stained with hematoxylin-eosin, and examined under light microscopy (Olympus BX41 photomicroscope, Tokyo, Japan). Histopathological changes were photographed. The dimensions of 10 gill lamellae were recorded in duplicate samples. According to a technique reported previously (Huges and Perry 1976), the length, width, and longitudinal area of gill lamellae were measured using image analysis software (Wuhan Image Technology Co., Ltd., Wuhan, CHN).

Statistical analysis

LC_{50} values for different exposure times were calculated using the Probit Analysis Statistical Method and the software SPSS, version 19.0 (IBM,Chicago, IL, USA, 2009). The safe concentration (SC) was calculated using the empirical formula $SC = 0.1 \times 96\text{ h-}LC_{50}$ (LC_{50} at 96 h of exposure). Data are expressed as mean ± standard deviation. Differences between experimental groups were analyzed using one-way analysis of variance (ANOVA). The least-significant difference multiple comparison tests were used to identify significant differences between treatments. Significance was set at $P < 0.05$ and $P < 0.01$(highly significant differences).

Table 1 Physical and chemical properties of experimental water

Water parameters	Mean ± SD
Temperature (°C)	22.58 ± 0.93
Dissolved oxygen (mg·L^{-1})	5.67 ± 0.43
pH	6.29 ± 0.27
Oxygen saturation (%)	64.98 ± 4.81

Results

The physicochemical parameters of the test water (temperature, dissolved oxygen, pH, and oxygen saturation) are shown in Table 1. No differences were found between different aquaria during the experiment.

Table 2 shows cumulative death and mortality of fish exposed to different concentrations of La (III) for 96 h. Fish exposed to different concentrations of La (III) behaved normally at the beginning of the trial. After about 4 h of exposure, fish in the higher concentrations of La (III) (6.31 and 10.00 mg L^{-1}) exhibited poisoning symptoms. Abnormal behavior was observed in fish exposed to La (III), in a dose dependent manner. Abnormal behavior included: sudden acceleration movement, reciprocating motion, water surface respiration, and the presence of distinct white floc attached to dorsal and caudal fins. None of these symptoms was observed in control fish, or fish exposed to other low concentrations of La (III).

In the course of the experiment, the fish body became slimy due to the secretion of excessive mucus, which increased in a dose-response manner. After 6 h of exposure to 6.31 and 10.00 mg L^{-1} LA (III), several fish showed rapid

Table 2 Cumulative mortality(%) of *Gobiocypris rarus* at different concentrations of La (III)after 96 h of exposure

Duration	24 h		48 h		72 h		96 h	
Concentration (mg L^{-1})	Cumulative deaths (num)	Cumulative mortality (%)	Cumulative deaths (num)	Cumulative mortality (%)	Cumulative deaths (num)	Cumulative mortality (%)	Cumulative deaths (num)	Cumulative mortality (%)
Control	0	0.00 ± 0.00	0	0.00 ± 0.00	0	0.00 ± 0.00	0	0.00 ± 0.00
1.00	0	0.00 ± 0.00	0	0.00 ± 0.00	0	0.00 ± 0.00	0	0.00 ± 0.00
1.58	0	0.00 ± 0.00	0	0.00 ± 0.00	1	4.16 ± 7.21	2	8.33 ± 14.43
2.51	2	8.33 ± 7.21	15	54.17 ± 26.02**	24	100.00 ± 0.00**	24	100.00 ± 0.00**
3.98	5	20.83 ± 19.09*	23	95.83 ± 7.21**	24	100.00 ± 0.00**	24	100.00 ± 0.00**
6.31	21	87.50 ± 12.50**	24	100.00 ± 0.00**	24	100.00 ± 0.00**	24	100.00 ± 0.00**
10.00	24	100.00 ± 0.00**	24	100.00 ± 0.00**	24	100.00 ± 0.00**	24	100.00 ± 0.00**

Asterisks (*) represent significant differences ($P < 0.05$); double asterisks (**) represent highly significant differences ($P < 0.01$)

opercula movements, hyperactivity, body imbalance, and increased oscillation frequency of the caudal fin. After the appearance of these symptoms,the fish exposed to the highest concentration (10.00 mg L^{-1}) gradually became lethargic and tended to settle at the bottom of the aquarium. At a later stage, the fish body attached some floc and became rigid and slimy. The fish kept their mouth and operculum wide open. After 8 h exposure, fish exposed to La (III) at concentrations >1.58 mg L^{-1}started to die, and continued dying until the end of exposure time. There were no deaths or behavioral changes in control fish, or fish exposed to 1.00 mg L^{-1} La (III).

After 24 h there were no deaths of control fish or fish exposed to La (III) at concentrations ≤ 1.58 mg L^{-1}. Mortality rates in fish exposed to 2.51, 3.98, and 6.31 mg L^{-1} La (III) were 8.33, 20.83 and 87.50%, respectively. The mortality rate in fish exposed to 10.00 mg $L^{-1}$was 100%.

After 48 h there were no deaths of control fish or fish exposed to La (III) at concentrations ≤ 1.58 mg L^{-1}. Mortality rates in fish exposed to 2.51 and 3.98 mg L^{-1} La (III) were 54.17 and 95.83%, respectively. The mortality rate of fish exposed to 6.31 mg L^{-1} La (III) was 100%.

After 72 h there were still no deaths of control fish or fish exposed to La (III) at 1.00 mg L^{-1}. The mortality rates in fish exposed to 1.58 mg $L^{-1}$and 2.51 mg L^{-1} La (III) were 4.16 and 100%, respectively.

After 96 h there were still no deaths of control fish or fish exposed to La (III) at 1.00 mg L^{-1}. The mortality rates of fish exposed to 1.58 mg L^{-1} La (III) was 8.33%. The 96 h-LC_{50} value of La (III) was 1.92 mg L^{-1} (Fig. 1) and the SC value was 0.19 mg L^{-1}.

Fish exposed to 0.80 mg L^{-1} La (III) had shorter gills than control fish (Fig. 2, $P < 0.05$). Gill lamellae were wider in fish exposed to 0.16, 0.32 and 0.80 mg L^{-1} La (III)than in control fish (Fig. 2, $P < 0.05$). Gill area was higher in fish exposed to 0.32 mg L^{-1} La (III) than in control fish(Fig. 2, $P < 0.01$).

Data on the histological analysis of gills is shown in Table 3. The gill tissue of control fish had an orderly arrangement, a clear boundary, and no abnormalities. The gill lamellae are composed of pavement cells, columnar cells, blood vessels containing blood cells, and other cell types. Representative light micrographs of gill tissue in control fish,and in fish exposed to La (III) for 21 d, are shown in Fig. 3. After 21 d of exposure to La (III), lamellar epithelium lifting was the most prevalent change at all doses of La (III). Congestion and desquamation necrosis were evident at 0.04 and 0.08 mg L^{-1} La (III). At 0.16 and 0.32 mg L^{-1} La (III), there was edema and epithelial hyperplasia in many areas of filament epithelium, as well as breakdown of the pillar cell system. In fish exposed to 0.80 mg L^{-1} La (III), tissue damage consisted of a large area of filament cell desquamation, prominent lamellar fusion, and focal cell

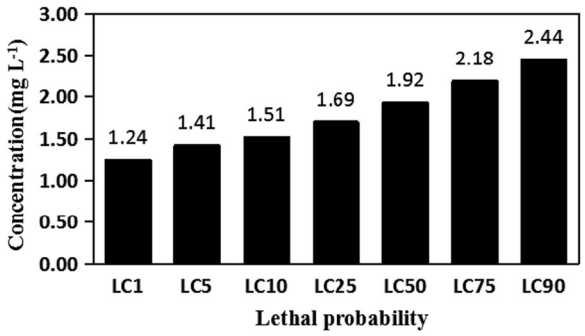

Fig. 1 The LC_{50} of La (III) in rare minnow after 96 h exposure was 1.92 mg L^{-1}, as calculated using probit analysis

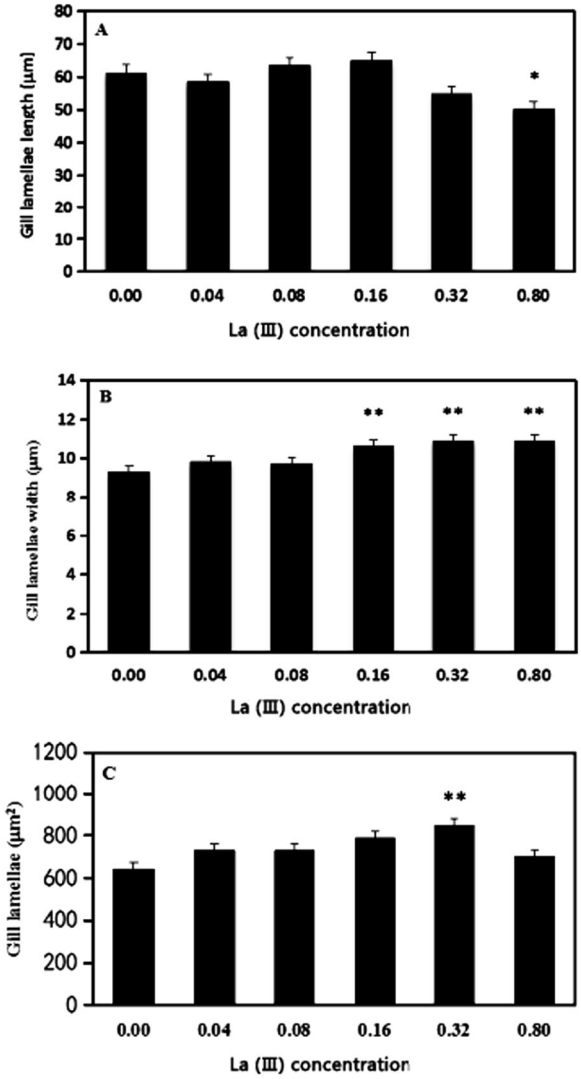

Fig. 2 The average length **a**, width **b** and area **c** of gill lamellae in rare minnow after 21 d exposure. Note: Asterisks (*) represent significant differences ($P < 0.05$); double asterisks (**) represent highly significant differences ($P < 0.01$), when experimental samples (La (III);) are compared to controls. Data are mean ± standard error (n = 120)

Table 3 Summarized histopathological changes in the gill of control fish and rare minnow exposed to La (III)

Concentration (mg L^{-1})	Duration	Epithelial lifting	Congestion	Edema	Epithelial hyperlasia	Desquamation necrosis	Lamellar fusion
control	21d	−	−	−	−	−	−
0.04	21d	+	+	−	+	+	−
0.08	21d	+	++	+	+	++	+
0.16	21d	+	++	++	++	−	+
0.32	21d	+	+++	+++	+++	+	+
0.80	21d	++	−	−	−	+++	++

Note: None (−), mild (+), moderate (++), and severe (+++)

necrosis. After histopathological analysis we conclude that the gill is one of the main targets of La (III).

Histopathology analysis of the liver after 21 d of La (III) exposure is shown in Fig. 4. In control fish, the hepatocytes, and other cells of the liver exhibited a normal structure, and systematically-arranged hepatocytes closely connected to the hepatic blood sinus with homogeneous cytoplasm and a centrally placed nucleus. After 21 d, in fish exposed to 0.04 and 0.08 mg L^{-1} La (III), there was dilation of blood capillaries, congestion of liver sinusoids, and presence of irregularly-shaped hepatocytes. Shrinkage of hepatocytes resulting in increased sinusoidal spaces was observed in fish exposed to 0.08 mg L^{-1} La (III). In fish exposed to 0.16 mg L^{-1} La (III), there was massive typical karyohexis and karyolysis of hepatocytes clustered around ductus hepaticus, or scattered in all corners. Prominent cell necrosis was only found in the other concentration groups. In fish exposed to 0.32 mg L^{-1} La (III), there was cytoplasmic vacuolization accompanied by infiltration of inflammatory cells and an increased number of macrophages, and unambiguous random distribution of local lesions were recorded accurately. Cathepsis and severe necrosis were observed in fish exposed to 0.80 mg L^{-1} La (III).

Discussion

The purpose of this study was to elucidate acute toxic effects of La (III) on freshwater fish behavior and histopathology. water's load capacity, pH, dissolved oxygen and temperature were in compliance with the design requirements of the experiment (the Guidelines for the Testing of Chemicals, the Ministry of Environmental Protection of China).

Available evidence indicates that a minute amount of some toxicants such as pesticide cause abnormal behavior in fish through impaired perceptive acuity (Kabir and Begum 1978). Therefore, during the 21-d toxicity test, approximately 1/3 of the test solution was replaced every 2 days, to ensure removal of toxic ammonia from metabolic waste.

Acute toxicity evaluation

According to the National Standard of China (GB/T21281-2007), the LC_{50} of La (III)at 96 h (1.92 mg L^{-1}) in our study was in the category "acute II" (high toxicity) and could cause severe harm to aquatic organisms.However, a static renewal acute test used to study the acute toxicity of lanthanum chloride ($LaCl_3$) on carp showed that the LC_{50} value of $LaCl_3$for carp at 96 h was11.49 mg L^{-1} (Zhang 2008). Likewise, Hu and Luo (1980) reported that the LC_{50} values of rare earth nitrate at 96 h were 0.937, 3.182, 3.600, and 6.575 mg L^{-1} for *Hypophthalmichthysmolitrix, Ctenopharyngodon, Carassius auratus*, and *Misgurnus anguillicaudatus*, respectively. These differences in toxic lanthanum concentrations might be due to the variations in species, individual size, and environmental factors.

Behavioral changes

Behavioral changes associated with exposure of fish to environmental contaminants, such as organophosphate pesticide, copper and zinc, and fertilizers, have been reported in fish (Chindah et al. 2016; Gabriel et al. 2006; Shah 2002). In our study, fish exposed to La (III) showed abnormal behavior, such as lateral swimming, slow opercula movement, and bradypragia.Similar behavioral changes were noted in *Cyprinus carpio* exposed to lanthanum (Zhang 2008). Avoidance responses of fish to the toxicant, such as hyperactivity, frequent surfacing, erratic swimming etc., have been reported in rainbow trout (Macleod and Pessac 2011), *Clarias gariepinus* (Gabriel and Kparobo 2002) and tilapia (Omoregie 1998). Loss of equilibrium and erratic swimming were also observed in our study, probably due to the impairment of the nervous system. Inhibitory and potentially sedative toxic effects of La (III) ions have been observed at concentrations comparable to those found in the plasma of patients with kidney disease being treated with lanthanum carbonate for hyperphosphatemia (Gramowski et al. 2011).

Surface mucus has protective effects on fish, such as reducing water friction, and preventing substances from

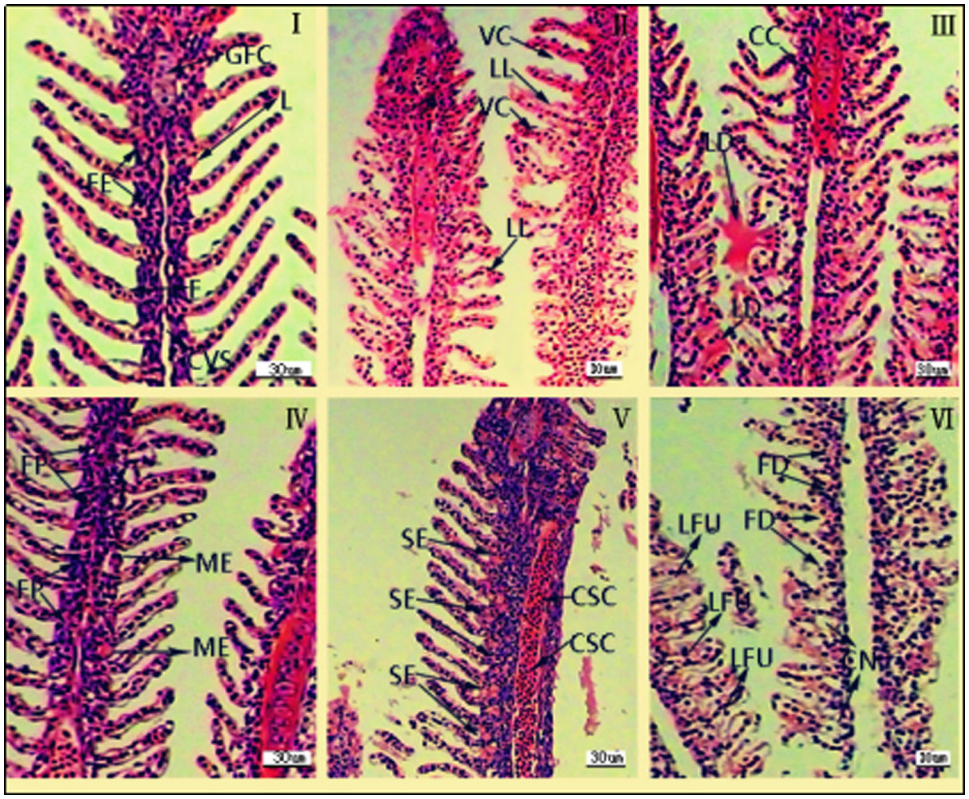

Fig. 3 Representative light micrographs of gill tissue in control (I) and La (III)- treated (II-VI) rare minnow for 21 d; (I) shows the normal appearance of gill filaments (F) and lamellae (L) in control fish: GFC, gill filament cartilage; FE, filament epithelium; (II) 0.04 mg L^{-1}La (III) in the gill lamellae: VC, vascular congestion; LL, lamellar epithelium lifting; (III) 0.08 mg L^{-1}La (III); LD, lamellar epithelium desquamation; CC, filament cartilage congestion; (IV) 0.16 mg L^{-1}La (III) in the filament near the lamellar axis; ME, moderate edema; FP, filament epithelium proliferation; (V) 0.32 mg L^{-1}La (III) on the basis of FP; SE, severe edema; CSC, central venous sinus congestion; (VI) 0.80 mg L^{-1}La (III) showing obvious necrosis in the gill tissues, FD, filament desquamation; LFU, fusion of adjacent lamellae; CN, cellular necrosis. Bars = 30 mm (100 × magnification)

entering arbitrarily into the body. The mucosa is not only a physical barrier, but also a site of local immune responses to pathogens. Therefore, attention has been paid to the mucosal immune system (Cain et al. 2000, Johnston et al. 1997). The composition and quantity of mucous are affected by the distribution, quantity, and developmental stage of mucous cells, as well as by the external environment (Zhang and Yue 2014). In our study, the reason for the large amount of white floc was most likely due to mucous cell destruction by La (III) (Yang and An 1999).

Histopathology changes in the gill

Our study also showed toxic effects of La (III) on organisms after 21 d of exposure. Carp exposed continuously to solutions containing 0–50 mg L^{-1} of lanthanum, gadolinium and yttrium for 45 d at pH 6.0 had a low ability to take up REE under the experimental conditions. The order of maximum bio-concentration was internal organs > gills > skeleton > muscle (Tu et al. 1994). Respiratory distress was one of the early symptoms of poisoning of fish exposure to other toxic chemicals. A high rate of absorption of arsenic through gills also contributes to toxicity (Ahmed et al. 2013).

Gill lamellae are the primary sites for oxygen uptake; only two thin layers of cells separate fish blood from external water. In this study, the average length of the gill lamellae of fish decreased significantly compared to fish of the control group ($P < 0.05$). There was congestion and swelling of the gill at low concentrations of 0.04 and 0.08 mg L^{-1}, with the top gill lamellae epithelia shedding at high concentrations of 0.16 and 0.32 mg L^{-1}. The width of gill lamellae increased with increasing doses of La (III) due to lamellar epithelium lifting and fusion. Similarly, hypsometric analysis documented the appearance of hemorrhage foci, as well as significant changes in blood vessel diameter, primary lamellae width, secondary lamellae length, in all concentrations tested from 0.01 to 1.00 mg L^{-1} (Marcon et al. 2016). Although a large surface area and short diffusion distances make fish gills well suited for gas exchange, these properties lead to exposure to toxic substances and pathogens. Thus, gill morphology is likely to compromise between opposing demands (Sollid and Nilsson 2006).

Histopathology changes in the liver

Liver is the main organ of various key metabolic pathways, and is involved in detoxification. The teleost liver is one of the most sensitive organs showing alterations in histoarchitecture, biochemistry, and physiology, following exposure to various types of environmental pollutants (Peddler et al. 2002; Katsambis and Iliopoulou-Georgudaki 1999). La (III) and Ca (II) induce fish liver mitochondrial

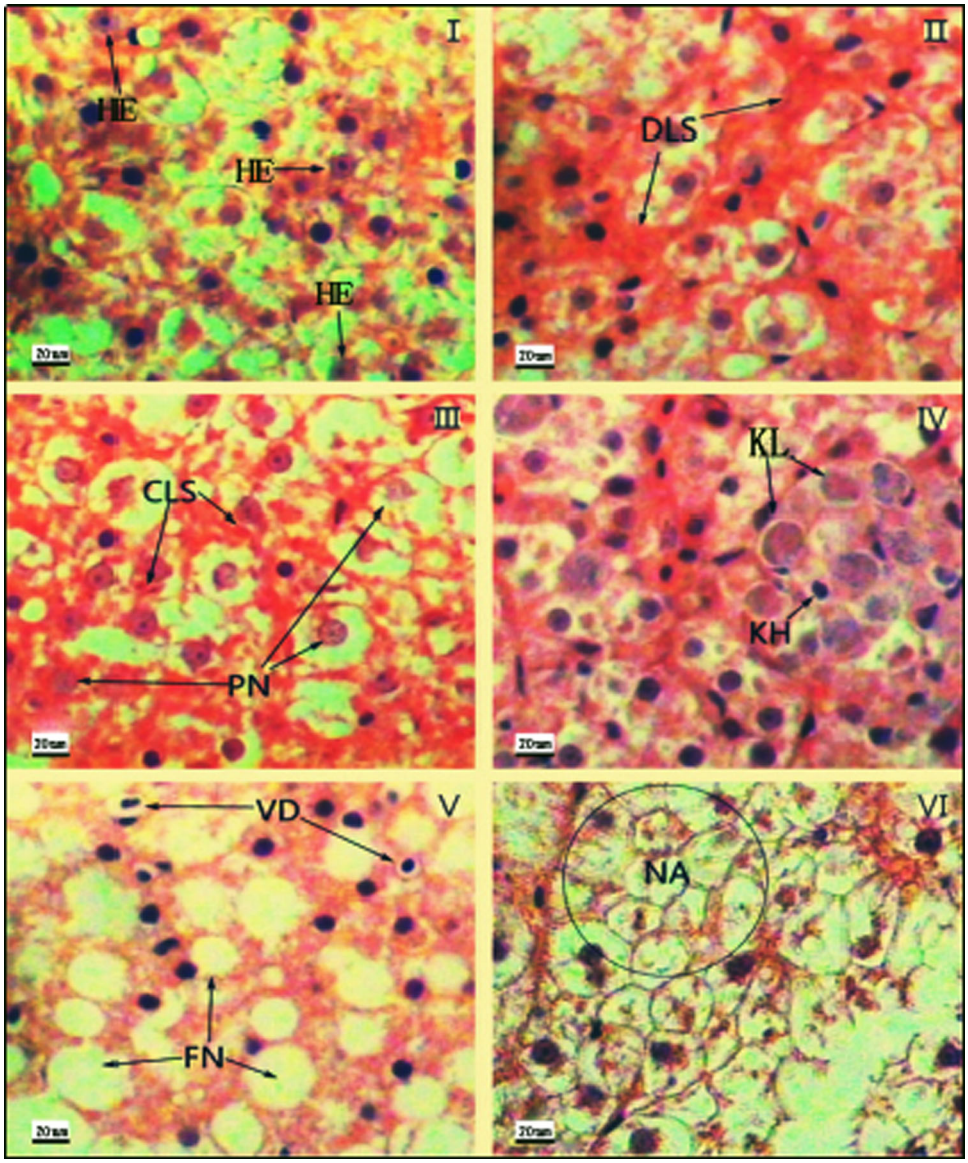

Fig. 4 Histopathology of liver tissue of rare minnow after 21 d of lanthanum exposure; (I) control fish showing regular round shaped nuclei of hepatocytes (HE); (II) 0.04 mg L^{-1}La (III) exposure; DBC, dilated blood capillaries; (III) 0.08 mg L^{-1}La (III) exposure; CLS, congestion of liver sinusoids; PN, pyknotic nucleus; (IV) 0.16 mg L^{-1}La (III) exposure; KH, karyohexis; KL, karyolysis; (V) 0.32 mg L^{-1} La (III) exposure; VD, vacuolar degeneration; FN, focal necrosis; (VI) 0.80 mg L^{-1} La (III) exposure appearing as large areas of necrosis in the liver tissues; NA, necrosis area. Bars = 20 µm (400 × magnification)

swelling and decrease mitochondrial membrane potential. The induction ability of La (III) is stronger than that of Ca (II) (Wu et al. 2015). In our study, the hepatocytes in the control group had a normal structure and homogeneous cytoplasm. Many layers of rough endoplasmic reticulum cisternae with abundant ribosomes were arranged regularly (Zhou et al. 2003). One study illustrated the influence of La $(NO_3)_3$ on rat liver at cellular and subcellular levels,providing an experimental basis for setting a reasonable safety standard for REE (Chen et al. 2003). The congestion of hepatic sinusoids, focal necrosis, and necrosis of hepatocytes, observed in our study were similar to pathological changes found in the liver of carp exposed to fenvalerate (Abraham et al. 2015).

Taken together,our findings indicate that freshwater organisms exposed to REE in polluted waters during growth and developmental stages face more survival pressure. Hence, we should protect the aquatic environment to alleviate the severe pressures to aquatic organisms derived from utilization of REE resources. According the results of our research, the content of REE in discharge water is lower than the SC (0.19 mg L^{-1}).

Conclusion

In the present study, the LC_{50} value of La (III) at 96 h was 1.92 mg L^{-1} for rare minnow. The SC value of La (III) was 0.19 mg L^{-1}. Numerous histopathological changes were observed in gills and liver of fish exposed to La (III) for 21 d. Abnormal behavior was observed in fish exposed to La

(III), in a dose dependent manner.. La (III) had severe acute toxic effects on fish, severely affecting vital organs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval The present research did not involve human participants. All applicable international, national, and, or institutional guidelines for the care and use of animals were followed.

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