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Effects of rare earth elements La and Yb on the morphological and functional development of zebrafish embryos

Jun'an Cui[1,2], Zhiyong Zhang[1,*], Wei Bai[1], Ligang Zhang[2], Xiao He[1], Yuhui Ma[1], Yan Liu[2], Zhifang Chai[1]

1. Key Laboratory of Nuclear Analytical Techniques, and Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, the Chinese Academy of Sciences, Beijing 100049, China. E-mail: qustcuijunan@163.com
2. Qingdao University of Science and Technology, College of Environment and Safety Engineering, Qingdao 266042, China

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Abstract

In recent years, with the wide applications and mineral exploitation of rare earth elements, their potential environmental and health effects have caused increasing public concern. Effect of rare earth elements La and Yb on the morphological and functional development of zebrafish embryos were studied. The embryos were exposed to La^{3+} or Yb^{3+} at 0, 0.01, 0.1, 0.3, 0.5 and 1.0 mmol/L, respectively. Early life stage parameters such as egg and embryo mortality, gastrula development, tail detachment, eyes, somite formation, circulatory system, pigmentation, malformations, hatching rate, length of larvae and mortality were investigated. The results showed La^{3+} and Yb^{3+} delayed zebrafish embryo and larval development, decreased survival and hatching rates, and caused tail malformation in a concentration-dependent way. Moreover, heavy rare-earth ytterbium led to more severe acute toxicity of zebrafish embryo than light rare-earth lanthanum.

Key words: zebrafish; embryo development; lanthanum; ytterbium; toxicity

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Introduction

Rare earth elements (REEs) are a collection of seventeen chemical elements in the periodic table, specifically the fifteen lanthanides plus scandium and yttrium. REE compounds frequently have valuable catalytic, chemical, electrical, metallurgical, nuclear, magnetic, and optical properties; therefore they are widely used in luminescent materials (Eliseeva and Bünzli, 2010), contrast agents in biomedical imaging (Laurent et al., 2009), automotive catalysts (He et al., 2010), high-performance permanent magnets (Goll and Kronmüller, 2000), anti-tumor medicine (Liang et al., 2010), nuclear radiation detector (Ding et al., 2008) and fertilizers (Li et al., 2007), etc. With the wide applications and mineral exploitation, REEs will inevitably enter the environment, especially aquatic environment. Their potential effects on aquatic species have recently attracted much attention (Lürling and Tolman, 2010).

It has been reported that low concentration of REEs has positive effects on fish, but relatively high dosage of REEs become toxic (Xu and Jiang, 2004). Toxicity studies have shown that REEs can cause liver damage in *Cyprinus carpio* (Zhang, 2008). Guo et al. (2001) reported that the activities of catalase (CAT) and glutamate-pyouvate transaminase (GPT) of crucian liver were significantly inhibited when the concentration of Yb^{3+} overtop 0.05 mg/L. Chen et al. (2000) observed a transient induction of the antioxidant enzyme CAT and an eventually significant induction of superoxide dismutase (SOD) in goldfish livers exposed to REE solutions. A biodistribution study indicated that REEs accumulated in viscera, bone and muscle of *C. carpio* (Wang et al., 1991; Jiang et al., 2008). In fish, the embryonic and larval stages are usually the most sensitive to pollutants. However, the toxicities of REEs on the developing embryo were poorly studied.

The objective of the present study is to determine the developmental toxicity of rare earth elements on the developing fish embryos. Zebrafish (*Dania rerio*), a small vertebrate species, can be rapidly and prolifically bred and easily maintained in the laboratory. Zebrafish eggs (1.0–1.2 mm in diameter) are transparent and develop quite rapidly, which facilitates direct optical observation of the toxic effects on their internal organs. Zebrafish embryo or larvae is demonstrated as one of normally used model organism for the toxicity examination, particularly sensitive to low-level environmental pollutants (von Westernhagen, 1998). In this study, zebrafish embryos were used as the model to evaluate the toxicity of rare earth elements La^{3+} and Yb^{3+}. The toxic mechanism of REEs was also discussed.

* Corresponding author. E-mail: zhangzhy@ihep.ac.cn

1 Materials and methods

1.1 Fish maintenance and eggs production

Zebrafish (*D. rerio*) were maintained in a closed flow through culture system filled with conditioned water (75 g $NaHCO_3$, 18 g sea salt, 8.4 g $CaSO_4$, per 1000 L; pH (7.0 ± 1.0); total hardness: 65 mg/L (as $CaCO_3$); temperature: (27 ± 1)°C; conductivity: 485 μS/cm) in a 14 hr:10 hr light:dark cycle. They were fed twice daily with live brine shrimps (*Artemia salina*). On the evening before spawning, male and female fish (the number ratio of 2:1) were placed in a hatching box. Spawning was triggered once the light was turned on in the next morning and was completed within 1 hr. Viable eggs were collected and rinsed for at least three times with E_3 medium (5 mmol/L NaCl, 0.17 mmol/L KC1, 0.33 mmol/L $CaCl_2$ and 0.33 mmol/L $MgSO_4$, pH (7.0 ± 1.0)), which is the standard hatchery water for zebrafish eggs. All of the other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. China, and were of analytical grade. To ensure the developmental synchronization at the beginning of exposure, the embryos at about 2.5–3.0 hr post fertilization (hpf) were sorted under a stereo microscope. Healthy embryos at blastula stage were then subjected to La^{3+} or Yb^{3+} exposure.

1.2 Preparation of exposure solutions

Lanthanum chloride heptahydrate ($LaCl_3{\cdot}7H_2O$) used in this work was purchased from Shantou Xilong Chemical Factory, China, which is analytical pure, purity is better than 45.0% with La_2O_3 of computational standard. Ytterbium chloride hydrate ($YbCl_3{\cdot}xH_2O$, $x \approx 6$), 99.9% (REO) was purchased from Alfa Aesar, UK. The stock solution of La^{3+} or Yb^{3+} (10 mmol/L) was prepared by directly adding $LaCl_3{\cdot}7H_2O$ or $YbCl_3{\cdot}6H_2O$ into Milli-Q water solution. A series of exposure liquid (0, 0.01, 0.1, 0.3, 0.5 and 1.0 mmol/L) were prepared by dilution with E_3 medium. The concentrations of La or Yb in the solutions were determined by a colorimetric method (Ding et al., 2008).

1.3 Embryo toxicity test

The assay is mainly based on the embryo test procedure developed by Schulte and Nagel (1994). Briefly, twenty-four eggs were transferred into a 24-well multi-plates with one embryo per well. Twenty wells were filled with 2 mL of La^{3+} or Yb^{3+} solutions, and the remaining four wells were filled with 2 mL E_3 (5 mmol/L NaC1, 0.17 mmol/L KC1, 0.33 mmol/L $CaC1_2$ and 0.33 mmol/L $MgSO_4$, pH = (7.0 ± 1.0)) medium to act as controls. All of the multi-plates were covered with transparent plastic films and placed in an illumination incubator at (27 ± 1)°C with a 14 hr:10 hr light:dark photoperiod. Direct observations were performed in the wells under a stereo microscope (20 × 1.5) connected to a camera device at specific timepoints (8, 24, 32, 48–60, 72, and 96 hr), during the period of 48–60 hr, and records were made every 2 hr (Bai et al., 2010). The toxicological endpoints included gastrula development, tail detachment, eye development, somite formation, circulatory system, pigmentation, hatching rate, malformations, length of larvae and mortality. At 96 hr, the larvae were positioned on the lateral side and photographed. The length of larvae was measured using digital image analysis (Olympus, ver.SZX12, Japan). All experiments were repeated at least 6 times independently.

1.4 Statistical analysis

Statistical analysis was performed using the statistical package SPSS 10.0 for Windows (SPSS Inc., USA). Data are presented as the (mean ± SD). The data were tested for homogeneity and normality. If these assumptions were met, one-way analysis of variance (ANOVA) was performed. Otherwise, the non-parametric Kruskal-Wallis test was performed. Significance level was set as $p < 0.05$.

2 Results

2.1 Actual La^{3+} and Yb^{3+} concentrations

The actual exposure concentrations of La^{3+} and Yb^{3+} in E_3 medium are listed in Table 1. The measured values agree well with the designed ones.

2.2 Hatching rate

Figures 1 and 2 show hatching rates at 72 hr and cumulative hatching rates from 48 to 96 hr, respectively. Compared to the control, the solution containing ≤ 0.1 mmol/L La^{3+} or Yb^{3+} did not significantly affect the hatching rates, but the toxicities of La^{3+} and Yb^{3+} increased as

Table 1 Concentrations of La^{3+} and Yb^{3+} in the exposure medium ($n = 3$)

Designed concentration (mmol/L)	0	0.01	0.1
Actual La (mmol/L)	0	0.0108	0.102
Actual Yb (mmol/L)	0	0.00995	0.0992
Designed concentration (mmol/L)	0.3	0.5	1.0
Actual La (mmol/L)	0.316	0.507	1.06
Actual Yb (mmol/L)	0.298	0.496	0.993

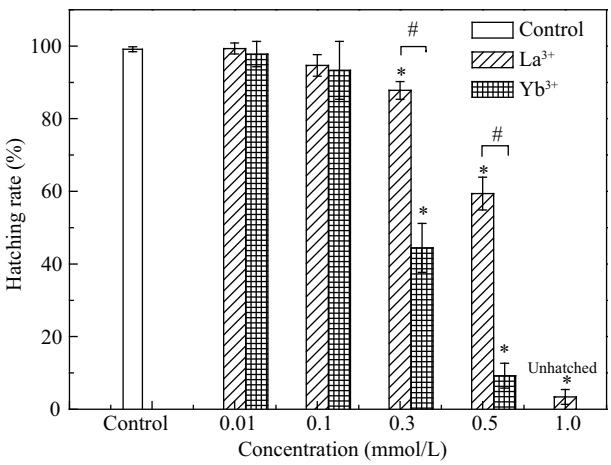


Fig. 1 Effects of La^{3+}/Yb^{3+} on the hatching rate of embryos at 72 hr ($n = 6$) * and # mean $P < 0.05$ compared with the control group and the Yb^{3+} group, respectively.

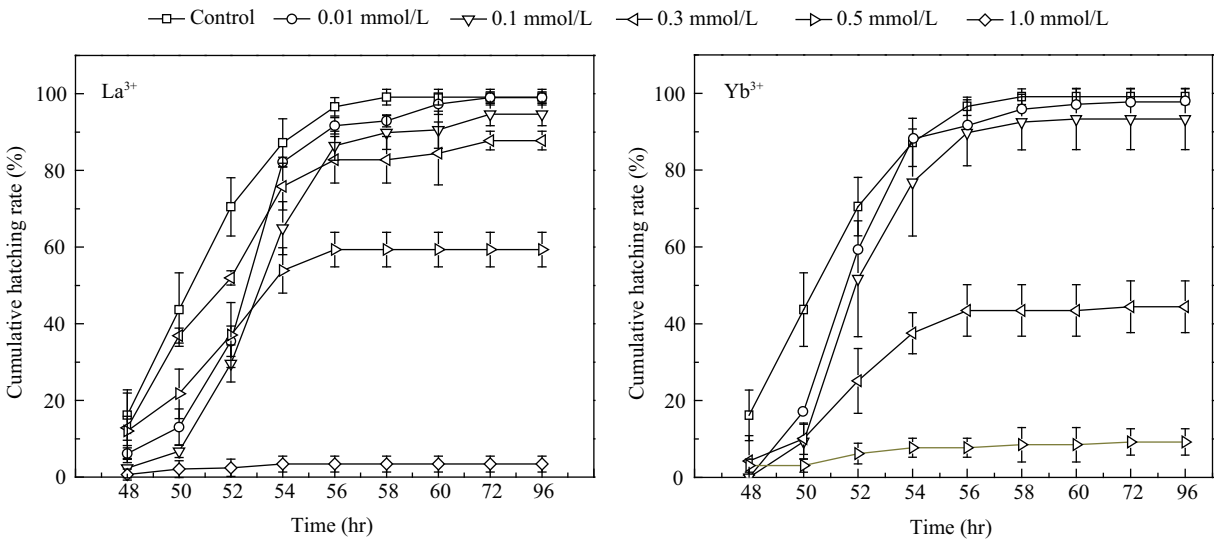


Fig. 2 Effects of La^{3+}/Yb^{3+} on the hatching rate of embryos from 48 to 96 hr (n = 6).

La^{3+} and Yb^{3+} concentrations increasing, which indicate the toxicities of La^{3+} and Yb^{3+} to the hatching of zebrafish embryos were dose-dependent. Moreover, Yb^{3+} led to more severe inhibitions of the embryonic hatching than La^{3+} in the concentration range from 0.3 to 1.0 mmol/L. The median lethal concentration (LC_{50}) calculated from the 72 hr hatching rate, La^{3+} and Yb^{3+} were 0.603 and 0.268 mmol/L, respectively. At the same condition, LC_{50} of Cd^{2+} was 0.0188 mmol/L, Cu^{2+} was 0.0019 mmol/L (Palmer et al., 1998; Blechinger et al., 2002). REE^{3+} was less toxic to the development of zebrafish embryos than Cd^{2+} and Cu^{2+}.

Table 2 lists the values of the median hatching time (HT_{50}) calculated from the cumulative hatching rate. There are no significant difference between the control and exposed groups. For 1.0 and 0.5 mmol/L Yb^{3+}, the hatching rate of the zebrafish embryos is lower than 50%, thereby, the TH_{50} can not be calculated out.

2.3 Mortality

The mortality of zebrafish larvae exposed to La^{3+} and Yb^{3+} at different concentrations was determined. It can be seen that the mortality of the larvae increased in a dose-dependent way. Compared to the control, 0.01 mmol/L La^{3+} showed no toxicity to the larvae while 0.01 mmol/L Yb^{3+} caused 1.41% zebrafish larvae dead. At the concentration of 0.1 mmol/L, the larvae mortalities were recorded as 30.9% and 65.6% for La^{3+} and Yb^{3+}, respectively (Fig. 3).

2.4 Body length and malformation

Figure 4 illustrates effects of La^{3+} and Yb^{3+} exposure on the length of larvae at 96 hr. Compared to control,

Table 2 Median hatching time (HT_{50}) of zebrafish embryos at different concentrations of La^{3+} and Yb^{3+} (n = 6)

Concentration (mmol/L)	0	0.01	0.1	0.3	0.5	1.0
HT_{50} for La^{3+} (hr)	50.3	53.1	53.2	50.6	54.00	–
HT_{50} for Yb^{3+} (hr)	50.3	51.2	51.6	53.1	–	–

–: no embryos hatched.

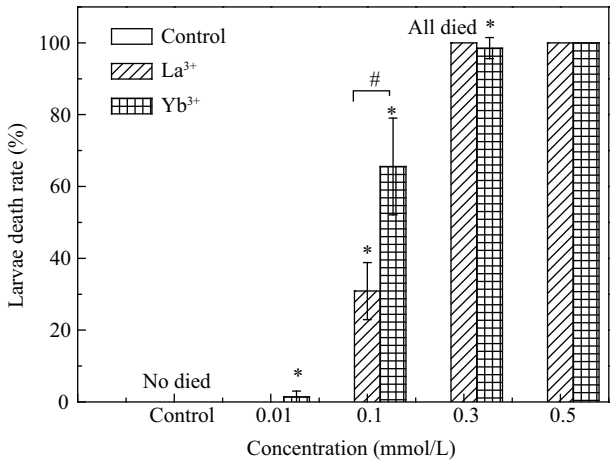


Fig. 3 Effects of La^{3+}/Yb^{3+} on the mortality of larvae at 96 hr (n = 6). * and # mean $P < 0.05$ compared with the control group and Yb^{3+} group, respectively.

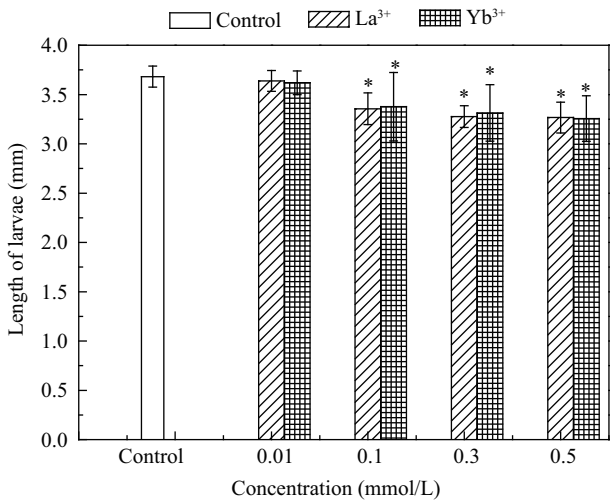
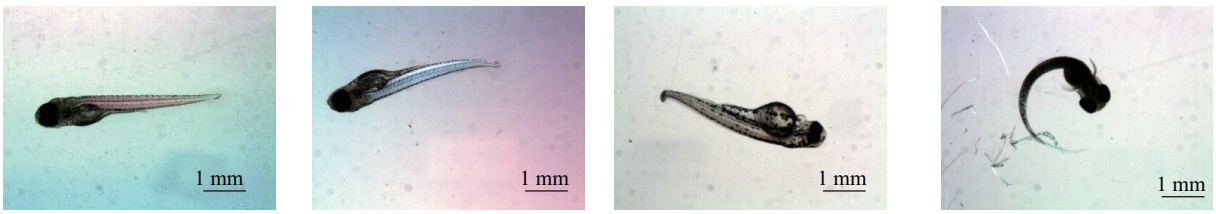


Fig. 4 Effects of La^{3+}/Yb^{3+} on the length of larvae ($n \geqslant 10$). * means $P < 0.05$ compared with the control group.

the solutions containing $\geqslant$ 0.1 mmol/L La^{3+} or Yb^{3+} significantly decreased the body length of larvae. There is no significant difference in the body length between La^{3+} and Yb^{3+} groups.



Normal larvae | Caudal curving | Yolk sac edema | Spinal column curving

Fig. 5 Malformation of larvae caused by La^{3+} or Yb^{3+} exposure.

La^{3+} and Yb^{3+} mediated malformation (e.g., body edema, bended tail, and yolk arc edema) in the embryos and larvae were observed, as shown in Fig. 5. In addition, no abnormality was observed in other developmental endpoints in the treated embryos, such as tail detachment, eye development somite formation, heartbeat, circulatory system, pigmentation, and otic capsule.

3 Discussions

The present study demonstrates that REE^{3+} is acute toxic to zebrafish embryos. The toxicity of REE^{3+} to zebrafish embryos development might include many reasons. However, at least in part, ascribe to the homeostasis of Ca^{2+} in zebrafish embryo. Calcium is an essential composition element for zebrafish cells. Because REE^{3+} have a similar ionic radius (9.6–11.5 nm) as the calcium ion (9.9 nm) but a higher valency (+3), it has been suggested that REE^{3+} might bind in place of calcium and affect physiological functions by regulating Ca^{2+} level in zebrafish embryos (Hu et al., 1996). In consequence, REE^{3+} may interact with many Ca-dependent biological systems, resulting in toxicity or impaired function in zebrafish embryos and larvae. In our previous research on the neurotoxicity of La and Yb, the Ca contents in tibia and serum of rats were significantly decreased, which suggests that La and Yb exposure could cause calcium deficiency and the impairment in spatial memory and the intracellular free calcium overload in the hippocampal cells were also found (He et al., 2007, 2008).

In this study, we found that the toxicity of Yb^{3+} on the development of zebrafish embryos or larvae was significantly stronger than that of La^{3+}. Considering the fact that the radius of Yb^{3+} is smaller than La^{3+} (Evans, 1990) while they have the same charge, complexes of Yb^{3+} with biological molecules will be more stable. This may produce more severe toxicity to embryos and larvae of the zebrafish. There are other possible reasons for the negative effect of REE^{3+} on the development of zebrafish embryos. During the normal hatching process of teleost embryos, the chorion is digested by the hatching enzyme, which is a proteolytic enzyme secreted from hatching gland cells of the embryo. The hatching enzymes have two proteases, choriolysin H and choriolysin L (Yamagami, 1996; Inohaya et al., 1997). Unfortunately, the enzyme activity of the hatching enzyme which exists transitorily is easily affected by environmental materials (Fan and Shi, 2002). The structure and the function of the protease might be destroyed by La^{3+}/Yb^{3+}. In addition, La^{3+} and Yb^{3+} might undergo hydrolysis at neutral pH conditions and REE hydroxides might block pore canals of the chorions, resulting in the shortage of oxygen supply to the development of embryos (Fan and Shi, 2002).

4 Conclusions

The embryo toxicity test revealed that La^{3+} and Yb^{3+} retarded the zebrafish embryos hatching (0.01–1.0 mmol/L), reduced the body length of larvae, killed the larvae died and caused malformation. Heavy rare earth Yb was more toxic than light rare earth La. Compared to the common heavy metal pollutants such as Cu and Cd, La and Yb caused lesser acute toxicity to zebrafish embryos.

Acknowledgments

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