Chemosphere 182 (2017) 617–623

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Toxic effects of NH_4^+-N on embryonic development of *Bufo gargarizans* and *Rana chensinensis*

Hongzhang Deng [a, b], Lihong Chai [a, b], Pingping Luo [a, b, *], Meimei Zhou [a, b], Daniel Nover [c], Xiaohong Zhao [a, b]

[a] Key Laboratory of Subsurface Hydrology and Ecological Effects in Arid Region, Ministry of Education, Chang'an University, Xi'an, 710064, China
[b] School of Environmental Science and Engineering, Chang'an University, Xi'an, 710064, China
[c] School of Engineering, University of California – Merced, Merced, CA, 95343, USA

HIGHLIGHTS

- Nitrogen fertilizers can damage ecosystems health while benefiting crop production.
- The effects of NH_4^+-N on two Chinese native amphibian species were compared.
- *B. gargarizans* and *R. chensinensis* embryos responded differently to NH_4^+-N.
- NH_4^+-N exposure may increase abnormality and inhibit embryo hatching and development.
- It has implication for fertilizer management and environmental standard for NH_4^+-N.

ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form
18 February 2017
Accepted 21 February 2017
Available online 3 April 2017

Handling Editor: David Volz

Keywords:
NH_4^+-N
Bufo gargarizans
Rana chensinensis
Embryo
LC_{50}

ABSTRACT

Although nitrogen fertilizer is commonly used worldwide, little information is currently available about NH_4^+-N toxicity on amphibians. This study determined the acute and chronic toxic effects of NH_4^+-N on two native Chinese amphibian species (*Bufo gargarizans* and *Rana chensinensis*), and compared the negative sensitivity of different embryos to NH_4^+-N. Static renewal aqueous exposures were performed using *B. gargarizans* and *R. chensinensis* embryos at Gosner stage 2 over 96 h. In terms of 96 h-LC_{50}, *B. gargarizans* and *R. chensinensis* embryos had significantly different responses to NH_4^+-N, and the latter was more sensitive to NH_4^+-N than the former. In the chronic toxicity test, exposure to 10 mg L^{-1} NH_4^+-N or higher significantly decreased the hatching rate of embryos in both species. Significant increases in the abnormality rate of embryos at 50 mg L^{-1} NH_4^+-N or higher were observed and morphological abnormalities were characterized by axial flexures, yolk sac edema, and hyperplasia in both species. Additionally, the total length of embryos decreased in a dose-dependent manner after exposure to NH_4^+-N. The results indicate that NH_4^+-N exposure can increase abnormality and inhibit the hatching and development of embryos in *B. gargarizans* and *R. chensinensis*.

1. Introduction

Use of fertilizers can improve soil fertility and provide nutrients required for crop growth, essential to increase crop yields (Liu, 2009). Nitrogen fertilizer is the fertilizer type with the highest application rate in Chinese farmland. The total arable land of China accounts for less than 10% of the world's total land, while the application of nitrogen fertilizer in this country is nearly 1/3 of the world's total application (Qun, 2014). However, the utilization rate of nitrogen fertilizer only ranges from 30 to 40%, and most nitrogen fertilizer applied is lost in the environment through rainfall, surface runoff, and soil leaching among other pathways. Eventually, nitrogen fertilizer leads to eutrophication and pollution of various water bodies (Zhang, 1981). Ammonium nitrogen (NH_4^+-N) concentrations of up to 2.284 mg L^{-1} have been reported in groundwater with agricultural nonpoint sources of nitrogen pollution on a small watershed scale (Ouyang et al., 2014); this value is nearly 5 times the Grade 5 standard for NH_4^+-N pollution of groundwater

* Corresponding author. Key Laboratory of Subsurface Hydrology and Ecological Effects in Arid Region, Ministry of Education, Chang'an University, Xi'an, 710064, China.
E-mail address: robertluoping@126.com (P. Luo).

http://dx.doi.org/10.1016/j.chemosphere.2017.02.156
0045-6535/

(NH_4^+-N >0.50 mg L^{-1}) (Gao et al., 2012).

Amphibians are an important component of aquatic and farmland ecosystems. Owing to their unique life-history and physiological characteristics, amphibian embryos and larvae show extreme sensitivity to water pollution (Yu et al., 2013; Watson et al., 2014). Meanwhile, the global amphibian population has shown major declines in recent years (Houlahan et al., 2000; Kieseker et al., 2001). Since the 1960s, approximately 160 amphibian species have gone extinct, and 32% of amphibian species are currently facing extinction (Stuart et al., 2004). Chemical pollution of the environment is one of the major risk factors leading to amphibian population decline (Kieseker et al., 2001; Gibbs et al., 2005; Hayes et al., 2010). Nitrogen fertilizer mainly exists as ammonia nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N and NO_2^--N) in water environments, with NH_4^+-N showing particularly high toxicity (Rabalais, 2002). There is evidence that aqueous exposure to NH_4^+-N can result in increased mortality of amphibian embryos and larvae (Ortiz-Santaliestra et al., 2006; Mann et al., 2009). The harm of NH_4^+-N to amphibian populations is reflected both in the aquatic embryonic and larval stages and in the terrestrial adult stage (Shufen, 2008). The toxic effects of nitrogen pollution on amphibians is therefore an important topic that must be better understood to safeguard ecosystem heath.

Region-specific NH_4^+-N toxicity tests are essential for understanding context specific amphibian response to nitrogen pollution. *Bufo gargarizans* and *Rana chensinensis* are two native amphibian species found widely in China. These two species generally reproduce by laying eggs at the end of winter and the beginning of spring. The oviposition site is generally adjacent to farmland and thus vulnerable to the stress of nitrogen pollution (Yu et al., 2000; Xia et al., 2012). Existing studies have shown that embryos and tadpoles of *B. gargarizans* and *R. chensinensis* are ideal study organisms with high sensitivity to copper ions and fluoride, among other water pollutants (Chai et al., 2016; Gosner, 1960). Thus far, no comparative studies have been reported on the acute and chronic toxic effects of NH_4^+-N on embryos of *B. gargarizans* and *R. chensinensis*. *B. gargarizans* usually live in agricultural environments with NH_4^+-N pollution, while *R. chensinensis* larvae generally live in slowly flowing water in mountainous areas and forests with rich vegetation. Thus, interspecific comparisons are possible between the response of *B. gargarizans* and *R. chensinensis* to pollution. The comparative toxic test with native species will inform fertilizer management strategies and the development of environmental standards for NH_4^+-N.

In this study, we investigate the acute toxic effect of NH_4^+-N on embryos of *B. gargarizans* and *R. chensinensis*. We also evaluate the chronic toxic effect of NH_4^+-N on these two amphibian species in terms of the hatching rate, abnormality rate, and total length of embryos. The results of this study provide the scientific basis for ecological risk assessment and protection of environmental health through improved understanding of pollution impacts on indicator species.

2. Materials and methods

Fig. 1 shows the detailed study design. First, *Bufo gargarizans embryos* and *R. chensinensis* embryos were exposed to NH_4Cl for the acute and chronic exposure tests. Mortality is the main indicator to judge acute toxicological effects in the acute exposure test. The median lethal concentration (LC_{50}) of ammonium chloride (NH_4Cl)

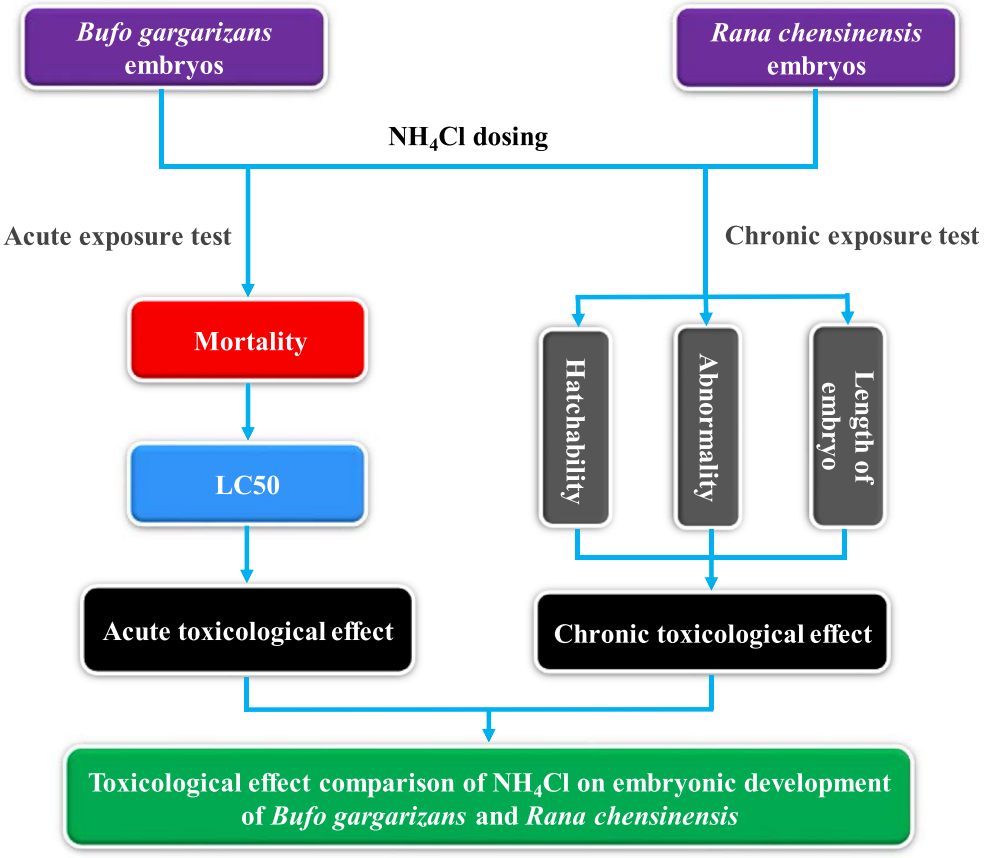

Fig. 1. Research flow chart of toxicological effect of NH_4^+-N on embryos of *B. gargarizans* and *R. chensinensis*.