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Limitation of spatial distribution of ammonia-oxidizing microorganisms in the Haihe River, China, by heavy metals

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A B S T R A C T

The Haihe River is characterized by high ammonia pollution. Therefore, it is necessary to determine how environmental factors, such as heavy metals in the river limit the spatial distribution of ammonia-oxidizing microorganisms. In this study, the relationships between five heavy metals and ammonia-oxidizing microorganisms were studied. The results showed that under high ammonia, low oxygen and high concentrations of suspended particles, ammonia-oxidizing bacteria (AOB) ranged from $10^{1.3}$ to $10^{4.8}$ gene copies/mL and ammonia-oxidizing archaea (AOA) ranged from $10^{2.7}$ to $10^{4.9}$ gene copies/mL. The average metal concentrations in water were 23.57 (Cr), 21.58 (Ni), 65.09 (Cu), 622.03 (Zn) and 10.16 (As) μg/L, with those of Zn, Cu and Cr being higher than the US EPA criteria. Scatter plots of microbial abundance and metals indicated that both AOA and AOB were limited by heavy metals, but in different ways. As had an inhibitory effect on AOB, while Ni and Zn promoted AOA, and the other metals investigated showed no significant correlation with microbial abundance. Overall, our results indicated that the effects of heavy metals on ammonia-oxidizing microorganisms in water are complex, and that the final effect is determined by the physiological role of each element in the microorganisms, as well as environmental conditions such as complexation of organic matter, not simply the total metal concentration.

Introduction

Ammonia-oxidizing microorganisms are key drivers of ammonia oxidation, which is an important process in the nitrogen cycle. Ammonia-oxidizing bacteria (AOB) have been studied for many years and are known to be widespread in soil, freshwater and marine systems. Until recently, it was assumed that autotrophic ammonia oxidation was restricted to ammonia-oxidizing bacteria. However, this changed with the detection of a unique ammonia monooxygenase gene on an archaeal-associated scaffold (Venter et al., 2004) and on genomic fragments of archaea (Treusch et al., 2005), and the discovery of ammonia-oxidizing archaea (AOA) greatly expanded our understanding of ammonia oxidation (Francis et al., 2007).

The distribution of AOA and AOB is related to environmental conditions such as ammonia level, dissolved oxygen, acidity, temperature and salinity. The AOB population has been found to be larger in fertilized ($(NH_4)_2SO_4$) than unfertilized soil (Okano et al., 2004); however, AOA have been shown to be more tolerant to low ammonium concentrations owing to their ammonia oxidation kinetics (Martens-Habbena et al., 2009). It is likely that AOA tolerate a wide range of oxygen levels and that some ecotypes are more suited to low-oxygen environments (Erguder et al., 2009). However, the abundance and community composition of AOB can change dramatically with the gradient of oxygen (Schramm et al., 2000). The role of pH in determining the distribution of AOA and AOB has been

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shown in many studies. Allison and Prosser (1993) found that *Nitrosospira* cluster 2 was more abundant in lower pH soil, while He et al. (2007) stated that archaeal amoA gene abundance decreased with increasing soil pH. Moreover, many studies have shown that salinity and temperature influence the abundance or community structure of AOA and AOB to a great extent (Erguder et al., 2009; Koops and Pommerening-Röser, 2006).

Heavy metals comprise a common environmental factor that plays a role in shaping the ecological niches of ammonia-oxidizing microorganisms; therefore, they have received increasing attention. The effects of Cu (Mertens et al., 2010; Li et al., 2009), Zn (Mertens et al., 2009; Ruyters et al., 2010), and Hg (Liu et al., 2010) on the ammonia-oxidizing community have been well documented. AOA and AOB have been found to possess different adaptabilities to environmental stress. It was reported that the recovery of nitrification after 3-year Zn contamination was meditated by AOB rather than AOA, which meant AOB can adapt to Zn contamination better than AOA (Mertens et al., 2009). Mertens et al. (2010) found the increased Cu tolerance of the nitrifying community in long-term Cu-exposed field sites was associated with changes in community structure. However, in a Cu inhibitory experiment, the abundance decrease of AOA was much less than that of AOB, indicating that AOA was more tolerant than AOB (Li et al., 2009). Hg pollution also changed the composition of soil AOB to some extent and inhibited the soil potential nitrification rate (Liu et al., 2010). It seems that the response of the nitrifying community to heavy metals relates to the metal content and metal type as well as the exposure time.

In fact, the mechanisms through which heavy metals impact microorganisms are complex. In addition to being toxic at high levels, low levels of heavy metals can inhibit the growth of microbes because some elements are necessary in certain physiological processes (Stumm et al., 1996). The ecological effects of heavy metals are also closely related to the speciation of the elements, which determines their bioavailability and mobility in the environment (Brümmer et al., 2007). Most studies that have been conducted to date have involved soil; therefore, the behavior of heavy metals in the liquid phase needs more attention. Metals in soil can be strongly bound by organic materials such as humic and fulvic acids, and their mobility is poor (Giller et al., 1998). In contrast, metals in aquatic habitats have weaker binding effects and stronger mobility, and interact more easily with microbes. Accordingly, it is necessary to increase our knowledge of the effects of heavy metals on ammonia-oxidizing microorganisms in aquatic environments.

The Haihe River is one of the most important systems in China. Beijing and Tianjin are located within its basin, which is characterized by high population density, rapid socioeconomic development and serious water pollution caused by the discharge of industrial and domestic wastewater. Water quality monitoring of the Haihe River has revealed high ammonia levels and indicated that this has become one of the most prominent water quality problems in the river in recent years. Because ammonia-oxidizing microorganisms play a significant role in the reduction of ammonia, it is essential to understand their distribution and how they respond to environmental factors such as heavy metals. In this study, the Fuyang River, a typical river in the Haihe River Basin, was selected as the study area. Heavy metal concentrations in the water and the abundance of AOA and AOB in the suspended solids were measured.

Our overall objective was to investigate the relationship between ammonia-oxidizing microorganisms and heavy metals through field survey data by statistical methods.

1 Materials and methods

1.1 Study area and sampling site

The Haihe River Basin is located in the north of China and has an area of about 300,000 km^2 and a population of 145 million. Beijing, Tianjin and many other cities are in this river basin and its urbanization level is over 45%. In 2007, the amount of ammonia discharged into the river by domestic sewage and industrial wastewater was as high as 61,700 tons, and ammonia is always the main factor found to be degrading the water quality. The Fuyang River (36°23′–38°14′N, 114°19′–116°7′E) is a well-known tributary of the Haihe River (**Fig. 1**) that receives the largest amount of ammonia. In addition, great differences in heavy metal concentrations may exist in the complex river system and large watershed of the Fuyang River. Therefore, the Fuyang River was selected as the sampling area.

The Fuyang River originates from mountains in Handan and ends at its confluence with the Hutuo River. The length of the main channel is 402 km. The New Fuyang River, which was constructed for the purpose of flood diversion, runs parallel to the downstream portion of the Fuyang River. Additionally, the Xiao River, Wangyang Ditch and Shaocun Ditch are tributaries of the Fuyang River that receive domestic wastewater, pharmaceutical wastewater and tannery wastewater, respectively, from Shijiazhuang. The Li River is another tributary of the Fuyang River system that receives a large amount of domestic and industrial wastewater from Xingtai. In addition, the mainstream of the Fuyang River primarily receives wastewater from Handan and Hengshui. In 2007, a total of 0.52 billion tons of wastewater were discharged into the river system, among which domestic sewage and industrial wastewater accounted for 50% each. This high pollution discharge results in deterioration of water quality, and ammonia is the primary pollutant in most rivers.

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