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Effects of Cd and Zn on oxygen consumption and ammonia excretion in sipuncula (*Phascolosoma esculenta*)☆

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Abstract

Physiological responses (oxygen consumption and ammonia excretion) of the sipuncula (*Phascolosoma esculenta*) exposed to four concentrations of Cd (0.45, 0.96, 2.04, and 4.46 mg L^{-1}) and four concentrations of Zn (1.09, 2.34, 4.96, and 10.91 mg L^{-1}) were monitored for 21 days, respectively. Oxygen consumption rates of sipuncula at all concentrations of Cd decreased from day 1 to day 6. At low concentrations of Cd (0.45 and 0.96 mg L^{-1}), the oxygen consumption rate was promoted. Time and concentration were significant in affecting oxygen consumption rate, respectively. Oxygen consumption rate decreased significantly with time for Zn-exposed individuals and also decreased significantly with the interaction between the concentration of Cd and time for Cd-exposed individuals. Changes occurred in the ammonia excretion rates and O:N ratios with individual sipuncula experiencing different metal concentrations over time. Although low O:N ratios (<30) were obtained in most of the treatments, no predictable correlation was found between concentrations of metals and values of O:N obtained. The value of using O:N ratio as a stress index is questioned.

Keywords: Sipuncula (*Phascolosoma esculenta*); Oxygen consumption; Ammonia excretion; O:N ratio

1. Introduction

Heavy metals are the most common pollutants appearing in many coastal areas worldwide, leading to losses in oceanic yield and hazardous effects on health when contaminants enter the food chain. During the last years, studies have been increasing to assess final fate of heavy metals in coastal environments, particularly in order to find methods to evaluate the environmental damage and to create models to predict the deleterious effects before it is already irreversible. In recent decades, due to industrialization and urbanization in coastal areas, many estuarine waters in the world have increasing contents of heavy metal (Flower, 1990; Li et al., 2007).

Jiulong River Estuary mangrove wetland of Fujian province is an important natural reserve of mangrove in China. In recent years, rapid economic growth and development in the region has led to excessive release of heavy-metal pollutants into the wetland. According to the standard quality of marine sediment in China, Zn and Cd polluted the mangrove wetland of Jiulong River Estuary (Liu et al., 2006).

The chemical concentration in the environment may not be enough to kill the organism. However, sub-lethal concentrations often affect the biochemistry of an organism. It is well known that, trace contaminants in aquatic ecosystem pose environmental hazard because of their great toxicity or persistence (Meador et al., 1995). In recent years, the emphasis on toxicity testing in marine organisms has been moving towards sub-lethal tests, as they can provide much more relevant information in assessing the long-term effects of pollutants imposed on the ecosystem. Various sub-lethal tests and indices from cellular to physiological levels within an organism have been developed

☆The experiments described in this article were conducted in accordance with national and institutional guidelines for the protection of animal welfare.

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(Bayne et al., 1985). Physiological parameters are considered to identify integrated responses to diverse stressors imposed by trace metals, other pollutants, and natural environmental factors such as temperature, salinity, and pH (Depledge et al., 1995). According to Hebel et al. (1997), many physiological variables must be measured to obtain the impact of toxicants in the whole organism which determine the survival potential of individuals. Two methods that have received much attention are the scope for growth and the O:N ratio.

Scope for growth represents the amount of energy available for growth and reproduction in an organism, while the O:N ratio purports to indicate the relative utilization of protein in energy metabolism (Bayne et al., 1985). The O:N values are discussed in relation to the various species' feeding habits. A high value of O:N is taken to represent a predominance of lipid and/or carbohydrate catabolism over protein degradation. In *Mytilus edulis*, O:N ratio values of 50 indicate a healthy condition, values less than 30 indicate a heavy reliance on protein as an energy source, and a value of 7 represents a total reliance on protein (Widdows, 1985). Correlations between the O:N ratio and pollution gradients have been reported (Widdows et al., 1990; Chinni et al., 2002), although other factors such as temperature (Saucedo et al., 2004; Wu and Sun, 2006), salinity (Wu and Sun, 2006), and starvation (Comoglio et al., 2005) may also influence the value of O:N.

Sipuncula *Phascolosoma esculenta*, a marine deposit-feeding benthonic invertebrate, is a special species of China. *P. esculenta* has a wide geographical distribution in the mangroves region in south China (Li, 1989). It is a very abundant intertidal macro-invertebrate along the rocky shore and it is likely to be among the first animals to be affected by anthropogenic sources of pollutants. It is an edible marine species and has long been used as a special dish. Although there was much research about *P. esculenta* on its distribution, classification (Li, 1989), nutritive composition (Zhou et al., 2006), embryo, and larval development (Wu et al., 2006), there has not been any published research regarding the effects of heavy metals on physiological responses of *P. esculenta*. Therefore, it is interesting to investigate the effects on oxygen consumption, ammonia excretion, and O:N ratio of *P. esculenta* exposure to cadmium and zinc.

The objective of the present study was to evaluate sublethal responses such as oxygen consumption, ammonia excretion, and O:N ratio of *P. esculenta* due to waterborne exposure to cadmium and zinc, which are major pollutants in the marine ecosystem (Chen, 1997), in short-term laboratory exposures. This information will provide valuable new data by which any increase in heavy-metal concentration in the study area can be properly evaluated and should form an integral component of any ecotoxicological risk assessment. It is helpful in predicting the ecological consequences of pollution in the mangroves region.

2. Materials and methods

2.1. Collection and maintenance

Specimens of *P. esculenta* (2–4 g body weight) were collected from the mangrove area of Jiulong River Estuary, Fujian. After transport to the Environmental Science Research Center of Xiamen University, animals were kept in tanks with a 15–20 cm layer of sediment from the original habitat. The experimental conditions are similar to environmental conditions. Salinity, water temperature, and dissolved oxygen levels observed were in ranges of 10–20‰, 20–30 °C, and 5.4–7.6 $mg\,L^{-1}$ in the environment, respectively. So, salinity, temperature, and dissolved oxygen levels were observed in the ranges of 13–17‰, 23–25 °C, and 6.1–7.6 $mg\,L^{-1}$, respectively. The animals were allowed to acclimatize in the laboratory for 3 days.

2.2. Toxicant preparation

The stock solution of heavy metals were prepared by dissolving in distilled water, respectively. Nine experimental treatments included four concentrations of Cd (0.45, 0.96, 2.04, and 4.46 $mg\,L^{-1}$), four concentrations of Zn (1.09, 2.34, 4.96, and 10.91 $mg\,L^{-1}$), and one control. Metal solutions were prepared using either cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) (analytical grade) or zinc sulfate ($ZnSO_4 \cdot 7H_2O$) (analytical grade) and artificial seawater. An atomic absorption spectrometer, Vario 6 (Analytik Jena AG) with a deuterium background correction, equipped with a transversely heated graphite furnace atomizer was used for this work. Zn was analyzed by a flame AAS, while Cd was analyzed using graphite furnace AAS. The monitored Zn and Cd wavelengths were 213.9 and 228.8 nm, respectively, while the slit bandwidth was set at 0.5 nm for Zn and 0.8 nm for Cd. The highest concentrations of metal solutions prepared were the LC_{50} values at 96 h obtained for this animal (Chen et al., 2007), and the lowest concentrations were one-tenth that of the LC_{50} values. Sixty individuals were maintained in each treatment and control, respectively. Each treatment and control had three replicates.

2.3. Oxygen consumption

The oxygen consumption rates (OCR) of the 60 individuals from each treatment and control were determined on days 1, 6, 13, and 20 by using bottle-water method. Two individuals were placed for 1 h inside a sealed container (500 mL) with the same solution of heavy metals. The dissolved oxygen (DO) in each container was measured at the start and after 1 h by the Winkler method. One container without sipuncula was used as control. Each treatment and control had three replicates. The oxygen consumption rate was calculated by the following formulation:

$$\mathrm{OCR} = \frac{[(\mathrm{DO}_0 - \mathrm{DO}_t) \times V]}{W \times t}\ \mathrm{mg\,g^{-1}\,h^{-1}},$$

where DO_0 is the DO of the water at the start of the experiment ($mg\,L^{-1}$), DO_t the DO of the water at the end of the experiment ($mg\,L^{-1}$), V the volume of the container (L), W the live wet weight of sipuncula (g), and t is the experimental time (h).

2.4. Ammonia excretion

From each treatment, the ammonia production of the 20 individuals was measured on days 1, 6, 13, and 20. Two individuals were placed inside a sealed container (500 mL) with the same solution of heavy metals, and the amount of ammonia produced in 1 h was determined measured using the phenol–hypochlorite method of Solorzano (1969). One container without sipuncula was used as control. Each treatment and control had three replicates. Ammonia excretion rate (AER) was calculated by the following formulation:

$$\mathrm{AER} = \frac{[(N_t - N_0) \times V]}{W \times t}\ \mathrm{\mu g\,g^{-1}\,h^{-1}},$$