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Influences of petroleum on accumulation of copper and cadmium in the polychaete *Nereis diversicolor*

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Abstract: Using the exposure simulation experiment, the action of petroleum affecting the accumulation of the trace metals including copper (Cu) and cadmium (Cd) in littoral polychaete *Nereis diversicolor* collected from the Shuangtaizi Estuary in Liaoning Province, China was examined. The results showed that there was a markedly non-linear relationship between the accumulation of Cu in worms and the experimental concentration of Cu in exposure solutions when the concentration of petroleum remained at 0, 100, and 220 μl/L, respectively. However, significantly non-linear relationship for worms exposed to Cd was observed only when the concentration of added petroleum was 0 and 220 μl/L. The accumulation of Cu in worms did not differ significantly among the three different levels of petroleum concentrations combined with various concentrations of Cu. So was the accumulation of Cd in worms ($p>0.05$). However, the addition of petroleum in exposure solutions brought about an increase in the accumulation of Cu in *Nereis diversicolor*, in comparison with single Cu pollution. On the other hand, when the concentration of added petroleum remained at 100 μl/L, the accumulation of Cd in worms was lower than that in worms exposed to various concentrations of only cadmium. However, the worms exposed to Cd and petroleum 220 μl/L did not show obvious and identical increase in the accumulation of Cd, compared with single Cd exposure. The accumulation of both Cu and Cd in worms did not increase significantly with the increases in concentrations of Cu or Cd in exposure solutions combined with petroleum (0, 100, and 220 μl/L) under the experimental conditions. Although *Nereis diversicolor* is exposed to very high Cu and Cd in exposure solutions, accumulation and detoxification mechanisms are sufficient to cope with the extra metal influx in order to survive.

Keywords: *Nereis diversicolor*; copper; cadmium; petroleum; combined pollution

Introduction

Estuaries are complex coastal regions with high productivity and biodiversity and crucial to the life history of many birds, fishes, prawns and invertebrates, thus they are of great importance to sustain ecological health of coastal areas. However, they are recently threatened by the contamination due to the anthropogenic discharge of toxic effluents from nearby and remote conurbation and industrial activities, as well as the precipitation of air contaminants. Such pollutants including toxic metals, for example, copper(Cu) and cadmium(Cd), result in the extinction of many living species or the decrease in the number of certain aquatic animals in those estuary ecosystems. Increased heavy metal contamination in estuary ecosystems has become a major concern in recent years (Hadjispyrou *et al.*, 2001; Xu *et al.*, 2004; Zhou *et al.*, 2004). Furthermore, as we known, many habitats of *Nereis diversicolor* are often polluted by petroleum from the leakage of oil tankers and by the exploration and exploitation of oils. According to a report by the Chinese State Bureau of Oceanography in 2003, the contamination of petroleum had taken place in more and more estuaries and the situation of contamination was more serious than before. Our previous study indicated that the concentration of petroleum in polluted seawater and sediments of the Shuangtaizi Estuary was up to 111—223 μl/L, and the highest concentration of Cu and Cd in seawater and sediments of the Shuangtaizi Estuary was up to 230 and 4.2 mg/kg, respectively. There is a similar situation of pollution in other countries. For example, the maximal concentration of Cu in the Bilbao Estuary, Northern Spain reached 740 μg/L (Irabien *et al.*, 2000), and a remarkable increase in the concentrations of heavy metals in the sediment of the Swartkops River Estuary, Port Elizabeth South Africa were revealed in comparison with the results made about 20 years ago (Binning and Baird, 2001).

The polychaete *Nereis diversicolor* is one of the commonest intertidal inhabitants of estuaries, to the extent that it is considered to be an ecologically keystone species (Zhou *et al.*, 2003). *Nereis diversicolor*, which is one of many species of sediment-dwelling organisms, plays a key role in the fate of chemicals as a consequence of its bioaccumulation

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capacity and of its influences on metal speciation in sediments through bioturbation(Francois *et al.*, 2002). It is also a key species of estuarine ecosystems functioning as a major food source for crustacean, fishes and waders, and a major component of the benthic biomass of mudflats. Then it can be concluded that the accumulation of trace metals in the bodies of *Nereis diversicolor* can bring about the transfer of heavy metals among food chain, or even biomagnification with high toxic effects at higher trophic levels. In addition, this species lives in the sediment of estuaries and serves as a good biological indicator of the contamination of the sediment by toxic trace metals, such as Cu and Cd (Bryan and Gibbs, 1983, 1987; Dallinger and Rainbow, 1993).

Although aquatic ecosystems often contain complex mixtures of contaminants, including petroleum and metals, such as Cu, Cd, Pb and Zn (Kennicutt *et al.*, 1996; Daskalskis and Oconner, 1995), the influences of contaminant mixtures on natural communities are poorly understood(Breitburg *et al.*, 1999) and little is known about their interaction. Up to now, there have been many studies focusing on the accumulation of Cu and Cd in worms, and most of them were carried out under the stress of single-factor pollution. However, there are no records about influences of petroleum on the accumulation of Cu and Cd in worms till now. It has been an accepted fact that, since chemical never occurs alone in nature, it is most important to investigate joint effects of more than one chemical on living organisms (Cheng and Zhou, 2002; Kungolos *et al.*, 1999; Zhou and Cao, 1994; Zhou, 1995, 1996; Zhou *et al.*, 2004). Therefore it is essential to evaluate influences of interaction between trace metals and petroleum on the accumulation of metals in worms and to determine the mode of interaction between them, antagonism or synergism (Guo and Zhou, 2003; Zhou , 1995; Zhou and Zhu, 1997; Zhou *et al.*, 2004).

In view of the fact that laboratory studies can frequently expound that the joint effects of contaminants with different modes and sites of toxicity, such as organic pollutants and heavy metals, equals to the sum of responses to the individual contaminants alone (Swartz *et al.*, 1995), the goal of this work was thus to compare differences in the accumulation of trace metals by the polychaete *Nereis diversicolor* under the stress of single Cu or Cd pollution, and of the combined pollution of petroleum and Cu and of petroleum and Cd in a series of exposure doses, consequently to determine whether petroleum promoted the accumulation of Cu and Cd in the worms or not.

1 Materials and methods

1.1 Sample collection

The polychaete *Nereis diversicolor* was collected from a relative clean site in the Shuangtaizi Estuary (41 ° 1 0'N, 122 ° 1 6'E) at low tide during the fall of 2004 (Fig.1). The Shuangtaizi Estuary neighbors on Panjin, Liaoning Province, China, which is the center of the Liaohe Delta and is a burgeoning city known as the exploitation of petroleum and the production of petrochemicals. Many oil wells are distributed along the Shuangtaizi Estuary. Worms were transferred from a field to our laboratory in cool boxes with sediments from the site of the origin. They were then maintained in sediments overlain by seawater in the laboratory at 10±1℃. Seawater from the site of origin was used as the medium of *Nereis diversicolor* in a field, and hence to eliminate disturbance factors that might affect uptake rate and toxicity of trace metals in the worms. The reagents used in this study were of analytical grade. The tested forms of Cu and Cd were $CuSO_4 \cdot 5H_2O$ and $CdCl_2 \cdot 2.5H_2O$, respectively. The tested petroleum was obtained from a gas station in Shenyang.

Fig.1 The geographical location of the sampling site for *Nereis diversicolor*

1.2 Exposure experiment

Collected worms were rinsed using seawater from the site of origin. Then every five worms were put to a 100 ml acid-washed glass beaker with 50 ml seawater containing one of the following pollutants whose tested concentrations were 0, 0.15, 1.5, 5.0, and 8.0 μmol/L for Cu, 0, 0.45, 4.5, 7.0, 9.0, and 27.0 μmol/L for Cd, and 0, 100, and 220 μl/L for petroleum. The exposure experiments were carried out in triplicate at 10 ±1℃ up to 21 d and exposure solutions were changed every three days. All beakers used in the experiments had been earlier soaked using 5% HNO_3, eliminating any adsorption of metals onto the beakers(Mouneyrac *et al*., 2003), and covered with glass utensils in order to reduce evaporation and heat dissipation.

1.3 Metal and petroleum analyses

After a 21-day exposure, all surviving worms were frozen at -20℃, and then dried to constant weight at 60℃ and later digested in concentrated HNO_3 and $HClO_4$ at 100℃. The digested solution was made up to known volumes with distilled water. The concentrations of Cu and Cd in the digested acidic solution were determined using the flame atomic absorption spectrophotometry (FAAS, WFX-120A, Ruili, Beijing). After an ultrasonic extraction using aether, the concentrations of petroleum in the extracted solution were determined using the gas chromatographic(Agilent 6890, USA) analysis.

1.4 Datum treatment

The statistical analyses of data obtained from this experiment were carried out using the SPSS 11.5, including the calculation of average values, standard deviation (*SD*), regression, variance analysis and multiple comparison. ANOVA was used to compare the accumulation of trace metals in the worms exposed to different pollutants as a function of concentrations of pollutants and statistical significance was set at $p<0.05$.

2 Results and discussion

2.1 Metal accumulation in the polychaete *Nereis diversicolor* exposed to Cu or Cd

The experimental results about the polychaete *Nereis diversicolor* which were exposed to a series of increasing Cu concentrations are shown in Fig.2. Under the single Cu pollution, it was indicated by the variance analyses that there were no significant differences in the concentration of Cu accumulated in worms among all the five concentrations of Cu in the exposure experiment. However, the Cu accumulation in worms exposed to 8.0 μmol/L Cu had a significant ($p<0.05$) difference from that exposed to one of the treatments 0.15, 1.5 and 4.5 μmol/L Cu, although the accumulated Cu by worms exposed to 0.15, 1.5 and 4.5 μmol/L Cu did not differ significantly each other. According to Fig.2, the accumulation of Cu in worms exposed to 8.0 μmol/L Cu in absence of petroleum was much lower than that in worms from exposure solutions with the concentration of Cu less than 8.0 μmol/L. Thus it was presumed that the elimination of Cu in *Nereis diversicolor* is probably due to the induction of metabolism and excretion under the stress of high Cu. Christensen *et al*.(2002) found that *Nereis diversicolor* excreted 50% of the body-burden of

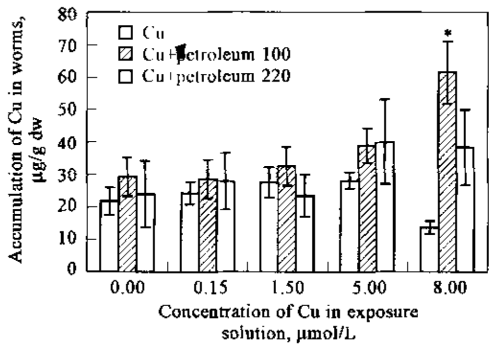

Fig.2 Accumulation of Cu in worms with the stress of Cu and petroleum(0, 100, 220 μl/L)
* indicates significance at the 0.05 probability levels between Cu and Cu+petroleum 100 treatment.

unmetabolized pyrene within 2 d after being transferred to an uncontaminated sediment. However, accumulated metals (Cu, Cd, Zn) in worms were not excreted in 21 d, when being transferred to 16 psu artificial seawater(Zhou *et al*., 2003).

The relationship between the accumulation of Cu in worms and exposure concentration of Cu in solution can be expressed as follows:

$$Y_1 = -0.675X_1^2 + 0.428X_1 + 22.72$$

$$(n=5,\ R^2 = 0.987,\ p<0.01) \quad (1)$$

Where X_1 is the concentration of Cu added to the exposure solution, μmol/L; and Y_1 is the concentration of Cu accumulated in worms, μg/g dw. From the Equation (1), the significant non-linear relationship between the accumulation of Cu in worms and the concentration of Cu in exposure solutions was observed.

The accumulation of Cd in worms increased with the concentration of Cd in exposure solutions(Fig.3). However, it was indicated by the variance of analysis that the concentration of Cd accumulated in worms did not differ significantly among all the 6 exposure concentrations, although Cd accumulation in worms exposed to 27 μmol/L Cd was markedly ($p<0.05$) higher than that in worms exposed to other concentrations. Furthermore, the relationship between Cd accumulated in worms and the exposed doses of Cd was also significantly ($p<0.05$) non-linear. The corresponding relationship can be expressed using the following regression equation:

$$Y_2 = 0.052X_2^2 - 0.965X_2 + 29.84$$

$$(n = 6,\ R^2 = 0.860,\ p<0.05) \quad (2)$$

Where X_2 is the concentration of Cd in the exposure solution, μmol/L; and Y_2 is the concentration of Cd accumulated in worms, μg/g dw.

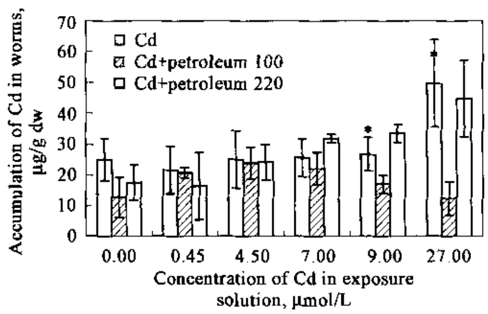

Fig.3 Accumulation of Cd in worms with the stress of Cd and petroleum(0, 100, 220 μl/L)
* indicates significance at the 0.05 probability levels between Cd and Cd+petroleum 100 treatment

2.2 Influences of petroleum on Cu accumulation in the polychaete *Nereis diversicolor*

The experimental results of *Nereis diversicolor* exposed to a series of increasing Cu concentration with the addition of 100 and 220 μl/L petroleum, are shown in Fig.2. It was indicated by the variance analyses that there were no significant differences in the accumulation of Cu in worms among the addition of two different levels of petroleum combined with all the concentration of Cu in exposure solutions, except 8.0 μmol/L Cu. In other words, there was a significant($p<0.05$) increase in the accumulation of Cu in worms exposed to 8.0 μmol Cu/L with the addition of 100 μl/L petroleum (Fig.2), compared with exposure solutions with the same concentration of only Cu. In addition, the statistical analysis showed that the Cu accumulation in worms did not differ significantly among all the 5 experimental concentrations of Cu in exposure solutions when the concentration of added petroleum was 100 and 220 μl/L, respectively. When the exposure concentration of petroleum was 100 μl/L, Cu accumulation in worms exposed to 8.0 μmol /L Cu was significantly($p<0.05$) higher than that in worms exposed to 0, 0.15, and 1.5 μmol/L Cu which themselves did not differ markedly. The significant non-linear relationships between the accumulation of Cu in worms and the experimental concentration of Cu in exposure solutions when the concentration of added petroleum was 100 and 220 μl/L can be expressed by the regression equations:

$$Y_1 = 0.463X_1^2 + 0.27X_1 + 29.44$$
$$(n = 5, R^2 = 0.990, p<0.01, X_3 = 100) \quad (3)$$

and

$$Y_1 = -0.257X_1^2 + 3.97X_1 + 25.03$$
$$(n = 5, R^2 = 0.718, p<0.05, X_3 = 220) \quad (4)$$

Where X_3 is the concentration of added petroleum, μl/L.

As shown in the Eq. (1), (3) and (4), the relationships between the accumulation of Cu in worms and a series of exposure concentrations of Cu were at the same significance level when the concentration of petroleum was 0 and 100 μl/L, but the significance level was low when the concentration of petroleum was 220 μl/L, which indicated that there were interactive effects between Cu and petroleum with the increasing concentrations of petroleum.

It was revealed in Fig.2 that the accumulation of Cu in worms under the joint stress of Cu and petroleum was higher than that in worms exposed to single Cu pollution, though the differences were not significant. This increase was especially obvious in the exposure solution with various concentrations of Cu and 100 μl/L petroleum. As shown in Fig.2, the addition of petroleum (100, and 220 μl/L) to the exposure solutions of Cu resulted in the increase in the accumulation of Cu in worms with the increasing concentration of Cu in exposure solutions. However, the increases were not significant. And the accumulation of Cu in worms exposed to various concentrations of Cu with the addition of 100 μl/L petroleum was higher than that in worms exposed to 220 μl/L petroleum and Cu at each of experimental doses of Cu (Fig. 2). Therefore, it can be concluded that the joint effects of Cu and petroleum on the accumulation of Cu in worms were extremely complicated and is dependent on the concentration of petroleum.

2.3 Influences of petroleum on Cd accumulation in the polychaete *Nereis diversicolor*

The accumulation of Cd in worms exposed to combined pollution of Cd and petroleum was described in Fig.3. It was indicated by the variance of analysis that the concentration of Cd in worms exposed to combined pollution of Cd and 100 μl/L petroleum differed insignificantly among all the 6 treatments. So was the concentration of Cd accumulated in worms exposed to a range of experimental doses of Cd with the addition of 220 μl/L petroleum. Nevertheless the worms living in the exposure medium with 27 μmol/L Cd and 100 μl/L petroleum had significantly ($p<0.05$) higher Cd concentration than that the worms exposed to the mixture of petroleum and Cd (4.5 and 7.0 μmol/L Cd), which were not significantly different from each other. Similarly, when the concentration of added petroleum was 220 μl/L and the concentration of Cd was 27 μmol/L, the worms had a significantly increase in the accumulation of Cd, compared with the mixed solutions with petroleum and 0, 0.45, 4.5 μmol/L Cd, respectively. Among the six experimental concentrations of Cd, only when the concentration of Cd remained at 9.0 μmol/L Cd, the concentration of Cd accumulated in worms brought about a significant difference($p<0.01$) across the three levels of petroleum exposure (Fig.3). That is to say, the accumulation of Cd in worms exposed to 100 μl/L petroleum and 9.0 μmol/L Cd differed significantly from the other two exposure solutions with 0 μl/L petroleum and 9.0 μmol/L Cd, and with 220 μl/L petroleum and 9.0 μmol/L Cd.

When the concentration of petroleum reached 220 μl/L, the accumulation of Cd in worms increased with the exposure doses of Cd in mixed solutions. However, the accumulation of Cd in worms under the combined pollution of 100 μl/L petroleum and every one of the six experimental concentrations of Cd increased at first, then decreased with the increases in the concentration of Cd in the exposure solutions. The Cd accumulation in worms exposed to only Cd was higher than that in worms exposed to every one of the six concentrations of Cd combined with 100 μl/L p etroleum, but the differences between both of exposure media were significant only at the concentrations of 9.0 μmol/L Cd, 27.0 μmol/L Cd (Fig.3). In addition, the accumulation of Cd in worms exposed to the mixture solutions of 220 μl/L petroleum and a range of Cd concentrations was higher than that in worms living in the exposure medium with 100 μl/L petroleum and each of experimental concentrations of Cd (Fig.3). When the concentration of petroleum reached 220 μl/L, the accumulation of Cd in worms increased with the increasing experimental doses of Cd under the condition of combined pollution, but they were still not higher than those in worms exposed to only Cd (Fig.3).

There was an insignificantly ($p>0.05$) non-linear relationship between the accumulation of Cd in worms and the concentration of Cd in exposure solutions when the concentration of added petroleum reached 100 μl/L. Neither was the accumulation of Cd in worms lived in exposure solutions of Cd combined with 220 μl/L petroleum. The corresponding regression equations can be expressed as:

$$Y_2 = -0.023X_2^2 + 0.36X_2 + 19.19$$

$$(n = 6,\ R^2 = 0.584,\ p>0.05,\ X_3 = 100) \qquad (5)$$

and

$$Y_2 = -0.038X_2^2 + 1.99X_2 + 18.66$$

$$(n = 6,\ R^2 = 0.982,\ p<0.01,\ X_3 = 220) \qquad (6)$$

According to the regression equations of Cu and Cd above described, our result is in line with the conclusion of Berthet (2003) that bioavailability of sediment-bound metal to *Nereis diversicolor* is not necessarily a simple direct relationship to total metal concentration in sediments.

3 Conclusions

The relationships between the accumulation of Cu and Cd in the polychaete *Nereis diversicolor* and the experimental concentrations of Cu and Cd in exposure solutions combined with petroleum (0, 100, 220 μl/L) were non-linear. The addition of petroleum resulted in the increase in the accumulation of Cu in worms exposed to Cu and petroleum, in comparison with the worms exposed to single Cu pollution. On the contrary, combined pollution of petroleum and Cd under the experimental conditions did not bring about a significant increase in the accumulation of Cd in worms, compared with only single Cd pollution. Moreover, the accumulation of Cu in worms exposed to various concentrations of Cu and 100 μl/L petroleum was higher than that in worms exposed to a series of concentrations of Cu combined with 220 μl/L petroleum. The worms lived in the mixture solutions of Cd and 220 μl/L petroleum had higher Cd accumulation than those lived in the mixture solutions of Cd and 100 μl/L petroleum. So it can be concluded that the addition of petroleum to the exposure solutions of Cu and Cd would exert extremely complicated and distinct effects on the accumulation of Cu and Cd in worms, and the influences of petroleum on the accumulation of Cu and Cd in worms are dependent on the concentration of petroleum.

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