



## Influences of Cu or Cd on the neurotoxicity induced by petroleum hydrocarbons in ragworm *Perinereis aibuhitensis*

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Received 21 May 2007; revised 13 July 2007; accepted 13 August 2007

### Abstract

The ecotoxicological effects of heavy metals and petroleum hydrocarbons (PHCs) on ragworms are still vague. The relationships between toxicological indices (mortality and acetylcholinesterase (AChE) activity) and concentrations of toxicants (Cu, Cd, and PHCs) were examined in the estuary keystone species *Perinereis aibuhitensis* in laboratory conditions. The results of single toxicant indicated that three toxicants had potentially physiological toxicity to *P. aibuhitensis*. The estimated 4-d and 10-d LC<sub>50</sub> for Cu, Cd, and PHCs was derived from the relationships between mortality and toxicants concentrations. Notable changes in the morphological signs and symptoms of *P. aibuhitensis* exposed to PHCs were observed. The AChE activity of *P. aibuhitensis* was more sensitive to the toxicity of PHCs than the others. The results of combined toxicants implied that the combined toxicity of Cu or Cd and PHCs to *P. aibuhitensis* was related to the concentration combination of toxicants. Compared to single PHCs treatment, the addition of Cu or Cd significantly mitigated the neurotoxicity of PHCs to AChE activity in *P. aibuhitensis*, which showed an antagonistic effect.

**Key words:** acetylcholinesterase (AChE); *Perinereis aibuhitensis*; Cu; Cd; petroleum hydrocarbons

### Introduction

Intertidal belt is the most sensitive component of littoral ecosystems, which is constantly threatened by contamination owing to its proximity to human settlements and highly developed economic and industrial activities. According to the China Marine Environment Quality Report (2003 to 2005, [http://www.soa.gov.cn/hyjww/hygb/A0207index\\_1.htm](http://www.soa.gov.cn/hyjww/hygb/A0207index_1.htm)) issued by the State Oceanic Administration (SOA), in the certain sea areas, the levels of Cu and Cd had exceeded the national standards. In addition, the coastal waters of the four sea areas of China were subject to the impact of petroleum hydrocarbons (PHCs). In the Yellow Sea, the petroleum concentration exceeded the national marine water quality standard by 8.0%. The pollutants enter the marine ecosystem through land-based activities, aquacultural activities, and even sea accidents, causing toxicity to marine invertebrates (George *et al.*, 2004; Zhou *et al.*, 2003). From an ecological point of view, therefore, understanding the environmental impacts of metabolise xenobiotics existing in the intertidal belts has been a crucial subject. The ragworms are marine invertebrates, which are the conspicuous elements of benthos in intertidal mudflats and estuaries with a worldwide distribution.

They form an important part of the food supply for various birds and bottom-dwelling fishes. Owing to the good adaptability to stressed environmental conditions, some researchers had regarded them as the robust sentinel and potentially ecological keystone species for monitoring programs (Bryan and Gibbs, 1987; Pérez *et al.*, 2004; Fourcy *et al.*, 2002).

Molecular indicators of anthropogenic pollutant effects, the so-called biomarkers are valuable tools in ecotoxicological monitoring. From the viewpoint of estimating pollution stress on marine invertebrates, researchers explored the ecotoxicological responses caused by xenobiotics in the target organisms (Pérez *et al.*, 2004; Fourcy *et al.*, 2002; Scaps *et al.*, 1997). Pérez *et al.* (2004) suggested that acetylcholinesterase (AChE) was the most sensitive biomarker in the investigated species, namely, the clam *Scrobicularia plana* and the polychaete worm *Nereis diversicolor* from the Cádiz bay (SW Spain) contaminated by untreated domestic discharges. AChE is a well-established biomarker of neurotoxic effects, which is present in most animals and is responsible for the rapid hydrolytic degradation of the neurotransmitter acetylcholine (ACh). AChE is abundant in invertebrates' nervous tissues, brain, red blood cells, and muscles. Scaps *et al.* (1996) characterized the cholinesterase (ChE) of the ragworm *N. diversicolor* and found that the ChE of this species turned out to be an

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AChE. Sibley *et al.* (2000) demonstrated in the microcosm study that AChE activity can be used as a reliable biomarker of exposure and mortality at the individual organism level. Martínez-Tabche *et al.* (1997) found that both Pb and crude oil can cause inhibition of AChE in the cladoceran *Moina macrocopa*, especially crude oil, which was the most potent anticholinesterase agent. Cd can also affect cholinergic transmission in the brains of adult male rats (De Castro *et al.*, 1996).

Although aquatic invertebrates were used routinely as biological indicators, the use of AChE inhibition as a biomarker in these species has largely been neglected (Ross and William, 2003). AChE inhibition was commonly used as a biomarker of pollution by pesticides including neurotoxic organophosphates (OP) and carbamates (CB) as effective compounds (Day and Scott, 1990; Jain-Rang *et al.*, 1998; Rao *et al.*, 1991; Yamin *et al.*, 1994). There is considerably less knowledge about the combined effects of multi-toxicants on the AChE activity of marine invertebrates. Most studies have focused on the uptake and bioaccumulation of heavy metals in the ragworm (Demuynck *et al.*, 2004; Grant *et al.*, 1989; Zhou *et al.*, 2003). The lack of systematic studies on the interactive effect of multi-toxicants on biomarkers prompted us to compare and clarify the changes of the AChE activity profile in the ragworm *Perinereis aibuhitensis* for the existence of Cu-PHCs or Cd-PHCs.

## 1 Materials and methods

### 1.1 Collection and acclimation of the ragworms

The ragworm *P. aibuhitensis* was collected from the coastal mudflat of the Ganyu County hatchery (34°84'N, 119°19'E) in Jiangsu Province near the Yellow Sea in Southeastern China at low tide during the autumn of 2005 (Fig.1). The ragworms had an average fresh filter-paper-dried weight of  $0.80 \pm 0.23$  g. Prior to any experiment, the ragworms were rinsed in sea water and held in 1.6% arti-

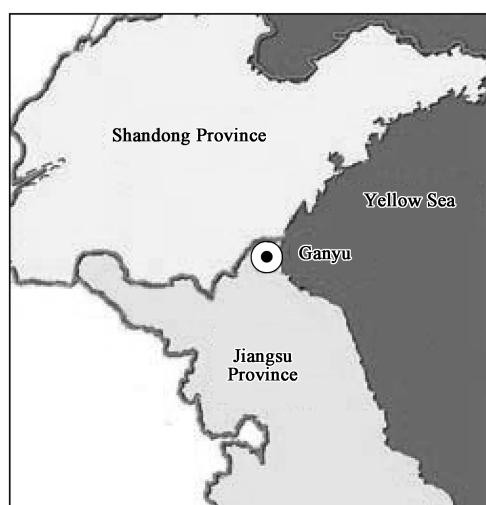


Fig. 1 Geographical location of the sampling site for the ragworm *Perinereis aibuhitensis*.

ficial seawater (Instant Seawater Crystal, China) for 2 d to acclimate to the test environment under starvation. The use of artificial seawater provides a medium of reproducible physico-chemical conditions.

### 1.2 Heavy metals and petroleum hydrocarbons background of the ragworm samples

The ragworm samples were dried to constant weight at 60°C and digested in the concentrated HNO<sub>3</sub> and HClO<sub>4</sub> at 100°C. 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 flame atomic absorption spectrophotometry (FAAS, WFX-120A, Ruili, Beijing). According to the method of the China marine monitoring criterion (GB17378.6-1998), the concentration of the total PHCs in the ragworm samples were determined using a fluorospectrophotometer (RF-540, Shimadzu, Japan).

### 1.3 Toxicity experiments and determination of single and joint toxicant concentration

After acclimation of the ragworms to laboratory conditions, every 15 ragworms were randomly divided into one treatment group and 5 ragworms were put into a 250-ml acid-washed glass beaker with 100 ml of 1.6% artificial seawater containing various concentrations of Cu, Cd, and PHCs described above for more than 10 d (15 d). The exposure experiments were carried out in triplicate at  $15 \pm 1^\circ\text{C}$  in continuous dark. Controls were also run in parallel with the artificial seawater alone. To maintain the toxicant concentration, the treatment solutions were renewed every day. No food was provided during the test period to avoid excessive activity of the ragworms. The behavioral and morphological abnormality and the percentage mortality of *P. aibuhitensis* were observed every day. The relationships between the toxicant concentration and mortality rate of *P. aibuhitensis* were subjected to nonlinear curve fitting for calculating the median lethal concentration (LC<sub>50</sub>) of the test toxicants.

The tested forms of Cu, Cd, and PHCs were CuSO<sub>4</sub>·5H<sub>2</sub>O, CdCl<sub>2</sub>·2.5H<sub>2</sub>O, and petroleum, respectively. The tested petroleum was obtained from a gas station in Shenyang. According to our previous study (Zhou *et al.*, 2003), the predetermined concentration levels of heavy metals were ranging from 0.1 to 3 (0.1, 0.2, 0.5, 1, 2, and 3) mg/L of Cu and from 0.2 to 15 (0.2, 0.5, 1, 5, 10 and 15) mg/L of Cd. The concentration levels of PHCs were ranging from 0.05 to 1 (0.05, 0.1, 0.2, 0.3, 0.5, and 1) ml/L based on the previous results (0.11 to 0.22 ml/L) of Shuangtaizi Estuary in Liaoning Province near the Bohai Sea in Northeastern China. After the calculation of LC<sub>50</sub>, the concentration levels of single toxicant acting on AChE in *P. aibuhitensis* were determined. The concentration levels were 0.05, 0.1, 0.2, 0.5 mg/L of Cu, 0.1, 0.5, 3, 8 mg/L of Cd, and 0.05, 0.1, 0.15, 0.2 ml/L of PHCs, respectively. The concentration levels of combined toxicants of Cu-PHCs and Cd-PHCs acting on AChE in *P. aibuhitensis* were also determined, which were 0.1, 0.2 mg/L of Cu, 0.5, 3 mg/L of Cd, and 0.1, 0.2 ml/L of PHCs, respectively.

### 1.4 Protein content and AChE activity

The ragworm tissues were homogenized individually at 4°C in one volume of ice-cold 0.1 mol/L, pH 8.0, phosphate buffer, including 0.5% TritonX-100 (1/20, w/v). The homogenates were then centrifugated at 10000×g for 30 min at 4°C. The supernatants stored on ice were used for AChE assay. Protein content was estimated by the method of Lowry *et al.* (1951).

AChE assay was carried out spectrophotometrically utilizing the method of Ellman *et al.* (1961). Reactions were performed in 300 µl of phosphate buffer, pH 8.0, containing (a) 20 µl of 0.01 mol/L DTNB (5,5'-dithio-(2-nitrobenzoic acid), (b) 20 µl of 0.075 mol/L ACTC (acetylthiocholine) iodide as substrate, and (c) 10 µl of protein. DTNB and substrate solutions were prepared in phosphate buffers at pH 7.5. The reactions were performed at 20°C and absorbance was recorded at 405 nm for 1 min using a microplate reader (Thermo Electron Multiskan MS, USA). The protein content and the AChE activity of the ragworms were estimated in triplicate after 2-d, 4-d, 6-d, and 10-d exposure. Enzymatic activity was corrected for spontaneous hydrolysis of the substrate and was expressed as nmol/(min·mg protein).

### 1.5 Statistical analysis

All the data were statistically processed by Origin 6.0, including the calculation of mean values, correlation, and regression. The results of AChE activity were expressed as mean ± standard deviation and were analyzed by a one-way ANOVA.  $P < 0.05$  was selected as the criterion for statistical significance.

## 2 Results

### 2.1 Cu, Cd, and total PHCs background of the ragworm samples

The concentrations of Cu, Cd, and total PHCs in the collected ragworm samples were determined using flame atomic absorption spectrophotometry and the fluorospectrophotometer, respectively. The results showed that  $9.73 \pm 0.92$  mg/kg dw of Cu,  $0.57 \pm 0.22$  mg/kg dw of Cd, and  $34.55 \pm 0.32$  µg/kg dw of total PHCs (equivalent to 0.005 ml/L) was in the ragworm samples, which was considered to be uncontaminated according to the China Marine Monitoring Criterion such as less than 10 mg/kg of Cu (up to the first grade), less than 2 mg/kg of Cd (up

to the second grade), and 15 mg/kg of the total petroleum hydrocarbons (up to the first grade).

### 2.2 Morphological signs and symptoms and toxic effects

The ragworms showed progressive signs and symptoms of toxicity such as coiling, curling, and excessive metabolic excretion at the initial exposure in all the treatments. 60% of the ragworms exposed to higher concentration levels of PHCs (0.15 and 0.2 ml/L) for 4 d extruded coelomic fluid and resulted in bloody lesions (Fig.2b). Morphological changes of the exposed ragworms, such as constriction, started appearing within 24 h. 40% of the ragworms exposed to PHCs presented constriction and cuticle rupture after 4-d exposure (Arrows in Fig.2b). 85% of the exposed ragworms after 8-d exposure in the treatments of PHCs showed degenerative changes (Arrow in Fig.2c). Most of the ragworms in all treatments were sluggish after 4-d exposure, and a lack of response to stimulus was also noted.

The toxic effects of Cu, Cd, and PHCs against the ragworm *P. aibuhitensis* were also recorded after 4-d and 10-d exposure. The corresponding relationships between the mortality of *P. aibuhitensis* and the amount of toxicants added to the artificial seawater can be described as the sigmoidal curve of the Boltzmann Function (Glantz, 2001). The equation of the Boltzmann Function is as follows:

$$Y = A_2 + (A_1 - A_2)/(1 + \exp(X - x_0)/x) \quad (1)$$

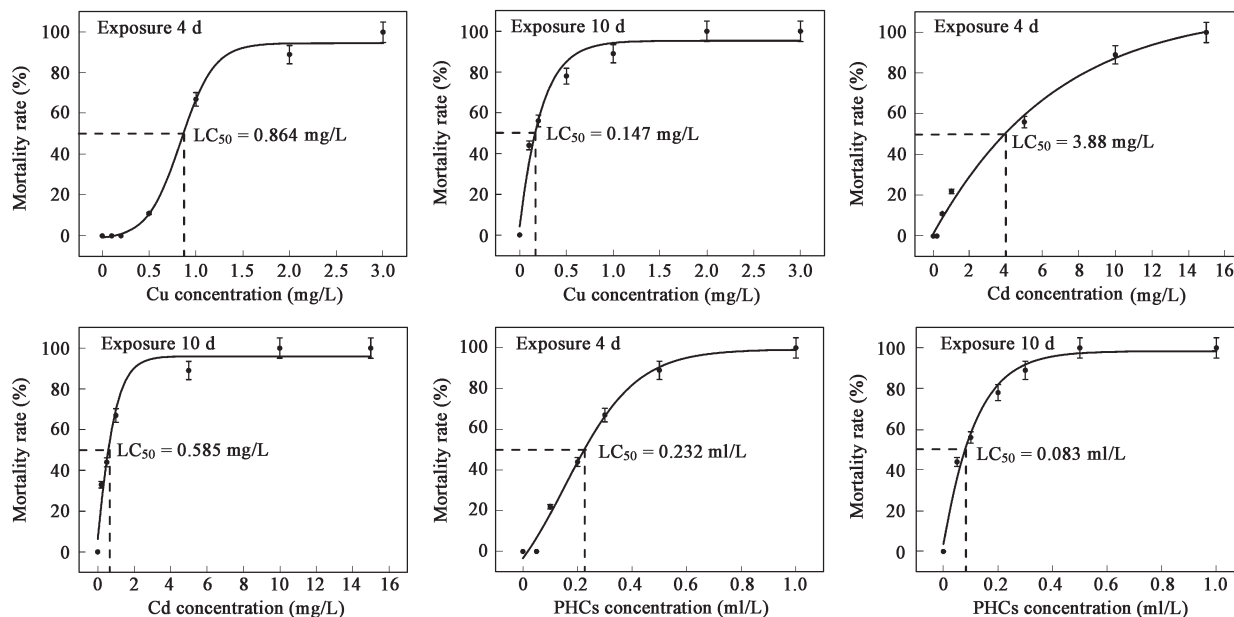
where,  $X$  is the concentration of single toxicant (Cu, Cd, and PHCs) added to the artificial seawater, mg/L or ml/L;  $Y$  is the mortality of the ragworms related to the action of toxicants;  $A_1$ ,  $A_2$ ,  $x_0$ , and  $x$  are coefficient or constant, respectively. The relationships and the median lethal concentrations ( $LC_{50}$ ) of Cu, Cd, and PHCs calculated are shown in Fig.3. 100% mortalities of the three toxicants were observed at the concentration of 3 mg/L of Cu, 15 mg/L of Cd, and 1 ml/L of PHCs on day 4. At the end of 10-d exposure, the next 100% mortalities were abruptly dropped at the concentration of 2 mg/L of Cu, 10 mg/L of Cd, and 0.5 ml/L of PHCs.

### 2.3 Influence of single Cu, Cd, and PHCs on the AChE activity in the ragworm *P. aibuhitensis*

The *in vivo* AChE activity of the ragworms exposed to single Cu, Cd, and PHCs was affected by the exposure dose of the toxicants. There was a negative linear-relationship between the AChE activity of the ragworms and the



**Fig. 2** Morphological abnormalities in the ragworm *Perinereis aibuhitensis* after 0-d, 4-d, and 8-d exposure of PHCs. (a) control; (b) 4-d exposure; (c) 8-d exposure.

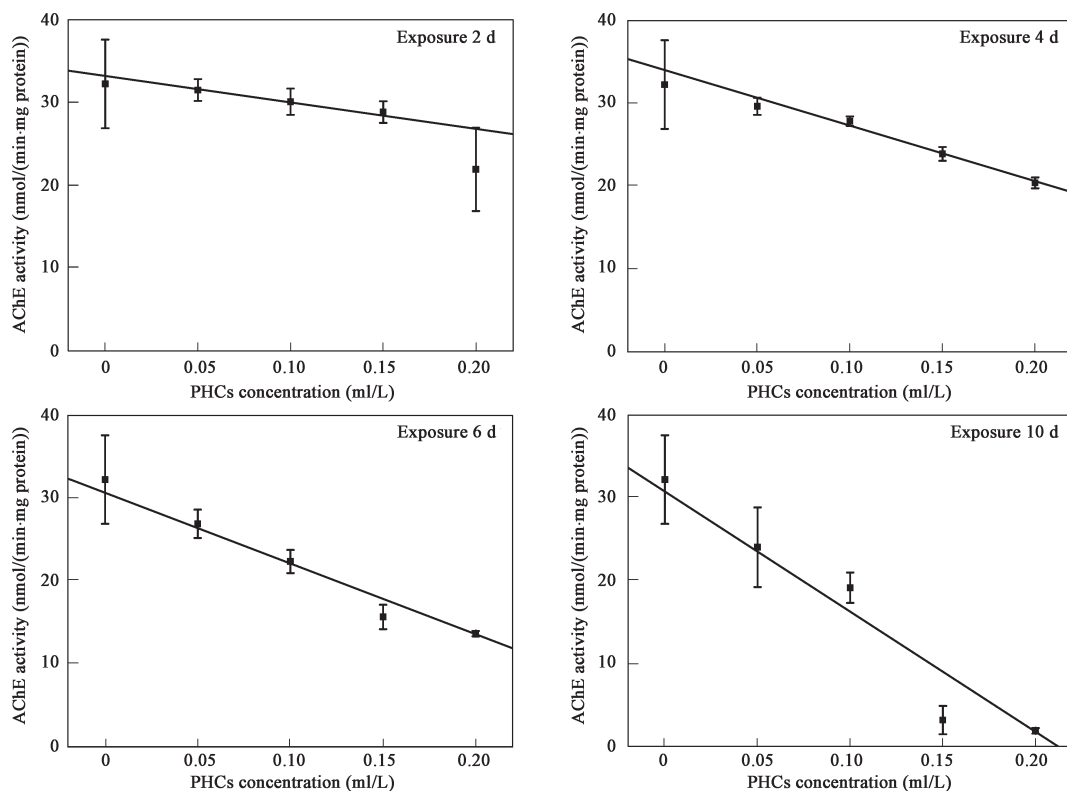


**Fig. 3** Toxicity curves, regression equations, and median lethal concentrations ( $LC_{50}$ ) of Cu, Cd, and PHCs to the ragworm *Perinereis aibuhitensis* in the artificial seawater test after 4-d and 10-d exposure.

concentrations of PHCs (Fig.4), while a negative non-linear correlation occurred in the Cu or Cd treatments. The inhibition of AChE in the ragworms exposed to Cu (Fig.5a), Cd (Fig.5b), and PHCs (Fig.5c) in artificial seawater for 2, 4, 6, and 10 d was measured. We observed that the percentage inhibition of AChE activity exposed to single Cu or Cd was less than 50% during the test period. When the given concentration exceeded 10-d  $LC_{50}$  (0.083 ml/L) and approximated 4-d  $LC_{50}$  (0.232 ml/L), the

percentage inhibition of AChE activity exposed to PHCs was more than 50% after 6-d exposure. Especially, the AChE activities of the ragworm *P. aibuhitensis* exposed to 0.15 and 0.2 ml/L of PHCs were significantly inhibited at the end of 10 d as compared with the control and the percentage inhibitions were 90% and 94%, respectively.

Exposure time is another factor that affects AChE activity. AChE activity of the ragworms tends to decrease with the exposure time increasing. When exposed to 0.15 and



**Fig. 4** Relationships between the AChE activity in the ragworm *Perinereis aibuhitensis* and the exposed concentration of PHCs after different exposure time.

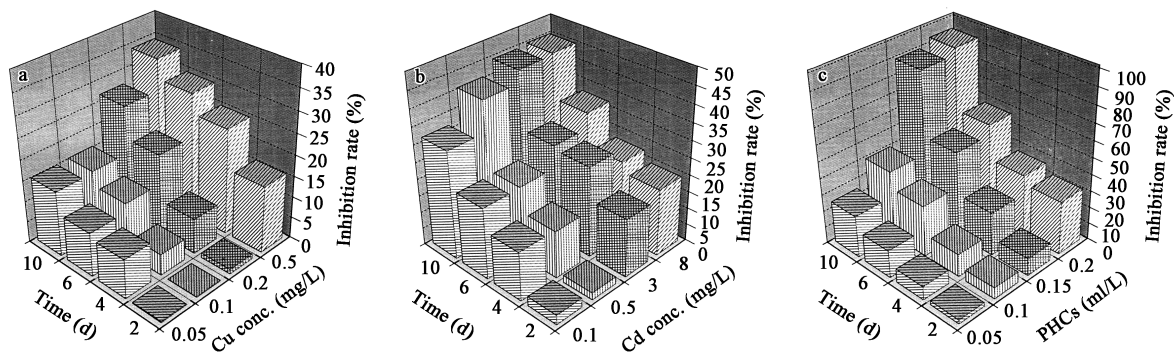


Fig. 5 Percentage inhibition of AChE in the ragworm *Perinereis aibuhitensis* exposed to single Cu (a), Cd (b), and PHCs (c) for 2, 4, 6, and 10 d.

0.2 ml/L of PHCs, the AChE activity was inhibited 10.5% and 32% after 2 d and this inhibition increased further to 26% and 36.8%, 51.4% and 57.6%, 94% and 90% after 4-d, 6-d, and 10-d exposure, respectively (Fig.5c). For 0.5 mg/L of Cu, the inhibition of AChE activity increased only from 15.9% to 34.3% during the entire tested period (Fig.5a). For 3 and 8 mg/L of Cd, the changes were from 17.2% to 44.7% and from 19.8% to 45.2%, respectively (Fig.5b).

#### 2.4 Influence of Cu-PHCs and Cd-PHCs on AChE activity in the ragworm *P. aibuhitensis*

The tested concentrations of Cu-PHCs and Cd-PHCs acting on AChE activity in the ragworms *P. aibuhitensis* were determined according to the single-factor toxic effects of Cu, Cd, and PHCs against the ragworms. The concentrations of Cu were 0.1 and 0.2 mg/L, the concentrations of Cd were 0.5 and 3 mg/L, and the concentrations of PHCs were 0.1 and 0.2 ml/L. Two chosen concentration levels of each toxicant were no less than 10-d  $LC_{50}$  and no more than 4-d  $LC_{50}$  values.

The AChE activity in the ragworms exposed to Cu-PHCs and Cd-PHCs for 10 d, during which time the ragworms still possessed vital signs, is shown in Fig.6. For 0.2 ml/L of PHCs (approximate 4-d  $LC_{50}$ ), the addition of Cu or Cd mitigated the toxicity of PHCs to the AChE activity in the ragworms. The AChE activities combined to be treated by Cu-PHCs were  $14.71 \pm 0.29$  (0.1 mg/L of Cu) and  $14.11 \pm 0.35$  (0.2 mg/L of Cu) nmol/(min·mg protein), respectively (Fig.6a). It was approximately 7 times relative to AChE activity single treated by PHCs

after 10 d ( $1.94 \pm 0.34$  nmol/(min·mg protein)) ( $P < 0.01$ ). The AChE activities combined treated by Cd-PHCs were  $16.58 \pm 3.67$  (0.5 mg/L of Cd) and  $12.55 \pm 0.39$  (3 mg/L of Cd) nmol/(mg protein·min), respectively (Fig.6b). It was approximately 6 to 8 times relative to the AChE activity single treated by PHCs after 10 d ( $P < 0.01$ ). In contrast, when the concentration of PHCs was 0.1 ml/L (approximate 10-d  $LC_{50}$ ), the addition of Cu or Cd did not mitigate the neurotoxicity of PHCs to AChE activity in the ragworms. The AChE activities combined to be treated by Cu-PHCs on day 10 were  $17.28 \pm 0.93$  (0.1 mg/L of Cu) and  $16.88 \pm 3.09$  (0.2 mg/L of Cu) nmol/(min·mg protein), respectively. The AChE activities combined to be treated by Cd-PHCs on day 10 were  $16.21 \pm 2.56$  (0.5 mg/L of Cd) and  $17.45 \pm 4.93$  (3 mg/L of Cd) nmol/(min·mg protein), respectively. These results were not statistically significant as compared with the AChE activity single treated by PHCs after 10 d ( $19.20 \pm 1.83$  nmol/(min·mg protein)) ( $P > 0.05$ ).

### 3 Discussion

It has been accepted that aquatic ecosystems often contain mixtures of toxicants, including petroleum and metals. Since a chemical never occurs alone in the environment, it is more important to investigate the joint effects of more than one chemical on living organisms (Zhou *et al.*, 2004). The influences of toxicant mixtures on natural communities are poorly understood and little is known about their interaction (Zhou, 1995). The joint effects of

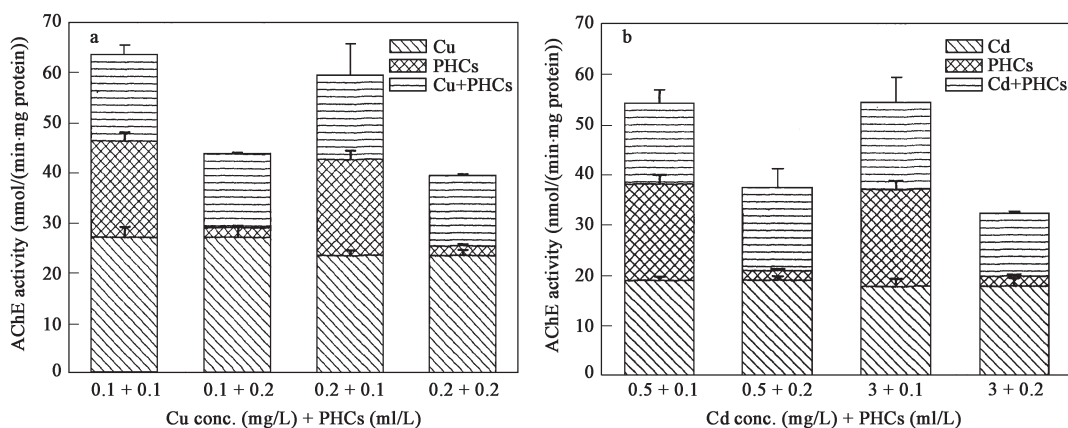


Fig. 6 Variation of AChE activity in the ragworm *Perinereis aibuhitensis* exposed to Cu-PHCs (a) and Cd-PHCs (b) for 10 d.

marine toxicants on the AChE activity in marine invertebrates was rarely investigated except for a few species (Forget *et al.*, 1999; Pérez *et al.*, 2004; Scaps *et al.*, 1997). According to Leiniö and Lehtonen (2005), there was an increasing evidence of the potential use of AChE activity in “stress screening”. The influences of heavy metals on the AChE activity in the ragworms exposed to petroleum hydrocarbons were not studied widely. The goals of this study were to explore the interactive effects of neurotoxic agent and heavy metals and to determine whether the combined toxicants can affect the AChE activity in the ragworm *P. aibuhitensis* so as to provide basal information for ecotoxicological evaluation of exposure to combined contaminations in estuarine areas.

The degenerative changes of the ragworms may meet their energy requirements to mitigate toxicity using a complete drain of utilizable levels of energy reserves and subsequent autolysis of their own tissues (Ramaswami and Subbram, 1992). Similarly, in the earthworms under the stress of toxicity, an autolysis from the posterior region was also observed (Ramaswami and Subbram, 1992; Rao and Kavitha, 2004). In highly polluted areas, several different species were able to cope with toxic chemicals and several different processes were involved in tolerance (Newman and Unger, 2003). In the polychaetes *Abarenicola pacifica* and *Neanthes virens* exposed in the field of oiled-enriched sediments, a depletion of glycogen had been observed (Augenfeld *et al.*, 1983; Carr and Neff, 1984). *N. diversicolor* from the Seine estuary, which were tolerant to raised zinc availabilities, exhibited a depletion of energy reserves. This energy depletion can correspond to the cost of tolerance to one or more of the toxicants' presence, or to the extra physiological cost of extra contaminant handling with or without enhanced tolerance to those contaminants (Durou *et al.*, 2005). Thus, it was hypothesized that the ability to resist a toxicant may be expensive in terms of energy, involving a decrease in the energy available for allocation to other processes (Holloway *et al.*, 1990).

Heavy metals can affect several different metabolism processes. Metal ions are most commonly bound to amino acids, which may be contained in proteins (including enzymes) or polypeptides. Several metals such as Cu and Zn play important roles in the functionality of some proteins as metalloenzymes, stress proteins, or redox activities (Camakaris *et al.*, 1999). Studies on marine mussels showed that Cu exposure strongly induces lipid peroxidation processes with marked decrease in the glutathione (GSH) level in several tissues (Regoli and Principato, 1999). The oxidative damage of membrane polyunsaturated lipids was characterized by free-radical mediated chain oxidations, which led to the production of hydroperoxides and carbonyl compounds (Regoli and Principato, 1999; Slater, 1984). On the other hand, induction of metabolism and excretion can eliminate the toxicity of heavy metals to some degree (Fernandez and Jones, 1989; Mouneyrac *et al.*, 2003). The polychaete *N. diversicolor* of different contaminated sites was shown to be tolerant to Zn, Cu, Cd, and Pb present in the estuary (Durou *et al.*, 2005; Mason and Jenkin, 1995; Zhou *et al.*, 2003). An efficient

regulation of the total Zn body burden by *N. diversicolor* was described (Amiard *et al.*, 1987; Bryan, 1976). Dennai *et al.* (1986) concluded that *Hediste (Nereis) diversicolor* was able to cope with the toxicant, and the mechanism of detoxification possibly involved the synthesis of non-metallothionein Cd-binding proteins, but not the formation of specific intracellular concretions. Thus, in the current case, AChE was more sensitive to the neurotoxicity of PHCs than that of Cu or Cd since it was not the target enzyme to heavy metals. It proposed that the pathological implications of Cu or Cd to the ragworm appeared only beyond a physiological level. In agreement with these assumptions, the activating effect of Cu or Cd addition on the AChE activity of the ragworms in the presence of PHCs was clearly detectable. The significant increase of activity and the catalytic efficiency of the enzymes suggested that the ragworm *P. aibuhitensis* possibly possessed the homeostatic mechanisms to modulate the intracellular traffic of Cu or Cd ions to lighten neurotoxicity induced by anticholinesterase agent. Metalloenzymes, stress proteins, and the detoxification enzymatic system were greatly possible to involve into this process. Some results presented that earthworms and the affiliative freshwater oligochaetes patently possessed the intrinsic capacity to synthesize one or more high affinity Cu-binding proteins and Cd-binding proteins (Klerks and Bartholomew, 1991; Mariño *et al.*, 1998), which may deliver  $\text{Cu}^{2+}$  or  $\text{Cd}^{2+}$  by specific carriers (chaperones), as previously observed for certain metalloenzymes as superoxide dismutase (Lippard, 1999; Rae *et al.*, 1999).

It can not be ignored that the concentration level of toxicants also influences the interactive effect of combined toxicants. Zhou *et al.* (2004) proposed that the concentration level played an essential role in the toxicological influences under the combined pollution of multi-contaminants and evidenced the supposition by several results in the study. According to the results of the present study, the median lethal concentration of PHCs significantly affected the AChE activity in *P. aibuhitensis*. The lower concentration level of PHCs (approximate 10-d  $\text{LC}_{50}$ ) and the different concentrations of Cu or Cd led to the synergic toxicity for the ragworm *P. aibuhitensis*, while the antagonistic effect presented the higher concentration level of PHCs (approximate 4-d  $\text{LC}_{50}$ ). Therefore, we set out to confirm that the addition of Cu or Cd can mitigate the neurotoxicity induced by PHCs in the ragworm *P. aibuhitensis*, presenting the antagonistic effect between PHCs and Cu or Cd in the given concentration level. In organisms, of course, interactions may occur not only between contaminants but also between contaminant and biological intrinsic component. Owing to this reason, various ecotoxicological effects can be observed even if in the same species.

#### Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20477049) and the Key Developing Planning Project of the National Fundamental Research Foundation of China (No. 2004CB418503).



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