Acute Toxicity of Cadmium and Nickel to Three Marine Copepods

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ABSTRACT

The present study aims to evaluate the toxicity of two heavy metals, cadmium (Cd) and nickel (Ni), to three marine copepods with different ecological habitats. Semi-static acute toxicity tests of Cd and Ni were conducted in our laboratory (Xiamen University) for 48 h with the harpacticoid copepod *Tigriopus japonicus*, cyclopoida copepod *Apocyclops borneoensis*, and calanoid copepod *Acartia pacifica*. Cadmium showed higher toxicity for the three copepods tested with 48-h LC50 values at 12.1, 1.6 and 0.1 mg L^{-1} Cd, respectively. While, that of nickel were 17.70, 13.05 and 2.36 mg L^{-1} Ni, respectively. Cadmium and Nickel were more toxic to the pelagic copepod *A. pacifica*, followed by *A. borneoensis* and *T. japonicus*. The results suggested that the pelagic calanoid copepod *A. pacifica* may be used as a benchmark species for assessing the toxic effects of heavy metals.

Key Words: Acute toxicity, Cadmium, Copepods, Heavy metals, Nickel

INTRODUCTION

Heavy metals are common environmental pollutants, because they are available through many diverse anthropogenic sources. They are considered very toxic to aquatic organisms because of their persistence, tendency to bioaccumulate (Fairbrother *et al*., 2007). Thus, environmental monitoring is essential to limit adverse effects of heavy metal pollution to aquatic organisms. Among major heavy metals, cadmium (Cd) and nickel (Ni), are known to have a toxic effects. Cd is a non-essential heavy metal and classified as human carcinogens (Waalkes, 2003). Previously, Cd is known to have adverse effects on marine organisms (Theede, 1980; Macken *et al*., 2009). Cadmium can reach high concentrations in coastal and estuarine waters (Chiffoleau *et al*., 2001). On the other hand, Ni is a dietary requirement to some animals, although it is toxic in higher concentrations (Denkhaus and Salnikow, 2002). Consequently, lack or excess of Ni can have adverse biological effects (Eisler, 1998). The toxicity of Ni to aquatic organisms has been the focus of relatively intense research in recent years. Previous studies showed that Ni is negatively affected marine organisms (Hunt *et al*., 2002; Hedfi *et al*., 2007). Nickel concentration in estuaries and streams generally ranges from 1 to 75 µg L^{-1} (Eisler, 1998) and could reach as high as 500 to 2000 µg L^{-1} in natural waters near industrial sites (Chau and Kulikovsky-Cordeiro, 1995).

The toxicity of heavy metals to marine invertebrates is related to a threshold concentration, which is different from one species to another (Rainbow, 2002). Once such threshold concentration is passed, the toxic effects will occur, initially sublethal but eventually lethal (Rainbow, 2002). Acute tests are often carried out before undertaking chronic or other tests when determining the toxicity of a substance or mixture and usually have a lethal endpoint such as mortality or survival. The LC50 (the concentration that causes 50% mortality) is the most frequency used endpoint (Mitchell *et al*., 2002). Marine copepods have recently been recognized as model organisms for ecotoxicology studies (Raisuddin *et al*., 2007). Several studies have been done on the lethal effects of heavy metals to marine copepods. Verriopoulos and Dimas (1988) study the acute toxicity of Cd and Ni on the copepod *Tisbe holothuriae*. Also previous studies showed that Cd and Ni have a lethal effect on copepod *Tigriopus brevicornis* (Forget *et al*., 1998; Barka *et al*., 2001).

The present study primarily aimed to determine the 48h- LC50 values of Cd or Ni to three different marine copepods *Tigriopus japonicus*, *Apocyclops borneoensis*, and *Acartia pacifica*. All three copepods were common species occurring in coastal waters around Xiamen city and adjunct Jiulong estuary, where, recent studies reported high heavy metal concentrations (Jian-qing *et al*., 2007; Weili *et al*., 2009). Moreover, these three copepods occupy different habitats, therefore, may display different sensitivity to the heavy metal pollution. The intertidal harpacticoid copepod *T. japonicus* is a benthic copepod; the cyclopoida copepod *A. borneoensis* adapt to brackish waters and often lives in the lower part of the water column; while the calanoid copepod *A. pacifica* is abundant and mainly distributing in the upper part of the water column.

MATERIALS AND METHODS

Copepod Collection and Maintenance

All species of the genus *Tigriopus* are dominant member of shallow supratidal rock pools (Raisuddin *et al*., 2007). In most cases the species belong to the genus *Apocyclops* are dominant in coastal brackish waters (Suantika, 2006). Copepods were collected from Xiamen bay, People's Republic of China (fig. 1). Since collection the copepods *T. japonicus* and *A. borneoensis* have been maintained in cultures in our laboratory. *T. japonicus* was maintained at 18 to 22° C and 24 to 26 ppt salinity. *A. borneoensis* was maintained at 28 to 31° C and 18 to 22 ppt salinity. Species of the genus *Acartia* are predominant and widespread in estuarine and coastal waters worldwide (Moon *et al*., 2008). *A. pacifica* was collected using 64 µm mesh size plankton net and was maintained at 24 to 26° C and 24 to 26 ppt salinity in the lab for at least 48 hours before the experiments began.

The copepod cultures were maintained under static-renewal conditions in 0.45 µm Millipore filtered seawater with 7 to 7.9 mg L^{-1} dissolved oxygen and a pH ranging from 7.90 to 8.25, under 12D: 12L photoperiod cycle. Copepods were fed a mixed algal diet of *Isochrysis galbana* and *Platymonas subcordiformis*. The algae were cultured in filtered seawater contain f/2 enriched media at 20° C.

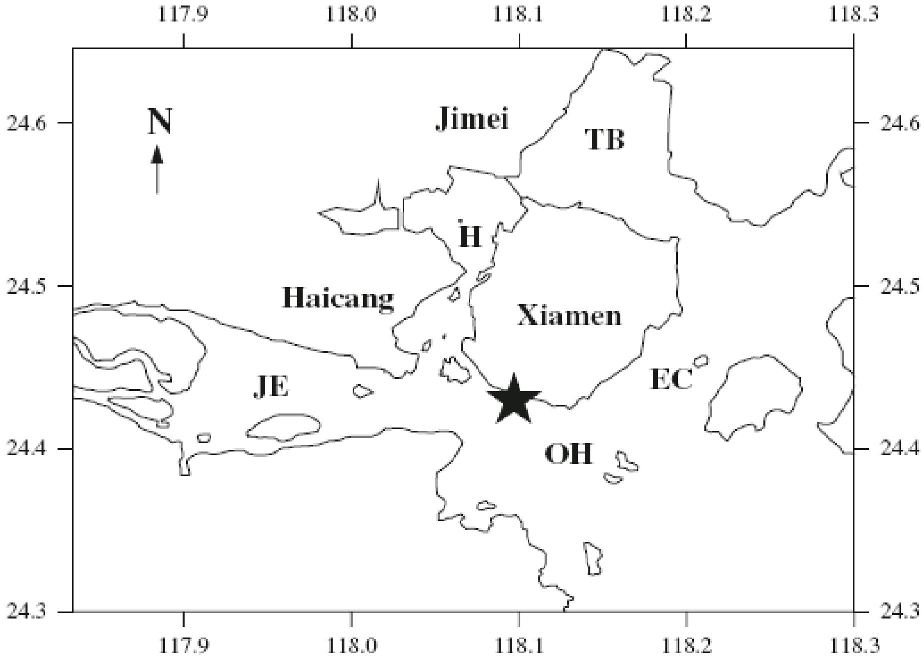

Fig. 1. Location of copepod sampling site as represented by the pentagram, Xiamen coastal waters, Fujian Province, People's Republic of China. H Harbor, OH Outer Harbor, JE Jiulong River Estuary, TB Tong'an Bay, and EC East Channel (from Wang and Wang 2010).

Test Solutions

Both heavy metals used in this study were provided in the form of chloride salts; i.e. $CdCl_2 \cdot 2.5H_2O$ (Sinopharm Chemical Reagent Co., Ltd, China; purity ≥ 99.0%) and $NiCl_2 .6H_2O$ (Guang Fu Chemical Institute, China; purity ≥ 98.0% pure). Stock solutions were prepared in double distilled water. The stock solutions were subsequently diluted in different volumes of 0.45 µm filtered seawater to create various Cd and Ni test concentrations.

Acute Toxicity Experiments

Semi-static 48-h acute toxicity tests were conducted using standard methods (Verriopoulos and Dimas, 1988; ASTM, 2004) with some modifications. The experimental conditions were broadly the same as copepod cultures, but temperature and salinity were different. The experimental temperature for *T. japonicus*, *A. borneoensis*, and *A. pacifica* was 20, 30, and 25 ° C, respectively, while the salinity was 25, 20, and 25 ppt salinity, respectively. Only active adult female copepods were used for the acute toxicity experiments. Copepods were exposed to different Cd or Ni concentrations for 48 h in a semi-static exposure system, with test solutions being replaced after 24 h. Five to six Cd or Ni concentrations and a control were used. Cd concentrations used for *T. japonicus*, *A. borneoensis*, and *A. pacifica* are range from 0 to 64 , 0 to 8, and 0 to 0.8 mg L^{-1}, respectively. Ni concentrations used for for *T. japonicus*, *A. borneoensis* experiments are range from 0 to 64 mg L^{-1}. However, Ni concentrations used for *A. pacifica* are range from 0 to 16 mg L^{-1}. Each treatment of test concentration was run in triplicate, with 10 active individuals in each replicate. Acute toxicity experiments on *T. japonicus* and *A. borneoensis* were conducted in six well culture plates (Corning Incorporated, USA), each well with 10 mL of test solution and five female copepods. For each treatment there are two well. While, *A. pacifica* acute toxicity experiments were carried out in 100 mL glass beakers containing 50 mL of solution and five female copepods. For each treatment there are two beakers. All glass materials used in the experiments had been acid washed and rinsed thoroughly with distilled water before their use. Animals were monitored every 6 hours and dead animals removed at 24 and 48 h. The criteria for mortality were lack of response after repeat touches with a probe during two minutes. Copepods were not fed during the short test period. The range of heavy metal concentrations used in acute toxicity tests was established by preliminary experiments.

Statistical Analysis

48 h LC50 values (concentration of a toxicant which kills 50% of the test animals after 48 h of exposure) of both Cd and Ni were calculated by means of a probit analysis (Finney, 1971) for all three copepod species. Survival curves of copepods after 48 h exposure to the two heavy metals were plotted from the relation between percent survival and concentrations of the heavy metals. Microsoft excel 2003 package software was used to analyze the data.

RESULTS

The three copepod species showed a same sensitivity trend toward Cd and Ni. For all three species, Cd was more toxic than Ni. Among three copepods tested, *T. japonicus* was least sensitive to both Cd and Ni exposure while the most sensitive species was *A. pacifica*, the sensitivity of *A. borneoensis* was between the two (Table 1 and 2, Fig. 2 and 3).

Acute Toxicity of Cadmium

48-h LC50 values of Cd to the copepods are summarized in Table 1. For all three copepods used for test, there was no mortality observed for the control. As expected, the survival rate of all three copepods decreased with the increase of Cd concentration (Fig. 2). Among three copepods tested, it was shown that *A. pacifica* was most sensitive to Cd exposure with a lowest LC50 value at 0.1 mg L^{-1}, which is followed by *A. borneoensis*

(1.6 mg L^{-1}). In contrast, *T. japonicus* was least sensitive to Cd exposure and has a highest LC50 value at 12.1 mg L^{-1}.

Acute Toxicity of Nickel

48-h LC50 values of Ni are summarized in Table 1. For all three copepods used for test, there was no mortality observed for the control. As expected, the survival rate of all three copepods decreased with the increase of Ni concentration (Fig. 3). Among three copepods tested, it was shown that *A. pacifica* was most sensitive to Ni exposure with a lowest LC50 value at 2.4 mg L^{-1}, which is followed by *A. borneoensis* (13.1 mg L^{-1}). In contrast, *T. japonicus* was least sensitive to Ni exposure and has a highest LC50 value at 17.7 mg L^{-1}.

Table 1: Summary of the 48-h LC50 values (mg L^{-1}) of the selected copepods (adult females) exposed to cadmium or nickel

Species	LC50 of Cd	LC50 of Ni
T. japonicas	12.1 (15.2 ~ 9.6)	17.7 (22.8 ~ 13.7)
A. borneoensis	1.6 (1.8 ~ 1.4)	13.1 (16.0 ~ 10.7)
A. pacifica	0.1 (0.11 ~ 0.09)	2.4 (2.7 ~ 2.1)

Note: The associated 95% confidence intervals were described in parentheses

Table 2: Comparison of cadmium and nickel LC50 values for the tested copepods with some other marine copepods

Metal	Species	LC50	Time interval	Reference
Cd	Tested copepods			
	T. japonicus	12.1 mg L^{-1}	48-h	Present study
	A. borneoensis	1.6 mg L^{-1}	48-h	Present study
	A. pacifica	0.1 mg L^{-1}	48-h	Present study
	T. japonicus	25.2 mg L^{-1}	96-h	(Lee *et al*., 2007)
	Other marine copepods			
	Tisbe battaglia	0.34 mg L^{-1}	96-h	(Hutchinson *et al*., 1994)
	Tisbe holothuriae	0.9 mg L^{-1}	48-h	(Verriopoulos and Dimas, 1988)
	T. brevicornis	47.9 µg L^{-1}	96-h	(Forget *et al*., 1998)
	Acartia tonsa	29.0 µg L^{-1}	96-h	(Toudal and Riisgard, 1987)
Ni	Tested copepods			
	T. japonicas	17.7 mg L^{-1}	48-h	Present study
	A. borneoensis	13.1 mg L^{-1}	48-h	Present study
	A. pacifica	2.4 mg L^{-1}	48-h	Present study
	Other marine copepods			
	Tisbe holothuriae	2.6 mg L^{-1}	48-h	(Verriopoulos and Dimas, 1988)
	T. brevicornis	206.9 µg L^{-1}	96-h	(Barka *et al*., 2001)

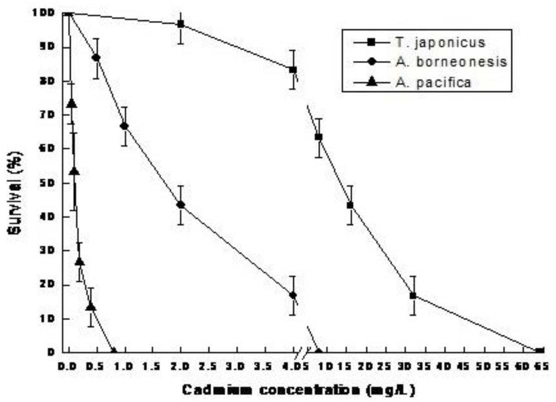

Fig. 2.The survival of copepods after 48 h exposure to cadmium

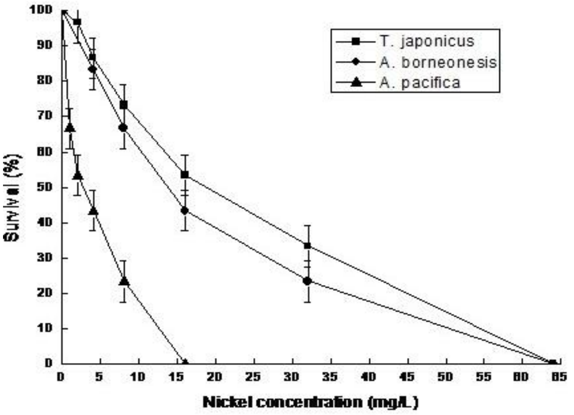

Fig. 3. The survival of copepods after 48 h exposure to nickel

DISCUSSION

Coastal and marine ecosystems worldwide are receiving a significant amount of pollutants from agricultural, industrial and municipal discharges due to rapid population growth and development of coastal areas (Islam and Tanaka,2004). Both acute and chronic toxicity tests play important role in ecological risk assessment of pollutants. Acute toxicity tests provide quick and relatively inexpensive estimates of the toxic effects of chemical pollutants and are useful for providing a measurement of the relative toxicity of different pollutants to a species, or assessing relative sensitivity of different species or different life stages of a same species to a particular pollutant. Furthermore, in the absence of chronic toxicity data on a particular species, acute toxicity data, with the use of appropriate application factors (AFs), can be used to predict "safe" environmental concentrations for the species, which may be used in establishing environmental standards and regulations (Hunt *et al*., 2002). Additionally, acute toxicity tests are often carried out before undertaking chronic or other toxicity tests, to determine the concentrations to be used for chronic experiments for a pollutant or their mixture. The LC50 is the most frequency used endpoint in acute toxicity tests (Mitchell *et al*., 2002). In the present study, acute toxicity tests of two heavy metals Cd or Ni were conducted with three marine copepods.

Comparison of Cd and Ni toxicity data for the three copepods tested in the present experiments with data from other marine animals showed that copepods are highly sensitive to heavy metals exposure. For instance, Eisler and Hennekey (1977) examined the acute toxicity of Cd, Cr, Hg, Ni, and Zn for several estuarine macrofauna. Their results showed higher values of metals as 96-h LC50, suggesting that copepods are good indicator species for heavy metals. Similarly, Wo *et al*. (1999) found significantly higher 96-h LC50 value of 16.22 mg L^{-1} Cd for the marine gastropod *Nassarius festivus* and, Petrich and Reish (1979) reported much higher 96-h LC50 values of 17, 49 and >50 mg L^{-1} Ni for three species of marine polychaetes.

Our results indicated that among the three copepods tested, *A. pacifica* was the most sensitive to Cd, followed by *A. borneoensis* and *T. japonicus*. Previous studies also showed that Cd is highly toxic to marine copepods (Table 2). For instance, Verriopoulos and Dimas (1988) found a 48-h LC50 value of 0.9 mg L^{-1} for the copepod *Tisbe holothuriae*. Thus, *T. holothuriae* appears to be more sensitive to Cd than *T. japonicus* (12.1 mg L^{-1}) and *A. borneoensis* (1. 6 mg L^{-1}), but not as sensitive as *A. pacifica* (0.1 mg L^{-1}). Previously, a 96-h LC50 value of 29.0 µg L^{-1}, 47.9 µg L^{-1} and 340 µg L^{-1} Cd is reported for marine copepods *Acartia tonsa* (Toudal and Riisgard, 1987), *Tigriopus brevicornis*

(Forget *et al*., 1998) and *Tisbe battaglia* (Hutchinson *et al*., 1994), respectively. However, direct comparison can not be made because of difference in LC50 time interval. Interestingly, while our 48-h LC50 value for *T. japonicus* is 12.1 mg L^{-1}, Lee *et al*. (2007) reported a 96-h LC50 value of 25.2 mg L^{-1} for the same species. The difference may be due to different experimental conditions, especially salinity. Our experimental salinity was 25 ppt while salinity of 32 ppt was used by Lee *et al*. (2007). It has been known that a significant decrease in LC50 values as salinity decreased which was most likely related to increase in free cadmium ion (Hall *et al*., 1995).

Previous reports on LC50 values for Ni on marine copepods are relatively scarce (Table 2). Barka *et al*. (2001) reported a 96-h LC50 value of 206.9 µg L^{-1} Ni for the copepod *Tigriopus brevicornis*, while Verriopoulos and Dimas (1988) found a 48-h LC50 value of 2.6 mg L^{-1} Ni for the marine copepod *Tisbe holothuriae*. In comparison, *A. pacifica* (2.4 mg L^{-1}) appears to be more sensitive to Ni than *T. holothuriae* (2.6 mg L^{-1}), *T. japonicus* (17.7 mg L^{-1}) and *A. borneoensis* (13.1 mg L^{-1}).

CONCLUSION

A comparison between the lethal response of tested copepods to Cd or Ni, revealed that among the three copepods, the pelagic copepod *A. pacifica* was the most sensitive to both Cd and Ni exposure. Thus, we suggest that the pelagic copepod *A. pacifica* as a useful indicator species for assessing the effect of the two toxic heavy metals. The benthic copepod *T. japonicus* was the least sensitive to both heavy metals. Raisuddin *et al*. (2007) also stated that *Tigriopus spp*. are relatively less sensitive to most common pollutants in acute toxicity test. Overall, the three copepod species showed the same trend, being more sensitive to Cd than Ni with, the 48-h LC50 value of Ni for *A. pacifica*, *A. borneoensis*, and *T. japonicus* about 24, 8, and 1.5 times higher than that of Cd, respectively. These results may be expected since Ni is an essential metal, involving in biological processes whereas, no biological function is known for Cd. Further studies on chronic effects of heavy metals on marine copepods in coastal waters will be evaluated.

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السمية الحادة لعنصري الكادميوم والنيكل لثلاث من مجدافية الأرجل البحرية

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الملخص

سجلت نتائج الدراسات الحديثة تراكيز عالية للمعادن الثقيلة في المياه البحرية الساحلية. تهدف الدراسة الحالية لتقدير السمية الحادة لعنصري الكادميوم والنيكل لثلاثة أنواع مختلفة من مجدافية الأرجل البحرية تشغل مواطن بيئية مختلفة.

أجريت تجارب السمية الحادة في المعمل (جامعة شيامن) لفترة (48) ساعة في نظام تربية مائي شبه ثابت بحيث يتم تغيير نصف الماء على مجدافية الأرجل البحرية التالية: *Tigriopus japonicas*, *Apocyclops borneoensis*, and *Acartia pacifica*.

أظهرت النتائج أن الكادميوم ذو سمية عالية بالنسبة لأنواع مجدافية الأرجل الثلاثة حيث كانت قيم التركيز المميت 48-h LC50 كالآتي: 1.6, 0.1, 12.1 ملجم/ لتر بالترتيب ، في مقابل 17.70 , 13.05 , 2.36 ملجم/ لتر لعنصر النيكل بالترتيب.

أشارت نتائج الدراسة أيضًا إلى أن مجدافية الأرجل *A. pacifica* هي الأكثر حساسية لعنصري الكادميوم والنيكل، تليها *A. borneoensis* ثم *T. japonicas*.

أوصت الدراسة بأنه يمكن استخدام *A. pacifica* كدليل ومؤشر لتقدير سمية العناصر الثقيلة.

الكلمات المفتاحية: سمية حادة ، عناصر ثقيلة، كادميوم، مجدافية الأرجل، نيكل.