ELSEVIER

Environmental Pollution, Vol. 91, No. 1, pp. 35–39, 1996

0269-7491/96 $09.50 + 0.00

0269-7491(95)00031-3

RELATIONSHIP BETWEEN COPPER SPECIATION IN SEDIMENTS AND BIOACCUMULATION BY MARINE BIVALVES OF TAIWAN

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(Received 25 October 1994; accepted 7 April 1995)

Abstract

*This paper evaluates the relationships between copper species in sediments and accumulation by the purple clam (*Hiatula diphos*) and venus clam (*Gomphina aeguilatera*) collected from the field and culture (aquaculture) ponds in the polluted coastal area of Lukang, Taiwan. Sediment was sampled along with the molluscs, including oysters (*Crassostrea gigas*), purple clams (*Hiatula diphos*), rock-shells (*Thais clavigera*), venus clams (*Gomphina aeguilatera*), and hard clams (*Meretrix lusoria*), from two unique environments of Lukang during the period from August 1993 to July 1994.*

The data indicate that the total copper concentrations in sediments from culture ponds (185 μg g^{-1}) was higher than those of the field (44·0 μg g^{-1}). Copper species in sediments were analyzed by a sequential leaching technique. Results show that concentrations of various copper species in the sediments are in the range of 1·14 ± 0·59 to 13·2 ± 22·4 μg g^{-1} and 0·36 ± 0·24 to 133 ± 36·7 μg g^{-1} for the two environments, respectively. Also the exchangeable copper in sediment from culture ponds was 15 times higher than that from the field. In addition, the sum of exchangeable and copper carbonates had the highest percentages of copper in both the pond sediment (86·6 %) and the field sediment (50·7 %).

Maximum copper concentrations (309 ± 35·1 μg g^{-1}) in oysters were much higher than those in the other benthic organisms by about 4–127 times. Similarly, the data also showed that copper concentrations in Thais clavigera *were 12–32 times higher than those in other benthic organisms. Copper concentrations in various benthic organisms differed significantly (*$p < 0{\cdot}05$*) from that in* Thais clavigera. *This capacity makes* Thais clavigera *a potential candidate for monitoring copper in marine sediments. In terms of copper species, the best correlation was generally obtained between copper carbonates in sediments and copper concentrations in* Hiatula diphos *(*r = 0·886*). A strong multiple regression correlation (*$p < 0{\cdot}05$, $r^2 = 0{\cdot}7894$*) also indicates that the copper carbonates may dominate as the available form of copper to* Hiatula diphos *from various environments in the Lukang coastal area under natural physicochemical conditions.*

Key words: Copper speciation, sediments, accumulation, bivalves.

INTRODUCTION

Taiwan is a modern industrialized island country with a population of about 21 million and a strong economy. Societal demand for protein increases when the standard of living rises. Oysters (*Crassostrea gigas*) and other molluscs (e.g. purple clams, *Hiatula diphos*) are the most popular seafood in Taiwan. However, due to dense population and rapid industrial expansion, the estuarine and coastal environments of Taiwan are suffering ever-increasing impact from all kinds of human activities (Hung, 1988; Han & Hung, 1990; Hung & Han, 1992; Han *et al.*, 1994*a*; Jeng & Han, 1994).

We monitored for heavy metals in the coastal environment of Taiwan and found that heavy metal pollution is the most serious and one of the most studied marine environmental problems in Taiwan. Moreover, most current health risks associated with seafood safety originate in the environment; for example, some alarm has been expressed concerning possible health implications of copper residues in oysters (Han *et al.*, 1994*a*). Because of the incident of 'green oyster' in the Charting coastal area (Han & Hung 1989, 1990), the green oysters, collected from the Erhjin Chi estuary in January 1989, gave copper concentration of 4401 ± 79 μg g^{-1} (Han & Hung, 1989, 1990; Han *et al.*, 1994*a*). The area around the Erhjin Chi estuary is especially affected by large discharges of heavy metals from acid cleaning of metal scrap on the riverbank. Besides, the purple clam (*Hiatula diphos*) incident was attributed to an aquaculture source resulting from polluted groundwater which caused abundant localized blooms of dinoflagellates (*Alexandrium tamarense*) when conditions were optimal (so-called 'red tides'). Dinoflagellates containing paraplytic shellfish poisoning (PSP) were eaten by purple clams which accumulated the toxins becoming themselves toxic to human consumers. Consumption of contaminated purple clams caused the death of one person and illness of six others during 1986 (Chang & Hong, 1986).

Metal speciation has become an important area of inquiry because of its importance in the understanding of the fate and effects of metals in the environment. As these different trace metal forms will generally exhibit different physical and chemical properties, measurement of the total metal concentration provides little indication of a metal's potential interaction with the biotic and abiotic components present in the aquatic environment (Tessier & Campbell, 1991). Relationship between speciation, bioavailability, and toxicity of metals to aquatic organisms has been clearly demonstrated under experimental conditions (Zamuda & Sunda, 1982; O'Donnel *et al.*, 1985; Hunt, 1987). Han *et al.* (1993) described the accumulation and depuration processes of metals in green oysters and blue mussels from Taiwanese waters. Furthermore, a multiple regression equation was developed on the basis of the results of field experiments relating copper accumulation by green oysters and various copper species in natural seawater. There has been some investigation exploring the role of acid volatile sulfide (AVS) in determining bioavailability and toxicity to benthic organisms (Carlson *et al.*, 1991; Ankley *et al.*, 1991,1993).

In effect, a major fraction of the trace metals introduced into the aquatic environment is found to be associated with the bottom sediments, where they constitute a potential danger for both autotrophic and heterotrophic benthic organisms. (Tessier & Campbell, 1991). Marine molluscs have a large capacity for the accumulation of metals such as Cu, Zn and Cd, and have therefore commonly been suggested as biomonitors of metal contamination (Phillips, 1990). In this context, Tessier and Campbell (1991) reported that there is much circumstantial evidence that speciation in sediments had an important influence on metal uptake, but there was a pressing need for further research. Furthermore, there is no information on the relationships between metal speciation in sediments and bioaccumulation by the benthic organisms of Taiwan. Especially, it is interesting to understand the bioaccumulation of various copper species by different benthic organisms under the same environmental conditions.

Therefore, this study presents the distribution of copper species in sediment collected from the Lukang culture (aquaculture) area of central Taiwan. The paper also evaluates the relationships between copper species in sediments and accumulation by purple clams (*Hiatula diphos*) and venus clams (*Gomphina aeguilatera*) collected from field and culture ponds of coastal Lukang. Finally, the purpose of this work is to better understand different copper species as indicators of metal bioavailability in the sediments of Taiwan.

MATERIALS AND METHODS

Five species of marine invertebrates including oysters (*Crassostrea gigas*), purple clams (*Hiatula diphos*), rock-shells (*Thais clavigera*), venus clams (*Gomphina aeguilatera*) and hard clams (*Meretrix lusoria*) were sampled from the same culture areas of Lukang during the period from August 1993 to July 1994. Oysters are a filter feeder; purple clams, venus clams, and hard clams are infaunal deposit feeders; and rock-shells are a carnivorous feeder.

Sediment was collected along with the molluscs and sieved (< 63 μm mesh) to obtain the mud fraction. Accurately, 0·200 g of the dried and homogenized sediment was placed in a 50 ml capped polypropylene centrifuge tube and was sequentially treated with different reagents as shown in Fig. 1 (Pai *et al.*, 1993; Han *et al.*, 1994*b*).

Field and pond-harvested molluscs were returned to the laboratory, individually scrubbed, shucked, and flesh placed in tared acid-washed Teflon beakers for weighing to obtain individual wet values. All biological samples were digested with a mixture of nitric and sulfuric acids (1/1, v/v), and the supernatant analyzed for copper by atomic absorption spectrophotometry (Hung *et al.*, 1982; Han & Hung, 1990).

Determination of metals was performed with a Hitachi Zeeman FAAS (model Z-8000 with an autosampler). NBS oyster tissue standard (NBS 1566, USA) was also used as a reference. The results generated were, in most cases, in good agreement with certified values. The influence of copper concentrations upon copper accumulation from various biological samples were statistically examined by *t*-test, analysis of variance (ANOVA), Shette's test, and multiple regression analysis (SAS, 1988).

RESULTS AND DISCUSSION

Copper concentrations in sediments and molluscs

Concentrations of copper in sediments are so much higher than those in the overlying water that even a minute fraction may represent an important source for uptake, especially in benthic filter-feeding and burrowing organisms (Bryan *et al.*, 1985; Han *et al.*, 1994*a*).

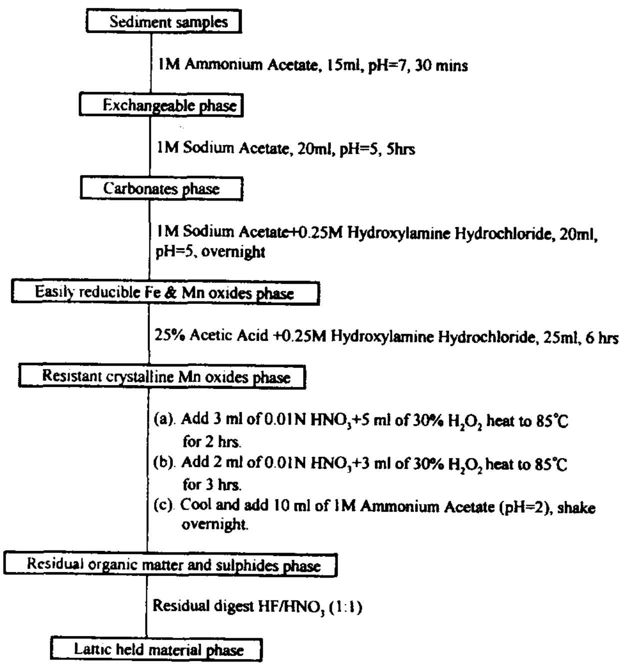

Fig. 1. Flow chart showing the sediment sequential extraction scheme (after Pai *et al.*, 1993; Han *et al.*, 1994*b*).

Table 1. Concentration of copper species ($\mu g\ g^{-1}$) in sediments collected from two different environments. Mean values are presented with standard deviations and high and low values in parentheses

Copper species	Phase	Number of samples	Culture environments	
			Field	Pond
Exchangeable phase	I	6	9·04 ± 14·1 (0·20–35·8)	133 ± 36·7 (0·20–604)
Carbonates phase	II	6	13·2 ± 22·4 (1·05–63·2)	27·2 ± 54·2 (0·20–137)
Easily reducible Fe and Mn oxides phase	III	6	1·14 ± 0·59 (0·20–2·08)	0·36 ± 0·24 (0·20–0·78)
Resistant crystalline Mn oxide phase	IV	6	9·03 ± 2·07 (7·15–13·1)	9·58 ± 2·85 (3·90–11·7)
Organic matter and sulphides phase	V	6	6·92 ± 2·19 (4·26–9·89)	10·8 ± 6·10 (4·72–17·0)
Lattice held material phase	VI	6	4·61 ± 1·47 (2·00–7·00)	4·00 ± 1·41 (2·00–5·50)
Total			44·0	185

Table 1 shows the average concentrations of copper species in sediments collected from two different environments in Lukang. The above data indicate that total copper concentrations in sediments from culture ponds (185 $\mu g\ g^{-1}$) were higher than those from the field (44·0 $\mu g\ g^{-1}$). From visual observation, culture pond sediments are muddy, and field sediments are sandy. It is possible that the higher copper concentrations reflect finer grain size in sediments. In general, with sediments the finer the particle size, the greater the concentration of metals observed (Hung *et al.*, 1993).

Results of the sediment sequential extractions show that the concentrations of various copper species are in the range of 1·14 ± 0·59 to 13·2 ± 22·4 $\mu g\ g^{-1}$ and 0·36 ± 0·24 to 133 ± 36·7 $\mu g\ g^{-1}$ for the two culture environments, respectively (Table 1). The highest copper concentration of 133 ± 36·7 $\mu g\ g^{-1}$ found in the exchangeable phase was obtained from the culture pond sediment. This result was 15 times higher than exchangeable copper from the field. In addition, the sum of phases I and II had higher percentages of copper both in culture pond (86·6%) and field sediments (50·5%) (Fig. 2). This suggests that the Lukang coastal environment is influenced by the local anthropogenic input of chemicals, especially copper. In general, the binding capacity of a sediment determines its trace metal capacity. Sediments with high percentages of smaller grain size fractions (e.g. clay or silt) and with high percentages of iron and manganese can scavenge trace metals very effectively (Timmermans, 1993).

Indicator organisms should be good accumulators of metals and their tissue concentrations must reflect metal availability. For this reason, organisms having an ability to regulate metals are clearly unsuitable (Bryan *et al.*, 1985). Figure 3 shows the copper concentrations in various marine molluscs (soft) collected for this study. Maximum copper concentrations (309 ± 35·1 $\mu g\ g^{-1}$, dry wt) in oysters (*Crassostrea gigas*) were much higher than those of other benthic organisms by about 4 to 127 times (Fig. 3). At the same time, it can be seen that

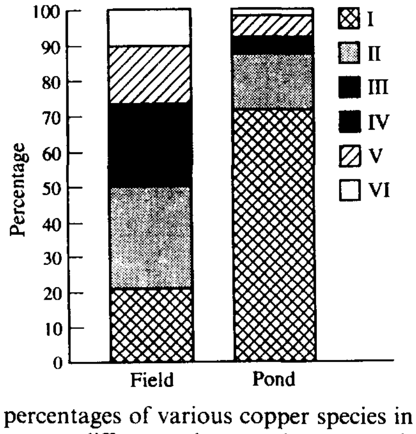

Fig. 2. The percentages of various copper species in sediments collected from two different culture environments (I) exchangeable phase; (II) carbonates phase; (III) easily reducible Fe and Mn oxides phase; (IV) resistant crystalline Mn oxides phase; (V) organic matter and sulphides phase; (VI) lattice held material phase.

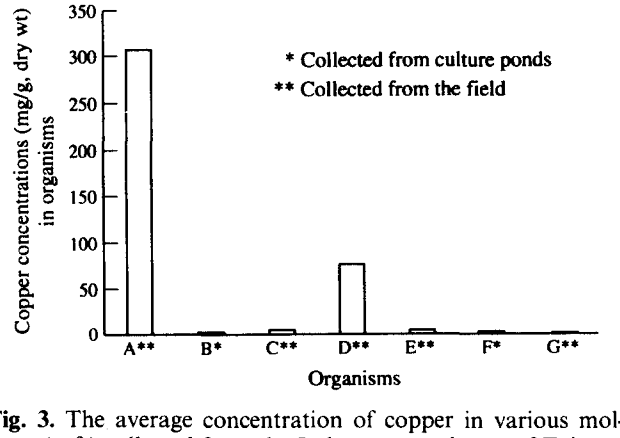

Fig. 3. The average concentration of copper in various molluscs (soft) collected from the Lukang coastal area of Taiwan. (A) *Crassostrea gigas*; (B) *Hiatula diphos*; (C) *Hiatula diphos*; (D) *Thais clavigera*; (E) *Gomphina aeguilatera*; (F) *Meretrix lusoria*; (G) *Meretrix lusoria*.

the range of copper concentrations in the oysters did differ significantly ($p < 0{\cdot}05$) from other organisms. This suggests the ability of oysters to concentrate copper is much larger than that of other species. In other words, the oysters can accumulate copper from surrounding water 4–127 times more efficiently than other species. However, copper concentrations in oysters were significantly correlated (at the level of $p < 0{\cdot}05$) with the concentration of particulate copper; this suggests that the food pathway from surrounding water may dominate the accumulation of copper by oysters (Han & Hung, 1990).

Figure 3 also shows copper concentrations in various benthic organisms collected from culture ponds and the field. The results reveal that relatively high copper concentrations ($77{\cdot}7 \pm 15{\cdot}2$ $\mu g\ g^{-1}$, dry wt) in *Thais clavigera* were found as compared with those in other benthic organisms (range from $2{\cdot}43 \pm 0{\cdot}21$ to $6{\cdot}30 \pm 1{\cdot}62$ $\mu g\ g^{-1}$, dry wt). Copper concentrations in *Thais clavigera* were 12–32 times higher than those in other benthic organisms which was significant ($p < 0{\cdot}05$).

The No Observed Effect Concentration for accumulation (NOEC accumulation) can be defined as the highest copper concentration in the sediment which did not result in a significant ($p < 0{\cdot}05$) increase of the copper concentration in the organisms (Kraak *et al.*, 1993); therefore, suggesting that *Hiatula diphos*, *Gomphina aeguilatera* and *Meretrix lusoria* were able to regulate (i.e. low efficiency in accumulation) their internal copper concentration. Conversely, *Thais clavigera* has a high capacity for accumulating copper at the same location (i.e. under the same physico–chemical conditions). This capacity makes *Thais clavigera* a potential candidate for monitoring copper in marine sediments.

On the other hand, relatively high copper concentrations were obtained for *Hiatula diphos* and *Meretrix lusoria* ($4{\cdot}98 \pm 1{\cdot}20$ and $3{\cdot}72 \pm 0{\cdot}42$ $\mu g\ g^{-1}$, dry wt, respectively) sampled from culture ponds as compared with those from the field ($3{\cdot}21 \pm 0{\cdot}45$ and $2{\cdot}43 \pm 0{\cdot}21$ $\mu g\ g^{-1}$, dry wt, respectively). The total copper concentrations in sediments from two different environments differed greatly, that is 44·0 and 185 $\mu g\ g^{-1}$, respectively (Table 1). However, copper concentrations in *Hiatula diphos* and *Meretrix lusoria* collected from culture ponds did not differ significantly ($p < 0{\cdot}05$) from those collected from the field. This also confirmed that *Hiatula diphos* and *Meretrix lusoria* were able to regulate their internal concentrations. In other words, copper concentrations in *Hiatula diphos* and *Meretrix lusoria* are not affected by copper concentrations in sediment.

Relationship between copper species and bioaccumulation

Bioavailable metals are defined according to Campbell *et al.* (1988) as biological available chemical states that can be taken up by an organism and can react with its metabolic machinery. High trace metal concentrations in one type of sediment can be quite harmless for organisms, whereas low concentrations in another type can be very available and result in a considerable accumulation (Timmermans, 1993). Currently, a simple relationship between external trace metal concentrations in sediment and internal concentrations in organisms is absent in Taiwan. Correlations of accumulation of various copper species have been calculated for *Hiatula diphos* and *Gomphina aeguilatera* (Table 2). The best correlations were generally obtained between copper carbonates and copper concentrations in *Hiatula diphos* ($r = 0{\cdot}886$). Additionally, significant regression correlations have also been found for resistant crystalline Mn oxides (phase IV) and copper concentration in *Hiatula diphos* ($r = 0{\cdot}798$) and for total copper in sediments and copper concentration in *Hiatula diphos* ($r = 0{\cdot}804$). The present result might suggest that copper carbonates gave better information about copper bioavailability than did either the total concentration in the sediment or any one of a range of other extractants (Table 2). Although *Hiatula diphos* is able to regulate copper (low efficiency in accumulation), this result indicates that at low copper body burden, there still exists some degree of correlation between copper species in sediment and copper concentrations in *Hiatula diphos*.

In a laboratory study of copper uptake from natural copper-spiked sediments by *Tubificid worms*, Diks and Allen (1983) also found a good correlation between accumulation and a dilute acid extractable fraction (which is similar to our carbonates phase). Our finding supports the conclusion of Tessier *et al.* (1984) that iron oxides and organic matter have a protective effect on the accumulation of copper by *Hiatula diphos* and indicate a similar role for carbonate.

The multiple regression correlation coefficient ($p < 0{\cdot}05$, $r^2 = 0{\cdot}7894$) was also obtained between the copper concentration of *Hiatula diphos* studied and the concentrations of various copper species in the sediments. From these results, the copper carbonates may dominate as a copper source to *Hiatula diphos* from several locations along the Lukang coastal area under natural physico-chemical conditions. Furthermore, a multiple regression equation was developed based on the results of field experiments relating copper accumulation by *Hiatula diphos* and the various copper species in natural sediments. This equation can be represented as follows:

$$Y = 0{\cdot}991 + 0{\cdot}0059X_1{}^* + 0{\cdot}0150X_2 \quad (r^2 = 0{\cdot}7984)$$

where Y is the copper concentration in *Hiatula diphos*;

Table 2. Relationships between copper accumulation by *Hiatula diphos* and various copper species

Copper species	Regression coefficient
Exchangeable phase	–0·490
Carbonates phase	0·886[a]
Easily reducible Fe and Mn oxides phase	0·175
Resistant crystalline Mn oxides phase	0·798[a]
Organic matter and sulphides phase	0·748
Lattice held material phase	0·117
Total copper	0·804[a]

[a]Significant at $p < 0{\cdot}05$.

X_1 is the copper carbonates concentrations in the sediments; X_2 is the lattice held material copper concentrations in the sediments; and * means significant at $p < 0.05$.

This demonstrates that the chemical characteristics of the sediment affect the extent of copper accumulation. Furthermore, it was the copper carbonates (phase II) and lattice held material (phase VI) which overwhelmingly gave the best prediction of accumulation in our studies. So, if the concentration of various copper species has been measured in natural sediment, these copper species can be used to estimate the copper concentration in *Hiatula diphos*. Unfortunately, reliable correlation between the concentrations of various copper species in the sediments and *Hiatula diphos* may not prove useful to monitoring requirements as *Hiatula diphos* has the ability of regulating copper which makes it clearly unsuitable for monitoring purposes.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Ming-Shiou Jeng of the Institute of Zoology, Academia Sinica, Republic of China, for providing chemical analyses and assistance in the collection of marine organisms for this study. The authors are grateful to one anonymous reviewer for constructive comments and suggestions. This study was supported by a grant from the National Science Council, Republic of China (NSC 83-0421-B-038-003-Z).

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