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Investigation of Zn, Cu, Cd and Hg Concentrations in the Oyster of Chi-ku, Tai-shi and Tapeng Bay, Southwestern Taiwan

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ABSTRACT

Metal concentrations in oyster, *Crassostrea gigas*, were investigated using 155 samples collected seasonally during 1996-2001 from the Chi-ku, Tai-shi, and Tapeng Bay areas in southwestern Taiwan. Seasonal variations as well as site difference were identified in the metal concentrations. The metal concentrations of oyster in southwestern Taiwan indicated a typical seasonal pattern of "winter-spring maximum" and "summer minimum". Among the three sites, Zn, Cu and Cd contents were found to be the highest in the March sample from Tai-shi, whereas Hg level was found to be the highest in the January sample from Chi-ku. The overall mean concentrations of Zn, Cu, Cd and Hg sampled from the three sites were 860 ± 375 , 267 ± 193 , 0.954 ± 0.484 and 0.097 ± 0.056 mg/kg dry wt., respectively, representing the baseline metal concentrations of oyster in Taiwan. In comparison with the 1970's survey, except the 1.8-fold increase in Cu, the other three elements were within the same ranges. After the transformation of the dry-weight-base data into flesh-weight-base data based on a ratio of 6.8 to 1, the mean metal levels, for the most part, closely agreed with many international food standards. However, extraordinarily high level of Cu (1115 mg/kg dry wt.) in oyster were found occasionally in wintertime. Therefore, it is strongly suggested that regulations with respect to food safety standards of metal concentrations need to be established as soon as possible.

Key words: baseline, zinc, copper, cadmium, mercury, summer minimum, cultured oyster, Chi-ku, Tai-shi, Tapeng Bay, Taiwan.

INTRODUCTION

Oyster is a major product of inshore mariculture in Taiwan. With a culture area making up over 85% of the total inshore mariculture field, its production, either in terms of quantity or economic value, ranks number one among all mariculture industries in Taiwan⁽¹⁻³⁾. Its annual production in 1998 to 2000 was NTD 25 to 34 billion consisting of 10-12% of the total aquaculture production in Taiwan. It is one of the most important fishery industries in Taiwan for coastal fishermen. Four western counties, Chiayi, Tainan, Changhua and Yunlin, make up 95% of the total oyster culture in Taiwan.

However, population and commercial activities have grown drastically in these areas over the last few decades. Untreated wastewaters have been allowed to flow through rivers into traditional oyster culture areas at estuary and into shallow coastal waters, thus often resulting in severe incidents of oyster pollution. The most notable case was the green oyster found at the Erhjen Chi estuary in 1987, which was attributed to acid-washed wastewaters from the nearby metal scraping and recycling factories⁽⁴⁾. Since the oyster has a high resistance to pollution, they are able to bioaccumulate various metals to a very high degree⁽⁴⁻⁵⁾.

In comparison with other coastal areas of Taiwan, the traditional oyster culture areas of Chi-ku, Tai-shi and Tapeng Bay, in Tainan, Yunlin and Pingtung respectively, are all relatively less industrialized and less urbanized. However, plans have been made, and in some cases projects completed for industrial development and national recreational ocean parks. This study was conducted to establish not only baseline data for the assessment of the environmental impact of these projects but also a database for the estimation and evaluation of acceptable safe metal concentrations in oyster in Taiwan.

MATERIALS AND METHODS

I. Materials

(1) Collection of specimens

A total of 155 oyster samples separated into sixteen batches were collected from Chi-ku, Tai-shi and Tapeng Bay along the southwestern coast of Taiwan (Figure 1). The oyster samples were collected from Chi-ku and Tapeng Bay during 1996 to 1997 and 1999 to 2000, respectively, and those from Tai-shi during 1998 to 2001.

Once the soft tissues of the oysters were removed, they were thoroughly washed with double distilled water. At least 30 individual oysters were randomly picked out

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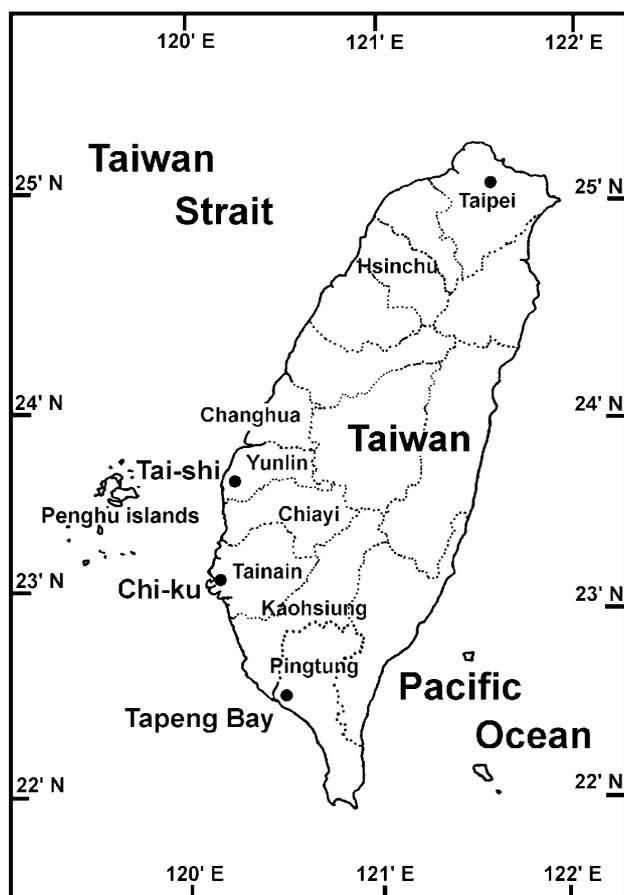


Figure 1. Map of the sampling sites in this study.

from each sample group and combined to make up a pooled sample⁽⁶⁾. Then, the oysters were homogenated and freeze-dried in an acid-washed, clean, white, plastic bottle. The flesh weights and dry weights of the samples were recorded.

(II) Reagents

All the chemicals used in this study, including HNO_3 , H_2SO_4 , SnCl_2 , KMnO_4 , were of GR grade, and including the Zn, Cu, Cd and Hg standard solutions of 1000 mg/L, were purchased from the Merck Company. Matrix matched standard reference materials, NIST SRM oyster 1566a from the National Standard Bureau, USA and DORM-2 dogfish muscle from the National Research Council of Canada, were used to control the analytical quality.

II. Methods

(I) Digesting methods

1. Sample digestion for Cd, Cu and Zn analysis

The sample digestion method for the analysis of Zn, Cu and Cd in this study was the same as in previous studies⁽⁷⁻⁸⁾, except that the amount of sample and acid were adjusted on the basis of the nature of the sample. In this

study, 0.5 g freeze-dried oyster samples were placed into 125 mL of conical flasks, and 15 mL nitric acid was added for digestion. After the digestion, a final volume of 25 mL was made up. Compared with fish tissues, oyster tissues contain a higher percentage of fat requiring longer time to breakdown the organic matter before complete digestion is achieved.

2. Sample digestion for Hg analysis

The methods employed in this study for total mercury analysis were also adopted from previous studies⁽⁹⁻¹¹⁾. Briefly, 1 g of the freeze-dried oyster tissues was weighed and placed into 75 ml graduated test tubes. 1 mL of HNO_3 and 4 mL of H_2SO_4 were added and heated to 80-90°C for 1 hour to ensure the tissues completely dissolved. Then 15 mL of 5% KMnO_4 was added for the final breakdown of the organic matter in the samples. Finally, the volumes were increased to 25 mL with the addition of double distilled water, and the samples were finally analyzed within 24 hours.

(II) Analysis of heavy metal concentrations

1. Measurements of Cd, Cu and Zn

A flame atomic absorption spectrophotometer (Hitachi Zeemen-8200) was used to measure Cu and Zn concentrations in the digested samples. However, Cd was measured using the standard addition method in the atomic absorption spectrophotometer with a graphite furnace. Details of the method are described in Chen and Chen⁽⁷⁾.

2. Measurement of Hg

The analysis of Hg was carried out using a cold vapor atomic absorption spectrophotometer following the modified system established in Chen's laboratory⁽⁹⁾. 2% of SnCl_4 was used as the reductant. The measurement was performed using the cold vapor-AAS method with an Hitachi Z-8200 AAS plus HFS-2 system with a T-joint device.

(III) Detection limits

The detection limits were determined following the same method described in previous studies^(7,9). The instrumental detection limits of Zn, Cu, Cd and Hg were 0.02, 0.03, 0.001 and 0.001 mg/L, respectively. The detection limits of Zn, Cu, Cd and Hg in oyster were 1, 1.5, 0.05 and 0.025 mg/kg dry wt., respectively.

III. Analytical Quality Control and Ensuring Accuracy

(I) Quality control measures

In order to achieve high quality in the analytical results, strict controls were implemented: (1) Each sample

Table 1. Analytical results of standard reference materials (SRM) in this study

SRM		Zn	Cu	Cd	Hg
Oyster SRM 1566a	Certified value	830 ± 57	66.3 ± 4.3	4.15 ± 0.38	0.0642 ± 0.0067
	This study	858 ± 22	67.2 ± 3.8	4.20 ± 0.10	0.0702 ± 0.0001
	Recovery (%)	103	101	101	109
Dogfish muscle DORM-2	Certified value	25.6 ± 2.3	2.34 ± 0.16	0.043 ± 0.008	4.64 ± 0.26
	This study	23.9 ± 0.9	2.26 ± 0.42	0.046 ± 0.003	—
	Recovery (%)	93	97	107	—

Unit: mg/kg

was digested in duplicate in order to determine its heterogeneity. (2) During the experiment, reagent blanks were inserted in every twentieth sample to detect any possible alien containments. (3) Duplicates of the standard reference materials were added simultaneously in each digesting batch. (4) The metal concentrations of NIST oyster 1566a and DORM-2 were then measured to verify the analytical quality.

(II) Analytical results of the standard reference materials

In this study, the recovery rate of Zn, Cu, Cd and Hg in the two standard reference materials were all within 100% ± 10% (Table 1).

IV. Statistical Analysis

The statistical analysis was performed with the SAS software ANOVA (one-way Analysis of Variance)⁽¹²⁾. The Duncan's multiple range test was also adopted so as to examine the differences in the metal concentrations among species ($p < 0.05$).

RESULTS AND DISCUSSIONS

The results of metal analyses showed that the concentrations of these four elements in the oysters varied seasonally (Figure 2). In Chi-ku oyster sampled in January showed the highest concentrations for all four elements, whereas the November sample contained the lowest Cu and Zn concentrations and the June sample had the lowest Cd and Hg concentrations. In Tai-shi, except for the Zn concentrations in the February samples, all the March and February samples showed either the highest or second highest Cu, Cd and Hg concentrations. In May, at this site, all the elements showed the lowest concentrations. These seasonal fluctuations of metal concentrations in oysters have been well documented⁽¹³⁻¹⁶⁾. The highest Zn and Cu were found at the end of the reproductive cycle of oysters, such as *Crassostrea iridescens*⁽¹³⁻¹⁴⁾ and *C. corteziensis*⁽¹⁵⁾ in October in Mexico. During that time, the oyster became skinny due to the repelling of gametes, which meant that Cd and Hg residues in the viscera were concentrated on a mass basis. Similar seasonal variations were also reported by Hsu *et al.*⁽¹⁶⁾ on an oyster study in Yunlin and Chiayi,

from southwestern Taiwan in the 1970's. The reproductive cycle of *C. gigas* in southwestern Taiwan varied geographically, demonstrating a trend whereby the oysters at the south of the island commenced their development of gametes earlier than did those of the northern populations, while the October to January period was the resting phase of oysters around the Island⁽¹⁷⁾. Therefore, the peak metal accumulation season may very well be highly related to the beginning of the reproductive cycle of the oysters. Another factor related to the bioaccumulation of metals in the oyster is the solubility of metal ions in the lower salinity runoff⁽¹⁸⁾, which normally contained more biological sources of metal. In addition, in southwestern Taiwan the dry season normally begins in November and ends in March. During this period of time, except for Zn, concentrations of Fe, Mn, Cu, Ni and Pb in the dissolved phase in water were significantly higher than those during the wet season⁽¹⁹⁾, which enriched the metal concentrations in the coastal waters and possibly caused the metal concentrations to become elevated in March in the Tai-shi samples, as well as in the April samples studied by Hsu *et al.*⁽¹⁶⁾. In the summer, during the pre-spawning period of oyster, the size and gonad growth in the oyster body diluted the metal concentrations in the whole body⁽¹⁴⁾. Thus, the lowest metal concentrations were found in May and June.

Table 2 shows the ranges of metal concentrations of oyster analyzed in the current study. For the summer samples, site differences were observed and their trends varied depending on the metal. Among the three sites, samples from Tapeng Bay showed the highest mean Cd and Hg concentrations but the lowest Zn and Cu levels. However, the Chi-ku samples contained the highest Zn and the lowest Cd concentrations, and the Tai-shi samples had the highest mean Cu and the lowest mean Hg (Table 2). Since oysters are known as very sensitive bivalves able to reflect geographical metal gradients in a very short time period⁽²⁰⁾, the metal concentrations in the summer samples were very low, but they did vary between sites. These fluctuations might be ascribed to natural variations due to geographical differences and environmental conditions.

A further look at the yearly mean concentrations of these metals clearly showed that the differences among the site-year groups were also significant ($p < 0.05$), with the exception of copper (Table 2). This is similar to the summer samples previously discussed, where group differences were metal-dependent. Except a significantly high level of

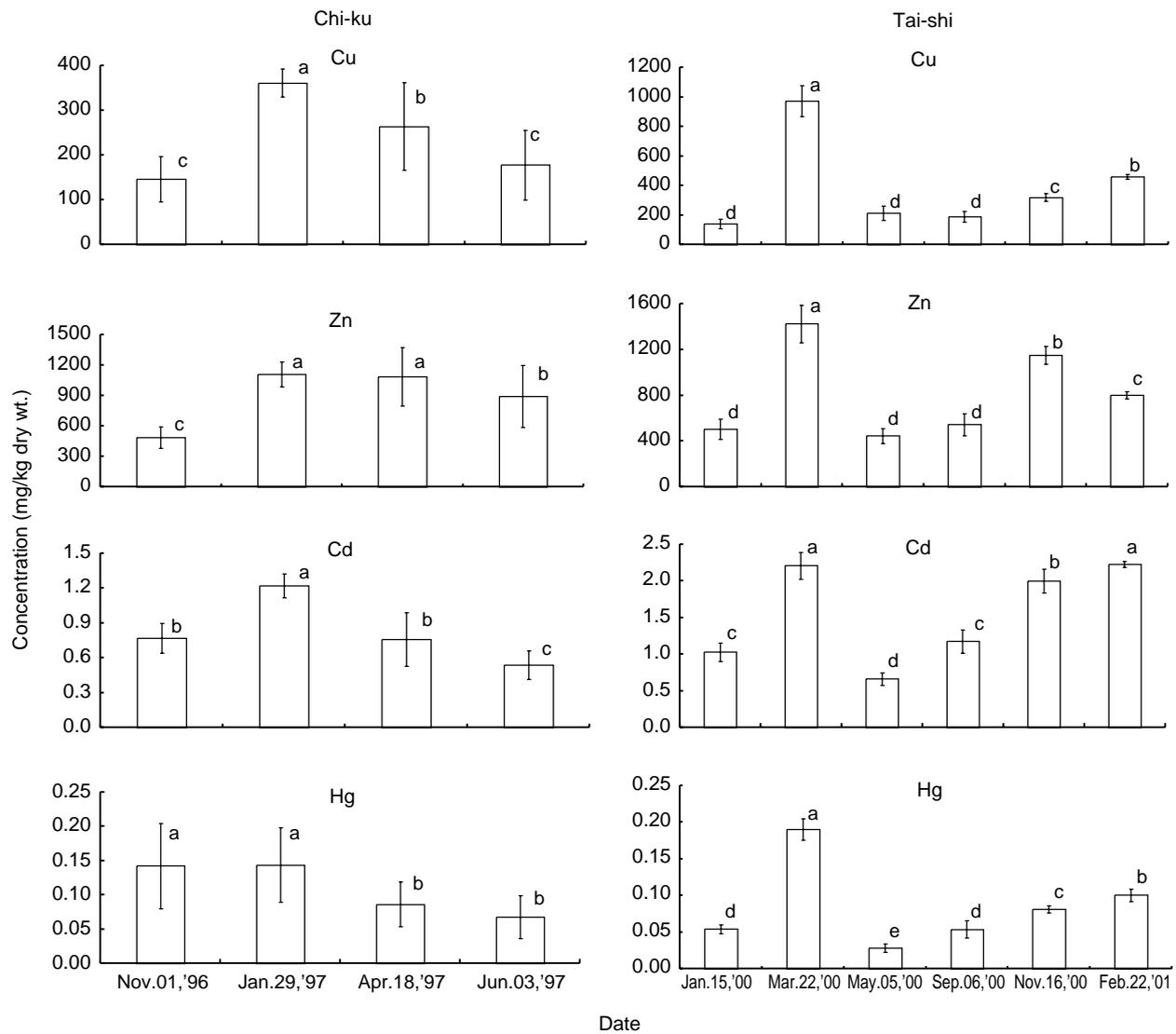


Figure 2. Comparisons of the seasonal variations of metal concentrations of oyster, *C. gigas*, in the Chi-ku and Tai-shi areas, southwestern Taiwan. The bars and vertical lines indicate the means and one standard deviation, respectively. The a, b, c, d and e notations marked above the bars show the results of Duncan's multiple range test at the significant level $p < 0.05$.

Table 2. Mean Zn, Cu, Cd and Hg concentrations (mg/kg dry wt.) in oysters sampled in summer from Tapeng Bay, Chi-ku and Tai-shi and the mean concentrations from whole year samples from Chi-ku and Tai-shi

Site	n	Zn	Cu	Cd	Hg
Summer					
Tapeng Bay	5	260 ± 15 ^c (243~275)	57 ± 17 ^b (34~71)	0.853 ± 0.130 ^a (0.685~0.992)	0.099 ± 0.028 ^a (0.058~0.132)
Chi-ku	29	893 ± 305 ^a (437~1281)	178 ± 78 ^a (91~321)	0.540 ± 0.122 ^b (0.312~0.734)	0.068 ± 0.031 ^b (0.027~0.158)
Tai-shi	15	482 ± 77 ^b (353~625)	205 ± 46 ^a (140~313)	0.827 ± 0.245 ^a (0.540~1.411)	0.037 ± 0.015 ^c (< 0.025 ~0.070)
Whole Year					
Chi-ku (1996-1997)	103	905 ± 332 ^{ab} (328~1533)	237 ± 108 (72~417)	0.808 ± 0.292 ^b (0.312~1.368)	0.104 ± 0.056 ^a (0.027~0.288)*
Tai-shi (1998-1999)	4	1175 ± 595 ^a (575~1998)	246 ± 53 (184~306)	0.958 ± 0.420 ^b (0.428~1.449)	–
Tai-shi (2000-2001)	34	774 ± 405 ^b (353~1606)	393 ± 319 (114~1115)	1.411 ± 0.683 ^a (0.540~2.452)	0.079 ± 0.059 ^b (< 0.025 ~0.213)**

Note: The notations a, b, c beside the mean values indicate the results of the Duncan's multiple range test or student's t-test (significant level, $p < 0.05$); and n indicates sample size. Sample size of *: 77 and **: 33.

Hg found in the Chi-ku oysters, the highest mean concentrations of Zn, Cu and Cd were found in the 1998-1999, 2000-2001 and 2000-2001 Tai-shi samples, respectively (Table 2). In comparison with the data reported in the 1970's^(16,21), it is interesting to note that a significant doubling of Cu and Cd concentrations were found in the 2000-2001 Tai-shi oyster, which was possibly associated with the extensive construction involved in the Island Industrial Camp off Yunlin. It is well known that digging and dredging of coastal areas greatly contribute to an increase in the precipitation of contaminants in adjacent waters⁽²¹⁾, and this could obviously increase the uptake of contaminants by the oyster.

In the current study, the ranking of metal concentrations in oyster was Zn > Cu > Cd > Hg, which reflects the typical metal richness of the *Crassostrea* species, like *C. commercialis*⁽⁶⁾, *C. gigas*⁽⁵⁾ and *C. corteziensis*⁽¹⁵⁾, as reported in many previous studies^(5, 6, 15-16, 21). The overall mean metal concentrations in the whole soft tissue found in this study (Zn = 860 ± 375, Cu = 267 ± 193, Cd = 0.95 ± 0.48 and Hg = 0.097 ± 0.056 mg/kg dry wt.) were fairly low and comparable to previous records for the coastal waters of Taiwan^(4, 5, 16, 21-24), except that the Cu concentration was higher (1.8 folds) than that of 1970's data^(16,21) investigating oysters in the same area. In general, the Zn, Cu, Cd and Hg concentrations of oysters in Taiwan were less than 1000, 500, 2.0 and 0.3 mg/kg dry weight. However, for those samples from hot spot areas, such as Hsian-san^(5, 23), Charting^(4, 23-24) and the Erhjin Chi estuary^(4, 23), the Zn and Cu concentrations of oyster reached 2000 and 4000 mg/kg dry weight. It is worth noting, that the Hg concentrations of oysters found in this study were only one-third of the level reported by Jeng *et al.*⁽²³⁾.

Further comparing the metal levels examined in this study with those of the genus *Crassostrea* from other parts of the world found that Zn, Cu, Cd and Hg concentrations in *C. gigas* from southwestern Taiwan are fairly low on a global scale, indicative of typical background level in an agriculture area⁽²⁵⁻³³⁾. On a global scale, the Zn, Cu, Cd and Hg concentrations of wild and cultured oysters, were similar to our previous conclusion, less than 1000, 500, 2.0 and 0.3 mg/kg dry weight. A further comparison was made with the background level of wild *Crassostrea* (as *Saccostrea commercialis*) from 27 locations in New South Wales, Australia⁽³³⁾. It was found that only the highest Cu and Hg concentrations detected in the current study exceeded the 85% of NSWBG (background metal concentrations of oyster in New South Wales, Cu = 170-394 mg/kg dry wt.; Hg = 0-0.1 mg/kg dry wt.) and that the Zn and Cd concentrations were all lower than those in the NSWBG (Zn = 2610-3904 mg/kg dry wt.; Cd = 5-11 mg/kg dry wt.). Our highest Cu level was still within the 85% concentration level of NSWAll (all metal concentrations of oyster in New South Wales, Cu = 390-1460 mg/kg dry wt.), and only the highest Hg concentration slightly exceeded the 85% NSWAll level (Hg = 0-0.2 mg/kg dry wt.). Accordingly, the metal concentrations of oyster in south-

western Taiwan, as determined in this study, resembled the records of less industrialized and urbanized areas all over the world.

Table 3. International Standards on metal concentrations (mg/kg flesh weight) in seafoods

Country	Standard	Zn	Cu	Cd	Hg	Reference
USA	FDA	-	-	2*	0.5	(34)
USA	NAS	-	-	0.5*	0.5	(35)
Australia	NHMRC	1000	30	2	1.0	(36)
Australia	TPHR	40	30	5.5	1.0	(37)
Canada	-	-	100	-	0.5	(38)
Japan	-	-	-	1	1.0	(38)
UK	MAFF	50	20	-	0.3	(39)
UK	FSC	50	-	-	0.3	(40)

Note: FDA: Food and Drug Administration; NAS: National Academy of Science; NHMRC: National Health Medical Research Council; TPHR: Tasmania Public Health Regulation; MAFF: Ministry of Agriculture, Fisheries and Food; FSC: Food Science Council; and * indicates dry weight base.

After transformation into flesh-weight based on a ratio of 6.8 to 1⁽³⁴⁾, the highest Cd and Hg concentrations (Cd = 0.361, Hg = 0.031 mg/kg wet wt.) were all within the regulatory food standards of different countries (Table 3). However, the highest Cu level (164 mg/kg wet wt.) was found to exceed all food standards, while the highest Zn (294 mg/kg wet wt.) exceeded most of the standards, with the exception of the NHMRC, Australia⁽³⁶⁾. These highest Zn, Cu and Cd concentrations of a single oyster sample were all recorded from the March, 2000 Tai-shi sample, whereas the highest Hg level was from the January, 1997 Chi-ku sample (Table 2). These reflect a geographical and environmental difference within the study area. However, the highest Zn and Cu concentrations were only two-third and one-fourth, respectively, of highest values previously reported⁽⁴⁻⁵⁾. The overall mean Zn and Cu levels were 126 ± 55 and 39 ± 28 mg/kg in wet wt., respectively, compliant with the food standards of various countries. So that the metal levels of oysters analyzed in this study represent the metal uptake of the general public through oyster-consumption in Taiwan. Based on these results, it is found that exceptionally high levels of Cu in oyster may occasionally occur in the wintertime. For public health concerns, it is therefore strongly suggested that regulations with respect to food safety standards of metal concentrations, limiting methyl Hg to be below 0.5 mg/kg in wet wt.⁽⁴¹⁾, should also be established for the other elements as soon as possible in Taiwan.

CONCLUSIONS

The investigation into metal concentrations of oyster in southwestern Taiwan showed a typical pattern of "winter-spring maximum" and "summer minimum". The pattern coincides with the reproductive cycle of oyster in the study area, where the spawning of oyster normally

occurs in January in the south and July in the north of Taiwan. In addition, the higher levels of metal concentrations in the oyster may be a result of the wet season when larger volumes of freshwater with more bioavailable sources of metal flows down to the estuary. The overall means of Zn, Cu, Cd and Hg concentrations in the oyster were 860 ± 375 , 267 ± 193 , 0.95 ± 0.484 and 0.097 ± 0.056 mg/kg dry weight, respectively, but the highest Zn, Cu, Cd and Hg concentrations were 1998, 1115, 2.45 and 0.288 mg/kg dry weight, respectively. The metal concentrations represent the metal levels found in a typical agricultural area, the major oyster culture areas and the background levels of oyster in Taiwan. These metal levels in oyster were similar to those reported in the literature. Using a conversion factor of 6.8 from wet to dry wt., high Cu concentrations in oyster were occasionally found in the wintertime, exceeding the regulatory standards of various countries.

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