Trace Metal Contents of the Pacific Oyster (*Crassostrea gigas*) Purchased from Markets in Hong Kong

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ABSTRACT / Arsenic, cadmium, chromium, and copper concentrations of the Pacific oyster, *Crassostrea gigas*, purchased from four different markets were determined in this project. In general, gill tissue had the highest proportion of metal contents (34%–67%) when compared with other tissue parts (mantle, viscera, and adductor muscle), except for arsenic, which showed the highest level in adductor muscle (44%). Smaller oysters (longitudinal length of soft body part less than 6 cm) had higher metal levels than larger ones (longitudinal length of soft body part more than 6 cm), except copper. None of the four metals examined showed an obvious seasonal trend, although cadmium levels seemed to be higher in autumn and winter months. Arsenic, cadmium, and copper levels in oysters purchased from different markets and different months obtained in the present study were higher when compared with past reports. Cadmium levels, as high as 10.98 mg/kg (dry weight basis) have been obtained. This approaches the safety limit that may be hazardous to human health. Continual monitoring of cadmium and other trace metals of toxicological significance to man in Hong Kong seafood is recommended.

Pacific oysters (*Crassostrea gigas*) have been cultivated in Deep Bay, at the northwestern part of the New Territories, for the past 170 years in Hong Kong (Figure 1). The distribution center has been set up at Lau Fau Shan Village and oysters are marketed fresh in this village.

The oysters are cultivated by the bottom-layer method. Rectangular tiles or concrete posts, acting as spat collectors are laid out on the surface of the intertidal mud prior to the fall of spat, which occurs in summer. Once the spat has settled, the collectors are taken offshore and placed in neat rows. Because of the high rate of sedimentation within Deep Bay, the collectors have to be periodically lifted out of the mud and relocated to prevent the spat from suffocating. The oysters are tended until they are 3–4 or even 6 yr old and are then cropped for sale.

Deep Bay receives water from streams and watercourses in the northwestern part of the New Territories. The most notable ones are the Shenzhen River and Yuen Long Creek (Figure 1). In recent years, the rapid industrialization and urbanization of Shenzhen and Yuen Long areas has resulted in an increase in the discharge of pollutants (including heavy metals) into the water-courses which, in turn, flow into the Deep Bay. Although the adverse water quality in Deep Bay has lowered the local oyster production, its production still accounts for 30%–40% of the oysters eaten annually in Hong Kong (Phillips and others 1982a).

Oysters have a great capacity to accumulate heavy metals (Boyden 1975) and accumulate three sources of heavy metals in seawater: dissolved inorganic metal ions, organometallic ion complexes, and metal ions preconcentrated on phytoplankton. Oysters are sedentary filter-feeders that cannot escape from the environment and hence accumulate high levels of metals in polluted environments and have been used as an environmental pollution indicator (Phillips and Yim 1981).

Cadmium is a well-known zootoxic metal (Klaassen and others 1986). Although the organic form of arsenic is less toxic, ingestion of its inorganic form (As^{3+}) will result in severe poisoning (LeBlanc and Jackson 1973). Cadmium accumulation in oysters had been explained by its affinity to bind with specific

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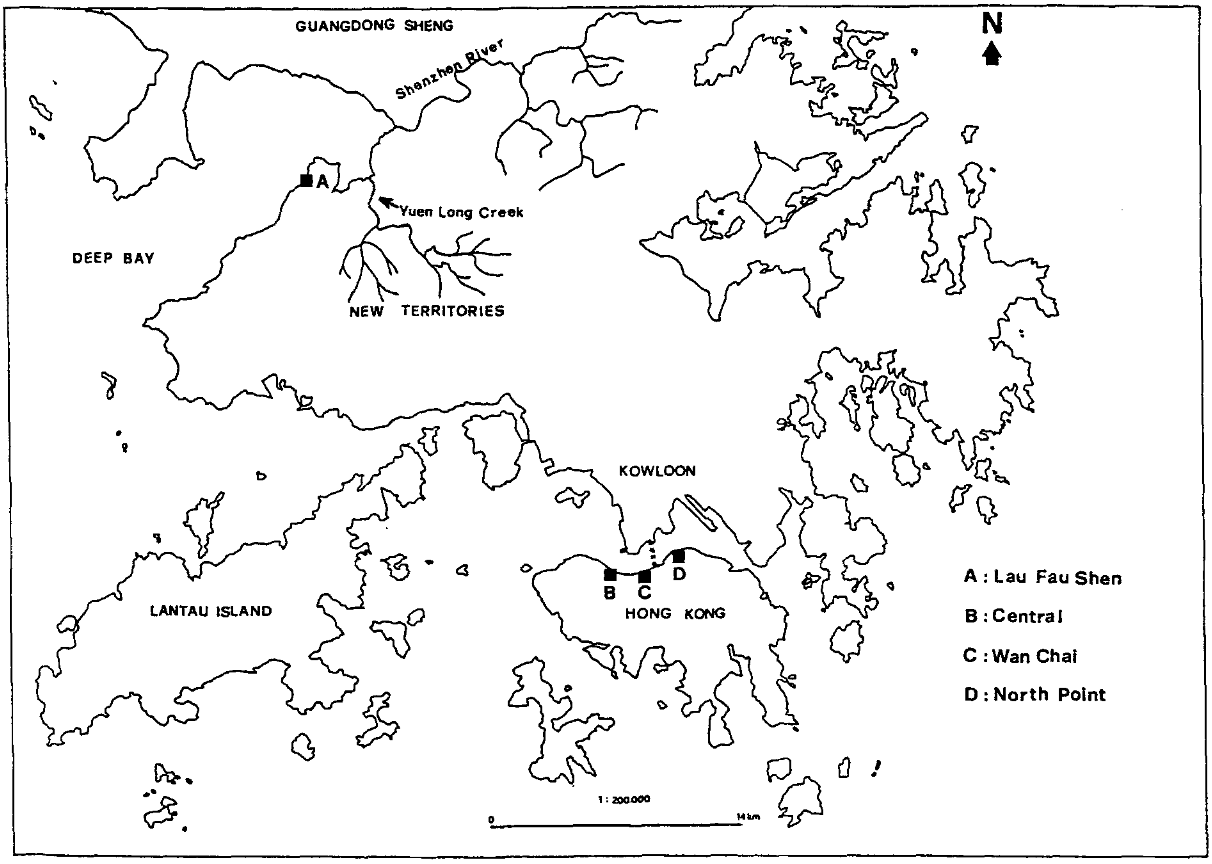


Figure 1. Map of Hong Kong showing the four sampling locations of the Pacific oysters, *C. gigas.*

proteins (metallothioneins) and metallothionein-like proteins (Bouquegneau 1984). This binding mechanism is thought to be a detoxification response of oyster to suppress the toxic effects of cadmium ions in its body. The carcinogenicity of hexavalent chromium to man and other mammals and its common usage in the leather and textiles industries has been revealed (Phillips and others 1982b). Chromium is found to form the essential part of the glucose tolerance factor and affects amino acid and nucleic acid synthesis in animals. Arsenic present in marine species is possibly a complex organic substance with very slight toxic properties and this organic complex may play a vital and necessary role in cell activity (LeBlanc and Jackson 1973). Copper, commonly considered as nontoxic, may accumulate in mollusks to substantial amounts. Biochemically, this metal resembles or even overlaps with that of iron in the role of metabolism of molecular oxygen in mollusks because the oxygen-carrying pigment of mollusks blood is not hemoglobin, but a copper-containing cuproprotein (hemocyanin). Furthermore, copper forms a vital part of a number of oxidase enzymes, sometimes in combination with other metal ions, as in cytochrome *c* oxidase, which contains both copper and heme iron. Furthermore, wandering leukocytes in mollusks will accumulate a high level of copper in order to detoxify and translocate the metal out of the body. This accumulation of copper metal on leukocytes contributes to the greening of such animals. Therefore arsenic, cadmium, chromium, and copper levels in the oyster are analyzed in the present project.

The purpose of this project is to monitor the heavy metal content of oysters purchased in Lau Fau Shan and other three retailing markets: Central, North Point, and Wan Chai (Figure 1).

The relationships of metal levels with different parts and sizes of the organism and the seasonal variations of the four metals in the oysters are also studied. Metal contents of oysters purchased from different markets are compared. Seasonal variations of the four metals in the oysters purchased from Lau Fau Shan market over a 1-yr period are also studied.

Table 1. Metal contents in different parts of *C. gigas* (mean for five replicates)[a]

Tissue	Dry weight (mg/kg)	As	Cd	Cr	Cu
Adductor muscle	Mean	2.2b	6.22a	0.928b	750.2c
	SD	1.284	0.677	0.696	50.73
Gill	Mean	0.903a	19.07d	3.03c	840.9d
	SD	0.744	0.542	0.46	35.25
Mantle	Mean	0.906a	8.67c	0.383ab	509.9b
	SD	0.44	0.293	0.505	7.93
Visceral mass	Mean	1.039a	7.45b	0.154a	384.0a
	SD	0.353	0.178	0.232	6.7

[a]SD: standard deviation. a,b,c,d: mean values with the same letter within each vertical column indicate no significant difference ($P > 0.05$).

Materials and Methods

Sampling and Pretreatment

Oyster samples were purchased from Lau Fau Shan retailing market and were transported to the laboratory in sealed polythene bags. The flesh of oyster samples were washed under tapwater and then dried on paper towels. They were then weighed and the corresponding longitudinal length was measured individually. The samples were frozen below 0°C if they were not analyzed immediately. After this pretreatment, each set of samples was treated differently according to the objectives.

Different parts of oysters. The oyster samples were purchased in September 1989 from Lau Fau Shan market and the pretreated samples were then dissected into four parts: adductor muscle, gill, mantle, and viscera. All dissected parts of the same type were pooled together and homogenized without addition of water.

Different sizes of oysters. The oyster samples were purchased in December 1989 from Lau Fau Shan market and the pretreated samples were divided into seven groups according to longitudinal lengths of their flesh: 5.1–6.0, 6.1–6.7, 6.8–7.5, 7.6–8.4, 8.5–9.3, 9.4–10.0, and 10.1–10.7 cm. Samples of each size group were then homogenized without addition of water.

Oysters purchased from different markets. Oyster samples were purchased from three other markets—North Point, Central and Wan Chai—in September 1989. All these samples were pretreated using the above method and grouped together according to their markets. The whole oysters were homogenized without addition of water.

Oysters purchased in different months from Lau Fau Shan market. From May 1988 to March 1990, oyster samples were purchased from Lau Fau Shan retailing market monthly. After pretreatment, the monthly samples were homogenized without addition of water.

Metal Analyses

After these specific treatments, the homogenized samples were weighed and then freeze-dried in order to prevent loss of metal at high temperatures. Dried samples were weighed again, and dry–wet weight ratios were calculated. Dry matter determination was also performed to check the efficiency of the freeze-dryer. Dried samples were then subjected to acid digestion.

Nitric acid digestion for cadmium, chromium, and copper. About 1 g of the dried samples were digested by 5 ml 65% nitric acid at 110°C until a clear solution had been obtained. The digested clear solution was then filtered through a Whatman No. 42 filter paper and diluted to 25 ml with distilled water. Cadmium, chromium, and copper contents were then determined by flame atomic absorption spectrophotometry (Allen and others 1974).

Specific digestion for arsenic. Arsenic is a volatile metal and a specific digestion method is needed. About 0.5 g of the dried samples were added to 5 ml 65% nitric acid and digested at 95°C for 30 min. Then 15–20 drops of 30% hydrogen peroxide were added and digested for the next 30 min. Addition of hydrogen peroxide was repeated twice at 30-min intervals to complete the digestion. The digested solution was then filtered through a Whatman No. 42 filter paper and diluted to 25 ml with distilled water. Arsenic content was then determined by graphite furance absorption spectrophotometry (Krynitsky 1987).

The results were subjected to Duncan's multiple-range test at 95% confidence limits.

Results and Discussion

Metal Contents in Different Parts of *C. gigas*

The results of this part of experiment are shown in Table 1 and Figure 2. Table 1 shows copper levels were the highest, on the order of hundreds of parts

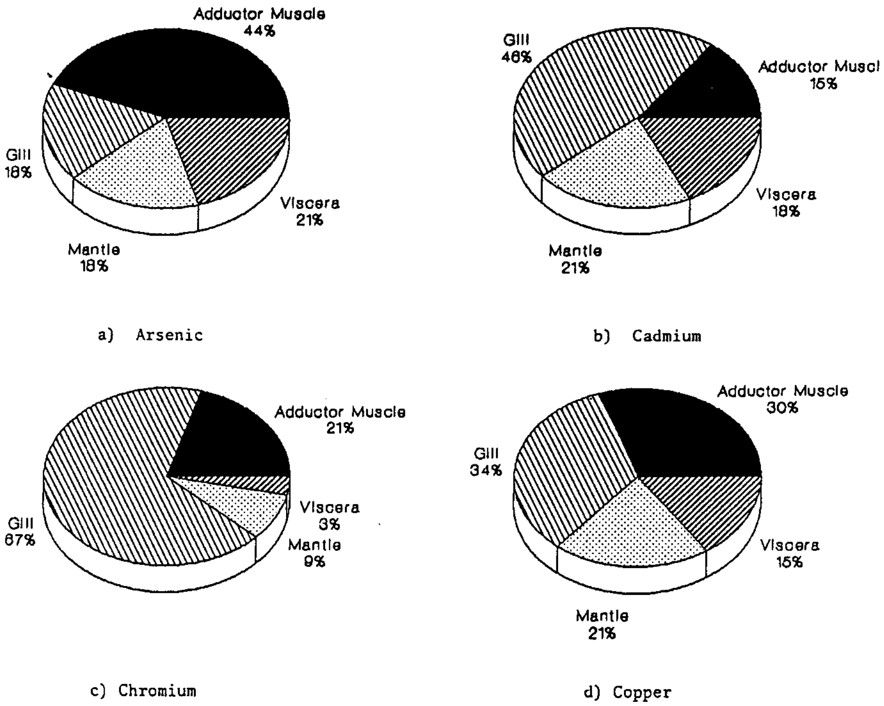


Figure 2. Metal content, expressed as percentage, in different parts of the Pacific oyster, *Crassostrea gigas*.

per million, because copper is linked to a variety of catalytic proteins. The other three metals (cadmium, chromium, and arsenic) show much lower levels in the oysters.

Generally, the results indicate that the gill tissue contained the highest proportion of metals (about 34%–67%). The contents of cadmium, chromium, and copper in the gill tissue were all significantly higher ($P < 0.05$) than that in the other three parts—the adductor muscle, the viscera, and the mantle. This agrees with our earlier finding (Wong and others 1981). This is due to the high surface area of the gill and the chemical nature of mucus on it (Cunningham 1979). Oysters are typical filter-feeders. Surrounding water is drawn through the inhalant siphon and is filtered through the gills. Small particles and food in the water are trapped there, and the filtered water is excreted through the exhalant siphon. The particles trapped are absorbed by mucus in the gill, and the large surface area of the gill tissue enhances this process. If the particles trapped are contaminated by metals, the gill tissue will then show the highest metal content.

As for the arsenic content, the adductor muscle had the highest level (2.2 mg/kg) among the parts tested (Table 1), whereas no significant difference ($P > 0.05$) was noted in arsenic content among other parts (0.9–1.0 mg/kg). The high metabolic activities of the muscles probably result in an accumulation of higher levels of arsenic (Moore and Ramamoorthy 1984).

The relatively low metal levels of the mantle and viscera can be explained by their rapid metal-eliminating mechanisms. There are two major mechanisms in the mantle for metal elimination: diapedesis and production of shell. The continuous process of diapedesis involves the slow migration of metal-laden amebocytes from the blood to surface epithelial tissue, and finally elimination from the body (Tripp 1963). The mantle is also a primary organ involved in shell production and metal is transferred to the shell from the mantle during this process.

The viscera have various means to eliminate metals since they consist of digestive, reproductive, excretory, and other biological systems. These systems can lose metals by means of feces, urine, and spawning. The unexpectedly low metal levels in the viscera may be due to the depuration of oysters by the retailers by placing the living oysters in clean water for several days to clear up the intestinal contents (Abbe 1982). As a result, lower metal levels are noted in the visera than other body parts.

Metal Contents in Different Sizes of *C. gigas*

Table 2 shows that, in general, the smallest-sized group, i.e., group A (5.1–6.0 cm) had the highest metal contents. Their arsenic, cadmium, and chromium contents were significantly higher ($P < 0.05$)

Table 2. Metal contents in different parts of *C. gigas* (mean of five replicates)[a]

Size	Dry weight (mg/kg)	As	Cd	Cr	Cu
Group A (5.1-6.0 cm)	Mean	16.10b	3.98b	3.05e	410.6b
	SD	5.18	0.318	0.041	8.59
Group B (6.1-6.7 cm)	Mean	0.510a	3.66b	1.82cd	412.6bc
	SD	0.146	0.403	0.20	4.86
Group C (6.8-7.5 cm)	Mean	1.066a	3.09a	2.00d	344.0a
	SD	0.781	0.057	0.20	11.51
Group D (7.6-8.4 cm)	Mean	0.162a	3.07a	1.60bc	418.0bc
	SD	0.112	0.111	0.237	1.41
Group E (8.5-9.3 cm)	Mean	0.128a	3.14a	1.27a	422.0c
	SD	0.103	0.112	0.018	8.51
Group F (9.4-10.00 cm)	Mean	0.618a	3.13a	1.44ab	410.2b
	SD	0.411	0.086	0.125	8.11
Group G (10.1-10.7 cm)	Mean	0.192a	3.04a	1.51b	412.4bc
	SD	0.125	0.061	0.219	7.20

[a]SD: standard deviation. a,b,c,d: mean values with the same letter within each vertical column indicate no significant difference ($P > 0.05$).

than the other six groups. As for the copper content, group A had an intermediate level. It has also been revealed that trace element concentrations in oysters were often inversely related to the dry weight of whole individuals (Boyden 1974). Mackey and others (1975) also noted that concentrations of copper and cadmium decreased with increasing wet weight and age in *Crassostrea commercialis*. Similarly, Phillips (1976) reported that accumulation of cadmium and copper in *Mytilus edulis* (mussel) was weight-dependent.

The fact that younger (smaller) bivalves accumulated higher metal concentrations in most cases is probably due to two factors. The more rapid growth rate in younger bivalves allows faster turnover of cellular materials and enhances more heavy metal uptake and incorporation into the tissues. Further increase in body weight would dilute the metal concentration in the body of larger individuals. Small bivalves also have a larger surface-to-volume ratio than larger ones; therefore, more surface area would be available for metal accumulation (Cunningham 1979). The low metal concentration in the larger groups may also be due to the maturation in larger individuals of some specific structures or organs that take part in the elimination of metals in the body.

In the present experiment, the oysters were divided into groups according to their size, based on the longitudinal length of the fresh tissue, instead of the weight as used in other reports. According to Figure 3, the longitudinal length of fresh tissue was generally proportional to the wet weight, so it was reasonable to conclude that heavy metal concentrations of oysters were inversely proportional to the longitudinal length of fresh tissue.

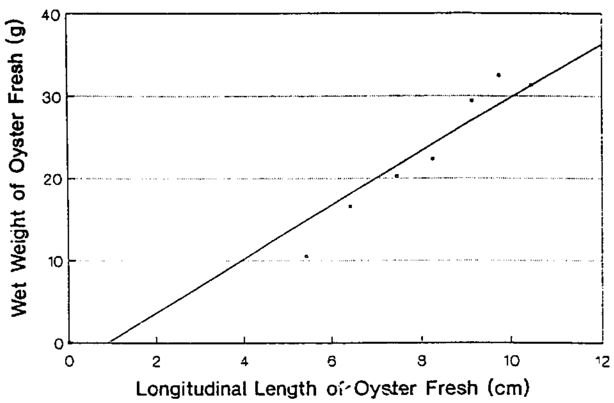


Figure 3. The relationship between wet weight and longitudinal length of oyster flesh.

Metal Contents in *C. gigas* Purchased from Different Markets

Analysis of oysters from retail markets allows no conclusion as to the possible areas of metal contamination, as no information regarding site of production is available. However, it acts as a direct check on the oyster products reaching the Hong Kong consumers, since many of them are derived from waters distant from Hong Kong. Table 3 shows that the levels of metals in oysters purchased from different markets were quite different due to their different sources of origin. Oysters are bioaccumulators of heavy metals, and their metal contents are greatly affected by the external environmental conditions, such as temperature, salinity, and contamination level (Phillips and others 1982a). Furthermore, the different practice of depurating oysters in different retailing markets may cause variations in metal levels.

Table 3. Metal contents of *C. gigas* purchased from different markets (mean of five replicates)[a]

Market	Dry weight (mg/kg)	As	Cd	Cr	Cu
Lau Fau Shan	Mean	2.952c	10.98d	1.25a	761.1b
	SD	0.163	0.151	0.392	23.84
North Point	Mean	1.326b	9.45c	1.82a	988.2c
	SD	0.742	0.337	0.183	26.26
Central	Mean	0.156a	7.44a	2.28a	588.8a
	SD	0.158	0.376	1.263	20.04
Wan Chai	Mean	0.116a	8.19b	1.97a	1071.2d
	SD	0.125	0.119	0.138	11.69

[a]SD: standard deviation. a,b,c,d: mean values with the same letter within each vertical column indicate no significant difference ($P > 0.05$).

The high cadmium levels in oysters, *Crassostrea gigas*, found in this study agree with the results of Boyden (1975). In that research, the level of cadmium in the oysters was even larger, on the order of magnitude of hundreds. These findings suggest that cadmium is rapidly accumulated by this species of oyster, even though a low concentration of cadmium was present in the surrounding water. The current legislative limit for cadmium in seafood is 1.0 mg/kg wet weight, or 5.5 mg/kg dry weight, in New Zealand. Oyster samples purchased from the four markets all exceeded this limit, especially those from Lau Fau Shan market, which was significantly higher ($P < 0.05$) than other samples and approached the limit imposed in Australia and United States (2.0 mg/kg wet weight and 11.0 mg/kg dry weight, respectively). These cadmium levels of Pacific oysters are also higher than those previously reported (0.6–5.4 mg/kg wet weight) in studies of the same species purchased from Lau Fau Shan market (Phillips and others 1982a). This implies that the metal contamination of our seafood has further deteriorated. This is also true for copper and arsenic levels where higher contents are noted in the present study.

The levels of arsenic and chromium noted in the Pacific oysters of the present study are relatively low when compared to standards (55 mg/kg and 5.5 mg/kg dry weight, respectively) imposed by the Public Health and Municipal Services Ordinance of Hong Kong (Hong Kong Government 1988). Arsenic and chromium are not significant contaminants of shellfish, and there is no evidence for extreme bioaccumulation in the majority of invertebrate species (Moore and Ramamoorthy 1984).

Metal Contents in *C. gigas* Purchased in Different Months from Lau Fau Shan Market

The results are shown in Figure 4 and the data were subjected to the moving average method (Conner and Morrell 1977) and the corresponding results are shown in Figure 5. With reference to Figure 4, arsenic content in the oysters from May 1988 to March 1990 was within the safety level (10 mg/kg, wet weight basis) while cadmium and chromium exceeded the safety levels (2 and 1 mg/kg, respectively, wet weight basis) in some months.

According to Figure 5, only cadmium showed a slight seasonal trend where higher contents were noted in autumn and winter months. These results were in line with other reports (Bryan 1973, Phillips and others 1982, Ramelow and others 1989, Wong and others 1981). These results are probably related to water salinity and the sexual cycle of oysters. During the cold season in Hong Kong, the amount of rainfall decreases and the volume of water flowing into Deep Bay also decreases, which results in high water salinity (26–32%). Salinity is considered to be an environmental parameter with a strong influence on the accumulation of metals by bivalves (Cunningham 1979).

Another reason for high levels of cadmium in the cold season is the sexual cycle. The spawning season of the Pacific oyster is in the warm season, between May and September, and a large number of eggs may be released during this period. Adult female oysters would transfer metals to their eggs (Greig and others 1975). As a result, a large amount of metals in adult oyster bodies may be lost through the eggs during spawning season and causing the depression of metal levels in the warm season.

No evident seasonal trend was found for the other three metals studied. For copper, a broadly defined rise and fall occurred for May/July 1988 to October/December 1989. For arsenic, the peak level appeared from November 1988 to March 1989, but the trend did not repeat in the next year. The failure in showing the seasonal trend may be due to the comparatively low level of arsenic uptake in oysters, resulting in changes in concentration within the bodies that were not easily detected. The trend shown by chro-

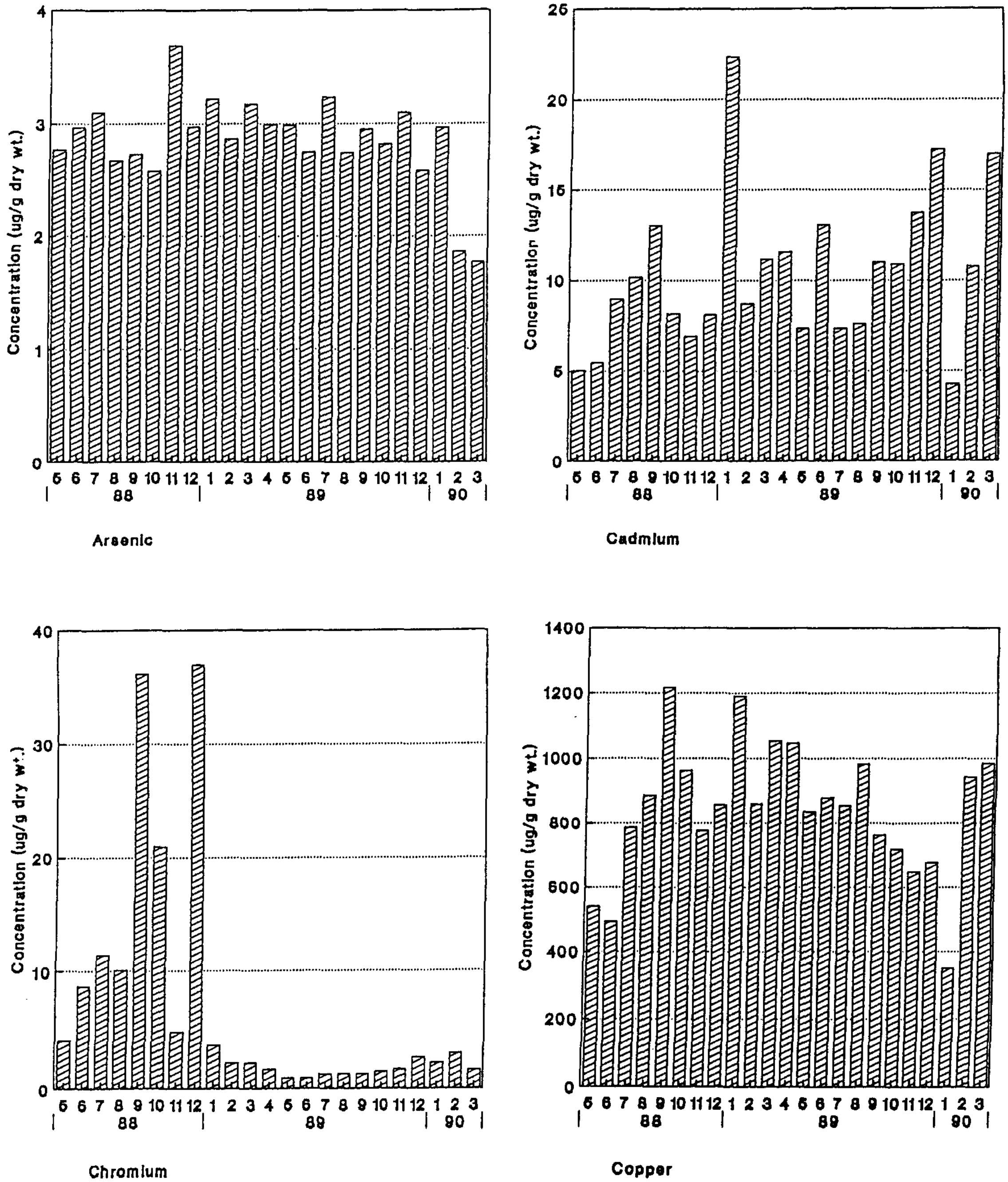
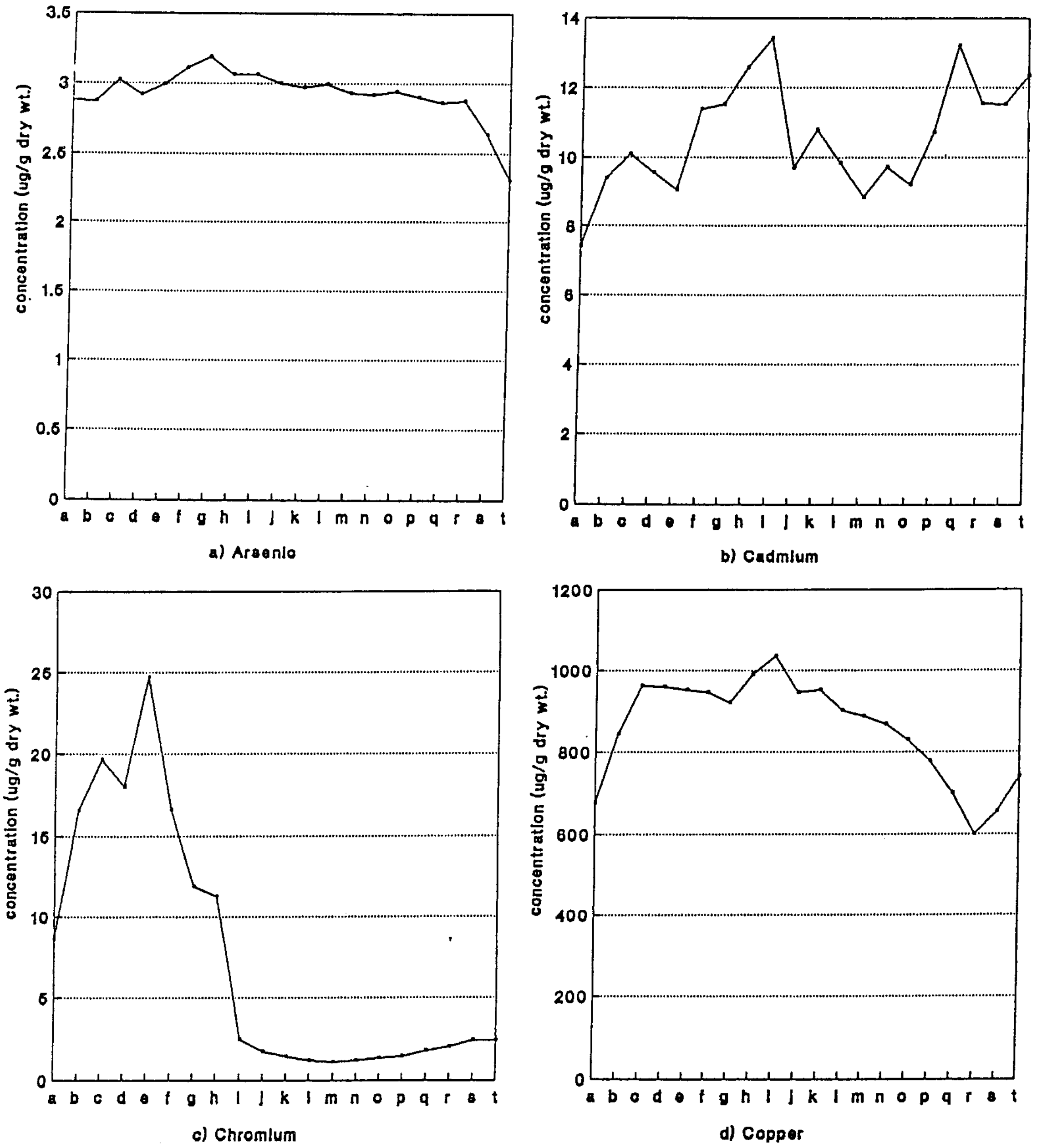


Figure 4. Metal contents, expressed as milligrams per kilogram, of the Pacific oyster, *Crassostrea gigas*, purchased in different months at Lau Fau Shan market.

mium was erratic. The comparatively high levels in 1988 and early 1989 may be due to the high level of contamination in oyster production sites, and the rapid decrease in 1989 and early 1990 may be due to the banning of some polluting industries, such as leather and textiles.

Conclusion

Highest levels of cadmium, chromium, and copper were found in gill tissues of the oyster, *C. gigas*. The reason for this may be the high surface area of gills and the presence of mucus on gills which adsorbed



Data pt. a - represents the average metal content from 5/88 to 8/88

Data pt. b - represents the average metal content from 6/88 to 9/88 and so on

Figure 5. Metal contents of the Pacific oyster, *Crassostrea gigas*, purchased in different months at Lau Fau Shan market after treating with the moving average method.

metals. Arsenic levels were highest in the adductor muscle. Arsenic usually accumulates in tissues with high metabolic rates, so the high metabolic rate in adductor muscles may be the reason for high arsenic accumulation.

Smaller-sized oysters have a higher metal concentration than larger oysters. The higher metabolism of smaller oysters may result in rapid turnover of cellular material and enhanced incorporation of metal in tissues. The higher surface-to-volume ratio in smaller-sized oysters also facilitates absorption of metals from surroundings.

A seasonal trend was only revealed in the cadmium level of oysters, with the highest level obtained in the autumn and winter months. This may be due to the sexual cycle of the oyster and the high water salinity during winter months.

Arsenic, cadmium, and copper levels in the oyster were elevated in the recent years and the cadmium level was approaching the safety limits of potential toxicity to humans.

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