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MACRO AND MICRO ELEMENTS IN SHRIMP *PENAEUS MONODON* COLLECTED FROM CULTURED POND OF MALAYSIA

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ABSTRACT

The concentrations of the macro elements Ca, Na, Mg and K and the microelements V, Cr, Co, Cu, Zn, As, Pb, Ti, Mn, Al, Fe, Se, Ni and Cd were determined in the whole body of black tiger shrimp *Penaeus monodon* collected from the shrimp culture ponds at Malacca, Malayasia. The mean concentrations (μg/g dry weight) of these were as follows: Ca: 19,137.3 ± 188.8; Na: 8,514.6 ± 99.6; Mg: 2,890.9 ± 76.7; K: 7,768.2 ± 82.9; V: < 0.01; Cr: 0.11 ± 0.01; Co: 0.01 ± 0.00; Cu: 0.63 ± 0.07; Zn: 0.53 ± 0.06; As: 0.05 ± 0.00; Pb: 0.02 ± 0.01; Ti: 0.38 ± 0.01; Mn: 0.38 ± 0.08; Al: 270.6 ± 5.91; Fe: 531.2 ± 30.8; Se: 0.02 ± 0.00; Ni: 0.02 ± 0.00 and Cd: < 0.01 μg/g. The concentrations of heavy metals viz. Cu, Zn, Pb, Ni and Cd were far below compared to other reported values elsewhere, whereas, Fe was comparable. The results suggest that the levels of As, Cd, Cu, Pb and Zn in shrimp body were below the maximum permissible limits set by the Malaysian Food Regulations 1985 and these indicated that the metal input from the surrounding marine coastal environment was not contaminated with these metals and their impact were therefore, negligible.

Keywords: *Elements; Heavy Metals; Penaeus monodon; Shrimp Farm; Malaysia*

INTRODUCTION

The shrimp pond established near the marine coastal area which is always influenced by ecotoxicological interest. This is due to two interesting possibilities of ecological impacts. Firstly, the changing water of the ponds withdrawn from the coastal waters that is already been contaminated by the riverine input and nearby anthropogenic inputs, and therefore could contaminate the growing shrimps in the ponds. Secondly, the ingredients and chemicals used in the shrimp ponds.

In the coastal waters, there is macro and microelements that can be transferred into any aquatic ecosystem via food chain trophic transfer concept. The chemicals found in the aquatic environment can be contributed by natural origins such as atmospheric inputs due to forest fires and other anthropogenic sources such as dumping and agricultural wastes. Besides, macroelements such as Ca, Na, Mg and K, some of the microelements are toxic nonessential elements such as Cd and Pb and some are very toxic even at trace levels such as Cu and Zn (Balkas *et al*., 1982).

In the coastal waters of Peninsular Malaysia, the effluents from industries and mining activities to rivers are the main sources of contamination (Ismail *et al*., 1995; Yap, 2003). Usually, this source of water is used for the culture of marine aquaculture species like fish and shrimp in captive condition in ponds near the coastal area. Besides, the aquaculturists deliberately manipulate the natural carrying capacity by increasing the stocking density, adding biological and chemical products such as artificial diet, antibiotics, algaecides, pesticides and probiotics into this complex aquatic ecosystem for higher shrimp production. Nevertheless, the high protein diets fed resulting in high levels of toxic inorganic compounds in the principle excreting products, which may cause water quality deterioration of culture

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pond. In order to evaluate whether these contaminants are a threat to aquatic organisms and to fish or shellfish as food, monitoring program were carried out within the framework of the Oslo and Paris Conventions (Guns *et al*., 1999). In addition, determination of trace metals in commercial shrimps started after 1990s in the coastal water of Malaysia (Awaluddin *et al*., 1992; Ismail *et al*., 1995).

Studies on accumulation of metals in shrimps were widely reported in the literature (Vazquez *et al*., 2001; Khan *et al*., 1989; Marcovecchio, 1994; Ruelas-Inzunza and Páez-Osuna, 2004; Jeckel *et al*., 1996; Karg *et al*., 2001; Mantelatto *et al*., 1999; Carbonell *et al*., 1998; Páez-Osuna and Tron-Mayen, 1995; Pourang *et al*., 2004; Visuthismajarn *et al*., 2005; Marx and Brunner, 1998). Studies on some metal concentrations have been carried out on the commercial shrimps *Penaeus vannamei*, *Metapenaeus ensis*, *Penaeus monodon* and *Crangon crangon* by earlier researchers in open waters (Paez-Osuna and Tron-Mayen, 1996; Che and Cheung, 1998; Guhathakurta and Kaviraj, 2000; Culshaw *et al*., 2002). However, the studies on the macro and microelement concentrations in farmed shrimp are scarce especially in the tropical climate. Therefore, the objective of the present study is to determine the concentrations of macro and microelements in the body of the commercially important black tiger shrimp *Penaeus monodon* collected from shrimp culture ponds throughout the culture period from Malacca, Peninsular Malaysia.

MATERIALS AND METHODS

The study ponds were situated at Kampung Tedong (2º 08´ 50" N and 102º 24´ 00" E) in Merlimau, Malacca of the west coast of Peninsular Malaysia. The ponds were managed by Farmers' Organization Authority Malaysia (Lembaga Pertubuhan Peladang). Two ponds were randomly selected for this study with the average area of 4,162 m^2. The soil type of the ponds was silty clay. Samplings were done between February 2002 and June 2002 during the culture period. Samples were collected between 10.00 am and 12.00 pm. All the culture ponds were dried by draining the water prior to start the shrimp culture. Tea seed cake (TSC) was used at 1.15-1.26 t/ha in the each culture pond to eliminate the predators and pests. At the beginning, the ponds were filled with about 20–30 cm seawater (filtered through 400–500 μm mesh net) from the reservoir and kept for 1 week, which allowed phytoplankton to grow. Water depth was then increased up to 1 m before the stocking. Stocking density was 26 PL_{15}/m^2 in each pond. A set of four paddlewheels was used during the whole culture period (12 h/d) in each pond. During the culture period, 50% of water was exchanged three times during the culture period. The water exchange was based on the water quality. The water was discharged through a canal to the adjacent water body and refilled by using pump from another reservoir. A commercial 35-40% protein of shrimp feed (Gold Coin, Singapore) was given at 10% body weight/day for the first month and 4–6% for the rest of culture period.

Five shrimps were collected in every three-week interval and brought to the laboratory of Department of Biology, Universiti Putra Malaysia for analysis. In laboratory, the fresh shrimp samples were dried for 24 hrs at 100°C in an oven to a constant weight. Afterwards, the dry shrimps were powered using mortar and sieved through 0.5 mm stainless steel sieve and kept in desiccators for further analysis. For this purpose, 0.5 g of dried shrimp sample was digested with 69% of HNO_3 (Allen, 1974). Then the samples were heated using the digestion block at 120°C for 1 hr and the temperature was increased up to 170°C for 3 hrs. The diluted samples were left to cool before adding double deionized water (DDW) to make the volume up to 50 ml. Samples were filtered through Whatman No. 1 filter paper and the filtrate was kept at 4 °C for further analysis. The element contents in shrimp body were detected (μg/g) using the ELAN 6000 ICPS-MS, Perkin Elmer at Malaysia Institute of Nuclear Testing (MINT), Bangi, Selangor, Malaysia. The recoveries of the elements were between 70-130%. Statistical Package for Social Science (SPSS) version-10 was used to analysis the data. One-way ANOVA Duncan Multiple Range Tests (DMRT) was used to compare the variation of element concentrations of shrimp within different sampling dates in the culture ponds.

RESULTS AND DISCUSSIONS

The elemental concentrations were not significantly different (*t*-test, p>0.05) between the two ponds. The concentrations of macro elements (Ca, Na, Mg and K) increased with increases of shrimp age (Table 1). Of the four macro elements measured, calcium showed the highest concentration in the shrimp body. Among the heavy metals detected, concentrations of Fe were the highest followed by Al. When compared to Cu and Zn concentrations, the level of Al in shrimp body was found higher with the value of 270.6 ± 5.91 μg/g, 0.63 ± 0.07 μg/g and 0.53 ± 0.06 μg/g for Al, Cu and Zn, respectively. Other microelements such as V and Cd were below 0.01 μg/g in the shrimp body.

Table 1: Element concentrations (mean±standard error) of shrimp *P. monodon* collected from the shrimp ponds throughout the culture period

Element	Culture period					
(μg/g)	Week 1	Week 4	Week 7	Week 10	Week 13	Week16
Macro						
Ca	1610±40.0[a]	21350±18.80[b]	22600±59.46[b]	21800±51.83[b]	18550±62.36[b]	20150±8.40[b]
Na	3530.5±0.84[a]	7765±41.79[b]	9400±11.89[b]	14250±25.22[b]	6400±16.81[a]	7250±22.24[b]
Mg	2599.5±0.84[a]	3050±8.40[a]	3000±11.89[a]	3100±16.81[a]	2700±20.59[a]	2750±8.40[a]
K	500±10.0[a]	8400±23.78[b]	8550±34.67[b]	7000±40.00[b]	9250±22.24[b]	9500±26.59[b]
Micro						
Ti	0.41±0.01[a]	0.38±0.08 [a]	0.42±0.13[a]	0.40±0.12[a]	0.33±0.08[a]	0.33±0.13[a]
V	<0.01	<0.01	<0.01	<0.01	<0.01	<0.00
Cr	0.21±[a]	0.11±0.03[a]	0.14±0.01[a]	0.07±0.01[a]	0.09±0.0[a]	0.09±0.0[a]
Mn	1.11±0.09[a]	0.45±0.13[a]	0.40±0.01[a]	0.18±0.03[a]	0.28±0.09[a]	0.19±0.04[a]
Ni	<0.01	0.02±0.0	0.02±0.0	0.01±0.0	0.02±0.0	0.02±0.0
Co	0.03±0.00	0.01±0.0	<0.01	<0.01	<0.01	<0.01
Cu	0.12±0.05[a]	0.55±0.09[a]	0.56±0.23[a]	0.54±0.09[a]	0.77±0.12[a]	0.96±0.21[a]
Zn	1.17±0.05[a]	0.42±0.05[a]	0.43±0.14[a]	0.42±0.10[a]	0.49±0.10[a]	0.56±0.12[a]
As	0.07±0.02	<0.01	0.04±0.01	0.05±0.02	0.06±0.0	0.04±0.0
Al	639.45±2.78[b]	310±13.55[a]	340±14.56[a]	80±3.76[ac]	155.5±5.88[a]	99±5.44[a]
Fe	1680±21.10[b]	385±10.96[a]	405±10.96[a]	230±20.36[a]	255±7.03[a]	230±3.76[a]
Se	0.21±0.0	<0.01	<0.01	0.02±0.0	0.02±0.0	0.02±0.0
Cd	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pb	0.12±0.0	<0.01	<0.01	<0.01	0.03±0.0	<0.01

Means in a row with different letter of superscripts are significantly different (Duncan, p < 0.05)

Among the microelements, Al and Fe were accumulated between 200-600 μg/g dry weight in the tissues of shrimps when compared to other microelements in which the concentrations were below 1.00 μg/g dry weight. With the exception of Fe and Al, the concentrations of some metals measured from shrimps in culture ponds were lower and comparable with the ranges of values reported in the literature (Eisler, 1981; Ismail *et al.*, 1995; Che and Cheung, 1998; Table 2). Boyd and Thunjai (2003) reported that the imbalance of ion in pond water probably not responsible for any problems on shrimp stress and shrimp body. Shrimp and other crustaceans absorb calcium ion from water during molting (Fieber and Lutz, 1982) and a calcium hardness (Ca plus Mg) at least 50 μg/g. This value was required for satisfying calcium requirements for molting (Boyd and Tucker, 1998). Exception of macro elements and a few microelements such as Fe and Al, the concentration of other microelements in the shrimp body were low probably due to regulation of essential metals like Zn, Cu and Mn (Rainbow, 1995a, 1995b). Bryan *et al.* (1986) stated that Zn is one of the important essential metals, constituent of more than 90 enzymes and proteins, and regulating the activity of many other enzymes. Zinc regulation has also been demonstrated in other decapod crustaceans (Rainbow, 1995a). Nevertheless, certain elements were bio-accumulated more than the other but, their accumulation strategy and function for certain metals such as V, Cr, As, Pb is not known in shrimp body or decapods.

Iron is an oligoelement and plays a vital role in the enzymatic and respiratory processes of crustaceans like shrimp. Tiger shrimp *Penaeus* sp. accumulates 50 μg/g of Fe in their body (Eisler, 1981). The concentration of Fe in the shrimp body was the highest among all metals in the present study. However, the higher concentration of this metal in pond soil probably accelerated the accumulation process of Fe in shrimp body (Che and Cheung, 1998). Since the Fe is adsorbed strongly to sediments, the presence of different amount of Fe rich sediments in the guts of the species may account for this difference through bioaccumulation. This suggests that shrimps have the ability to be selective in accumulating and excreting unwanted elements through physiological processes including molting. It is also indicated that high Fe level in shrimp body was due to bioavailability, which adsorbed strongly into the culture pond sediments.

Table 2: Comparison of element concentrations (mean ± standard error) in shrimp (*Penaeus* sp.) body from regional studies with the present results

Element (μg/g)	This study	Ismail *et al.* (1995)*	Paez-Osuna and Tron-Mayen (1996)	Guhathakurta and Kaviraj (2000)
Ca	19137.27±188.79	NA	NA	NA
Na	8514.55±99.56	NA	NA	NA
Mg	2890.90±76.73	NA	NA	NA
K	7768.18±82.85	NA	NA	NA
V	<0.01	NA	NA	NA
Cr	0.11±0.01	NA	NA	NA
Co	0.01±0.00	NA	NA	NA
Cu	0.63±0.07	0.8-24	543.9-601.1	NA
Zn	0.53±0.06	5-16	321-375	7.3-4809
As	0.05±0.00	NA	NA	NA
Ti	0.38±0.01	NA	NA	NA
Mn	0.38±0.08	NA	97.6-100	NA
Al	270.57±5.91	NA	NA	NA
Fe	531.18±30.82	NA	769-1407	5.0-495.0
Se	0.02±0.00	NA	NA	NA
Ni	0.02±0.00	NA	3.8	NA
Cd	<0.01	0.09-0.8	<0.30	0.11-3.22
Pb	0.02±0.01	0.06-5.9	NA	22.9-42.1

NA = Not available; * = wet weight

According to Jeckel *et al.* (1996), trace-metal distribution in tissues of the shrimp *Pleoticus muelleri* from the Patagonian region, Argentina, was related to sex, size and physiological condition. Concentrations of cadmium, copper, manganese and zinc were determined in the digestive gland, male reproductive system and muscle of adult specimens. Pourang *et al.* (2004) reviewed distribution and redistribution of trace elements in various tissues of different shrimp species, especially genus *Penaeus*. The possible roles of metallothionein in this regard are emphasized. Factors affecting heavy metals uptake and distribution have also been reviewed separately. Moreover, patterns of metal bioaccumulation and their order of occurrence have been evaluated. Another part of this paper deals with comparison of the related data from different aquatic environments as well as existing guidelines and limits for human consumption. The research finding of Visuthismajarn *et al.* (2005) indicated a need for further ecological risk assessment at a more detailed level to focus on the bioavailability and effects of metals from abandoned shrimp farms, with manganese the highest priority. Jones *et al.*

(2001) studied the biological impact of shrimp farm effluent, and to compare and distinguish its impacts from treated sewage effluent.

According to Marx and Brunner (1998), Cd, Pb and Hg contents of common shrimp (*Crangon crangon* L.) from the German mud flats in the North Sea were low: 0.043-0.026 mg; Cd, 0.019-0.011 mg Pb and 0.033-0.016 mg Hg (mean-SD) per kg wet weight. All of the samples contained lower amounts of the investigated elements than the levels of concern of the Federal Institute for Health Protection of Consumers and Veterinary Medicine, i.e. 0.5 mg Pb/kg, 0.1 mg Cd/kg and 0.5 mg Hg/kg wet weight; most of the samples showed significantly lower levels of contamination. Therefore, no risk to the consumer arises from the Cd, Pb and Hg contents of the shrimp caught in this area. The main reasons for the overall low levels of contamination might be the short period of feeding of the shrimp, and with respect to Pb, an active mechanism of secretion.

CONCLUSIONS

Since the shrimps are highly consumed by humans locally and are considered as a seafood delicacy in Malaysia, the monitoring of toxic metals especially should be conducted regularly. Based on the present results, expansion of shrimp aquaculture farm at the coastal area of the west Peninsular Malaysia is recommended because all the toxic metals of ecotoxicological concerns such as As, Cd, Cu, Pb and Zn were low when compared to reported data and permissible limits set by Malaysian Food Regulations 1985. The data obtained from this study could be used for the safety level of farmed shrimp in the tropical eco-region.

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