# Heavy Metal Concentrations in Water, Sediment and Shrimp From Grow-out Farms in Johore

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*Abstract:* A study was carried out to identify the heavy metals contamination in the aquatic environment and its effect on concentrations in cultured shrimp *Penaeus monodon.* Six sampling locations in Johore were chosen i.e. Minyak Beku, Parit Balak, Sungai Danga, Pasir Putih, Tanjung Buai and Tenglu Laut. Sampling was carried out monthly for a period of four months. Heavy metal concentrations in water (estuaries and ponds), sediment and shrimp were determined using atomic absorption spectrophotometer. The concentrations of Cd, Cu, Pb and Zn in water from the estuaries ranged from 0.02-0.05, 0.02-0.09, 0.04-0.15 and 0.06-0.38 µg/ml respectively whilst in ponds ranged from 0.02-0.06, 0.03-0.10, 0.06-0.17 and 0.13-0.36 µg/ml respectively. These concentrations were within the optimal range recommended for shrimp culture. All the heavy metal concentrations showed significant differences between locations. The concentration ranges of Cd, Cu, Pb and Zn in sediment samples were 0.28-1.14, 0.68-3.53, 12.37-21.47 and 13.11-24.32 µg/g dry weights respectively. The heavy metal concentrations were generally within the ranges reported by the previous researchers for shrimp pond sediment. Significant differences among locations were found only for Pb and Zn. Concentrations of Cd, Cu, Pb and Zn in shrimp ranged from 0.02-1.21, 8.21-49.45, 0.09-2.91 and 21.43-91.41 µg/g dry weight respectively. The results showed that shrimp cultured in ponds accumulated all the metals studied. In general, the level of heavy metal concentrations in shrimp did not exceed the maximum permissible limit. Except for Pb, analysis of variance showed that concentrations of Cd, Cu and Zn in shrimp varied significantly among locations. Correlation analysis showed significant relationships between Cd concentrations in shrimp and in pond water. The results showed that only Cd concentrations in the pond water directly influenced its concentrations in shrimp.

**Keywords**: heavy metals, water, sediment, shrimp, farms

*Abstrak*: Kajian dijalankan bagi mengenalpasti pencemaran logam berat dalam persekitaran akuatik dan kesannya ke atas kandungan logam-logam tersebut dalam udang harimau *Penaeus monodon* yang diternak. Sebanyak enam lokasi di negeri Johor iaitu Minyak Beku, Parit Balak, Sungai Danga, Pasir Putih, Tanjung Buai dan Tenglu Laut telah dipilih bagi kajian ini. Pensampelan dijalankan tiap-tiap bulan selama empat bulan. Kepekatan Cd, Cu, Pb dan Zn dalam air (muara dan kolam), sedimen, udang dan makanan udang ditentukan menggunakan spektrofotometer serapan atom. Kepekatan Cd, Cu, Pb dan Zn dalam air sungai (muara) adalah masing-masing dalam julat 0.02-0.05, 0.02-0.09, 0.04-0.15 dan 0.06-0.38 mg/L manakala bagi air kolam pula, adalah masing-masing dalam julat 0.02-0.06, 0.03-0.10, 0.06-0.17 dan 0.13-0.36 mg/L. Kepekatan logam-logam tersebut adalah dalam julat optima yang disyorkan bagi ternakan udang. Kesemua kepekatan logam dalam air sungai dan air kolam menunjukkan perbezaan yang signifikan antara lokasi. Julat kepekatan Cd, Cu, Pb dan Zn dalam sedimen adalah masing-masing 0.28-1.14, 0.68-3.53, 12.37-21.47 dan 13.11-24.32 µg/g berat kering. Kepekatan logam-logam berat tersebut adalah dalam linkungan kepekatan yang dilaporkan oleh penyelidik-penyelidik terdahulu bagi sedimen kolam ternakan udang. Hanya Pb dan Zn dalam sedimen sahaja menunjukkan perbezaan yang signifikan antara lokasi. Kepekatan Cd, Cu, Pb dan Zn dalam udang adalah masing-masing dalam julat 0.02-1.21, 8.21-49.45, 0.09-2.91 dan 21.43-91.41 µg/g berat kering. Keputusan menunjukkan udang yang diternak berupaya mengakumulasikan semua logam-logam yang dikaji. Secara umumnya, kepekatan logam berat dalam udang adalah masih dibawah paras maksima yang dibenarkan. Kecuali Pb, analisis varian menunjukkan terdapat perbezaan yang signifikan bagi kepekatan Cd, Cu dan Zn dalam udang antara lokasi. Bagi makanan udang pula, julat kepekatan adalah masing-masing 1.13-2.34, 12.96-20.06, 0.84-5.42, 13.04-46.30 µg/g berat kering. Ini menunjukkan kandungan Cu dan Zn dalam makanan udang adalah tinggi. Ujian korelasi menunjukkan terdapat hubungkait yang signifikan antara kepekatan Cd dalam udang dengan kepekatan Cd dalam air kolam. Keputusan kajian ini menunjukkan bahawa hanya kepekatan Cd dalam air kolam mempengaruhi secara langsung kepekatan logam tersebut dalam udang.

## Introduction

Concern has been raised over the past few decades regarding the increased input of heavy metals to aquatic environments that has resulted from anthropogenic activities. The impact of the anthropogenic perturbation is most strongly felt by estuarine and coastal environments adjacent to urban areas. In aquatic ecosystem, heavy metals received attention due to their toxicity and accumulation in biota. Some marine organisms concentrate certain metals above the level found in the surrounding environment.

Crustaceans have high sensitivity to metals, including those with a high commercial value such as shrimp, prawns, lobster or crabs (Bambang *et al.*, 1995). Crustacean, under some circumstances, are potential source of toxic metals and several countries have developed maximum acceptable concentration for commercialization and consumption of these organisms.

To date, comparatively few studies of trace metals in cultured tiger shrimp and shrimp culture areas in Malaysia have been published (Awaluddin *et al.*, 1992; Patimah and Dainal, 1993; Hashmi *et al.*, 2002). Since tiger shrimp (*Penaeus monodon*) is a commercially important cultured species, consideration should be taken in terms of metals pollution. This paper presents the concentrations of Cadmium (Cd), Copper (Cu), Lead (Pb) and Zinc (Zn) in water, sediment and tissues of tiger shrimps from some brackish water ponds in Johore. Comparisons were made in terms of concentrations of metal pollutants in shrimp tissues with those allowed by Malaysia Food Regulation (1985).The relationship between metals concentrations in shrimp and in pond water and sediment were also evaluated.

## Materials and Methods

*Study sites*

Samples for heavy metals analyses were obtained from brackish water ponds at six different locations in Johore (Fig. 1).

*Sampling and Analyses*

Random samples of water, sediment and shrimps (*Penaeus monodon*) were collected from two ponds of each locality and were pooled together to make duplicate set of each sample for each locality. Sampling was carried out monthly for a period of four months. All samples were stored on ice in an insulated container and transported to laboratory.

Water samples from estuaries and ponds were collected in acid-washed polyethylene bottles, filtered using 0.45 μm membrane filter and acidified with concentrated nitric acid (1 ml $HNO_3$/L). Water samples were preserved at 4°C until digestion. Before digestion, water samples were brought to room temperature and a well-mixed 50 ml aliquot was digested with 5 ml concentrated nitric acid to the minimum volume possible (approximately 10 ml). Subsequently it was cooled, filtered, diluted with deionized distilled water and was stored in an acid washed polyethylene bottles (APHA, 1995). Concentrations of metals (Cd, Cu, Pb and Zn) in the filtered digested samples were determined by flame atomic absorption spectrophotometer (GBC Avanta System 3000). Blanks were prepared from deionized water. The performance of the method was evaluated by recovery tests outlined by Nafde *et al.* (1998). Results of the recovery tests were within acceptable ranged. All metal concentrations are expressed as μg/ml.

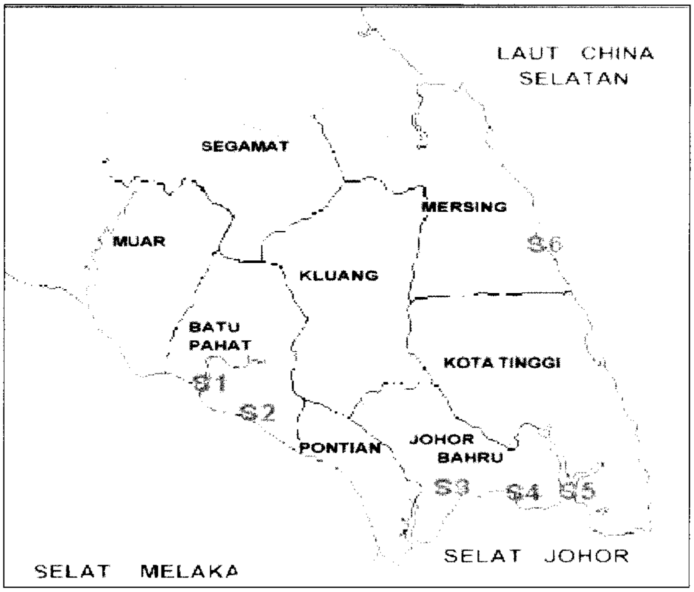


**Figure 1**: Sampling stations in Johore, Malaysia

Sediment samples (upper 5 cm) were collected randomly from a pond using corers made up of PVC tubes approximately 6 cm in diameter and 2.5 m in length. The samples collected from ponds were kept in clean acid-soaked polyethylene packets. In the laboratory, the sediment samples were dried to 105°C to constant weight, ground and sieve through a 250 µm stainless steel sieve. The samples were stored in clean acid-soaked polyethylene packets at 20°C. Before digestion, sediment samples were brought to room temperature. About 1 g samples and blank were digested in a digestion tube with 10 ml aqua-regia solution (3:1 mixture of concentrated HCl and concentrated $HNO_3$) following FAO (1975). After digestion, the cooled acid digest were filtered and diluted to 50 ml with deionized water. Concentrations of heavy metals were determined by flame atomic absorption. The performance of the method was evaluated by analyzing a standard reference material SRM 1646 (Estuarine Sediment Standard Reference Material) supplied by National Bureau of Standards, USA. Results of the standard reference material analyses were within 10% of the certified values. All metal concentrations are expressed as µg/g dry weight.

Samples of shrimp collected from ponds were kept in clean acid soaked polyethylene packets and stored on ice in an insulated container and transported to laboratory, where they were thoroughly rinsed with deionized water and stored at 20°C until further analysis. Preserved samples were brought to room temperature, thawed and were washed with deionized water. The exoskeletons of shrimps were removed and the shrimp muscles were dried at 105°C to constant weight. Pulverization and homogenization were achieved by grinding the tissues samples in a teflon mortar. Samples and blanks were prepared by digesting 2 g of dry material with 10 ml 65% nitric acid and left overnight to reduce excessive foaming. The samples were then heated at 90°C in a water bath for 3 hours. After digestion, samples were filtered and diluted to 25 ml with double distilled water (Duquesne and Riddle, 2001). Concentrations of metals in the filtered digested samples were determined by flame atomic absorption spectrophotometer. The performance of the method was evaluated by analyzing standard reference material TORT-2 (Lobster Hepatopancrease Reference Material) supplied by National Research Council, Canada. Results of the standard reference material analyses were within 10% of the certified values. All metal concentrations are expressed as µg/g dry weight.

Analysis of variance (ANOVA) was employed to check significant differences between sampling locations. The relationships between heavy metal concentration in shrimp and in water and sediment were determined by simple linear regression analyses. All statistical analyses were performed using SPSS software. Statistically significant differences were expressed as $p < 0.05$.

## Results and Discussion

*Heavy metals in water (estuaries and pond)*

Concentrations of heavy metals in water samples collected from the estuaries and ponds are presented in Table 1. The concentrations of Cd, Cu, Pb and Zn in water from the estuaries ranged from 0.02-0.05, 0.02-0.09, 0.04-0.15 and 0.06-0.38 µg/ml respectively whilst in ponds ranged from 0.02-0.06, 0.03-0.10, 0.06-0.17 and 0.13-0.36 µg/ml respectively. All heavy metals concentrations in both estuaries and ponds showed significant differences ($p<0.05$) among locations (Table 1). The concentrations of Cd, Cu and Zn in estuaries and ponds were higher at Pasir Putih compared to the other areas. Pasir Putih is situated within the vicinity of housing, industrial and port areas. Heavy contamination in these areas may contribute to a high level of metal concentrations in water at this station. The highest concentrations of Pb in estuaries and ponds were found at Tanjung Buai. Tanjung Buai is situated well within the bay near the mouth of Johore river.

**Table 1:** Concentrations of heavy metals in water samples from estuaries and pond

| Location | Concentration of heavy metals (µg/ml) | | | |
|---|---|---|---|---|
| | **Cd** | **Cu** | **Pb** | **Zn** |
| Estuary | | | | |
| S1 | $0.02 \pm 0.01^{a}$ | $0.02 \pm 0.01^{a}$ | $0.10 \pm 0.03^{a}$ | $0.11 \pm 0.03^{ab}$ |
| S2 | $0.03 \pm 0.01^{ab}$ | $0.04 \pm 0.01^{a}$ | $0.10 \pm 0.04^{ab}$ | $0.11 \pm 0.02^{ab}$ |
| S3 | $0.02 \pm 0.01^{a}$ | $0.04 \pm 0.01^{a}$ | $0.10 \pm 0.04^{ab}$ | $0.06 \pm 0.03^{a}$ |
| S4 | $0.05 \pm 0.01^{b}$ | $0.09 \pm 0.03^{b}$ | $0.11 \pm 0.04^{ab}$ | $0.38 \pm 0.09^{c}$ |
| S5 | $0.02 \pm 0.01^{a}$ | $0.03 \pm 0.01^{a}$ | $0.15 \pm 0.02^{a}$ | $0.21 \pm 0.04^{b}$ |
| S6 | $0.02 \pm 0.01^{a}$ | $0.05 \pm 0.01^{a}$ | $0.04 \pm 0.03^{b}$ | $0.16 \pm 0.03^{ab}$ |
| Pond water | | | | |
| S1 | $0.03 \pm 0.01^{a}$ | $0.03 \pm 0.00^{a}$ | $0.16 \pm 0.03^{ab}$ | $0.14 \pm 0.07^{ab}$ |
| S2 | $0.04 \pm 0.01^{a}$ | $0.04 \pm 0.01^{a}$ | $0.12 \pm 0.06^{abc}$ | $0.14 \pm 0.08^{ab}$ |
| S3 | $0.03 \pm 0.03^{a}$ | $0.07 \pm 0.02^{ab}$ | $0.16 \pm 0.04^{ab}$ | $0.13 \pm 0.04^{a}$ |
| S4 | $0.06 \pm 0.01^{b}$ | $0.10 \pm 0.05^{b}$ | $0.15 \pm 0.05^{ab}$ | $0.36 \pm 0.29^{b}$ |
| S5 | $0.03 \pm 0.01^{a}$ | $0.04 \pm 0.02^{a}$ | $0.17 \pm 0.05^{ab}$ | $0.30 \pm 0.20^{ab}$ |
| S6 | $0.02 \pm 0.01^{a}$ | $0.07 \pm 0.02^{ab}$ | $0.06 \pm 0.05^{c}$ | $0.17 \pm 0.03^{ab}$ |
| Optimum level for shrimp culture (Chien, 1992) | <0.15 | <0.10 | NA* | <0.25 |

Stations with different superscript are significantly different ($p<0.05$)
* NA - not available

The high level of Pb at this station is probably due to anthropogenic inputs including domestic, industrial and agriculture discharge from either the tidal or the inland fresh water. Previous studies also showed that these areas were contaminated by certain heavy metals (Ahmad and Ismail, 1992; Yusof and Wood, 1993). However, heavy metal concentrations in estuaries in this study areas were lower than those reported for estuaries from other areas (Ahmad and Ismail, 1992; Mokhtar *et al.*, 1994; Mazo-Gray *et al.*, 1997). All the heavy metals concentrations in pond were within the optimal range recommended for shrimp culture except Zn at Pasir Putih and Tanjung Buai (Table 1). In general, the concentrations were within the range reported by other researchers (Awaluddin *et al.*, 1992; Guhathakurta and Kaviraj, 2000; Hashmi *et al.*, 2002).

Correlation analyses showed significant relationship ($p<0.05$) for all metals studied between estuaries and water in shrimp pond. It was found that heavy metals concentrations in estuaries directly influenced these metals concentrations in the ponds.

*Heavy metals in sediment*

Concentrations of heavy metals in sediment samples collected from shrimp ponds are presented in Table 2. The concentrations of Cd, Cu, Pb and Zn in sediment ranged from 0.28-1.14, 0.68-3.53, 12.37-21.47 and 13.11-24.32 µg/g respectively.

**Table 2**: Concentration (mean ± S.D) of heavy metals in sediment (µg/g dry weight) from different sampling stations

| Location | Concentration of heavy metals (µg/g) | | | |
|---|---|---|---|---|
| | **Cd** | **Cu** | **Pb** | **Zn** |
| S1 | 0.46 ± 0.27 | 3.15 ± 1.62 | 14.94 ± 1.30$^{a}$ | 19.86 ± 5.92$^{ab}$ |
| S2 | 0.82 ± 0.45 | 2.98 ± 1.99 | 15.12 ± 3.11$^{a}$ | 21.79 ± 8.05$^{a}$ |
| S3 | 0.28 ± 0.14 | 3.54 ± 2.44 | 21.47 ± 2.18$^{b}$ | 21.66 ± 6.38$^{ab}$ |
| S4 | 1.14 ± 1.23 | 2.35 ± 1.41 | 19.20 ± 3.05$^{b}$ | 24.32 ± 6.51$^{a}$ |
| S5 | 0.57 ± 0.65 | 2.82 ± 2.67 | 12.72 ± 2.45$^{a}$ | 21.03 ± 3.64$^{ab}$ |
| S6 | 0.28 ± 0.15 | 0.68 ± 0.63 | 12.37 ± 2.11$^{a}$ | 13.11 ± 1.07$^{b}$ |

Stations with different superscript are significantly different ($p<0.05$)

Analysis of variance (one way) showed a significant difference ($p<0.01$) among locations for Pb and Zn concentrations in sediment. However, no significant differences ($p>0.05$) were found for Cd and Cu concentrations (Table 2). The highest concentrations of Pb and Zn were found at Sungai Danga and Pasir Putih respectively. Several factors may contribute to this. Since sediment acts as integrators and amplifiers of metals concentrations, discharges from various industries and domestic wastes over the years may cause the elevated levels observed in sediment from these areas. As mentioned earlier, Pasir Putih is situated within the vicinity of housing, industrial and port areas and Sungai Danga is situated nearby the highly populated areas.

In addition to direct disposal of solid and liquid wastes, river runoff and ship-related inputs contributed to heavy metal contamination, particularly Pb and Zn (Tam and Wong, 2000). Previous studies by Yusof and Wood (1993) also showed elevated levels of Pb and Zn in these areas. However, an important reason for the high metals concentrations in pond sediment could be the enclosed nature of the ponds together with irregular access to tidal flushing. Overall, concentrations of Cd, Cu, Pb and Zn from the study areas were comparable to the concentrations reported in pond sediments from other part of the world (Awaluddin *et al.*, 1992; Ong-Che and Cheung, 1998; Carbonell *et al.*, 1998; Guhathakurta and Kaviraj, 2000).

With the exception of Pb, analysis of variance showed that metals in shrimp muscle varied significantly among localities (Table 3). Analysis of variance showed that there was a significant difference ($p<0.01$) in Cd concentrations among locations. The highest concentration of Cd in shrimp was at Pasir Putih (0.80 µg/g). For Pb, no significant differences ($p>0.05$) in concentrations were found among locations. The highest Pb concentration in shrimp was found at Tanjung Buai (1.57 µg/g). However, the highest values of these metals were well below the maximum permissible levels (Malaysian Food Regulation, 1985). The maximum permissible level for Cd and Pb are 4.5 and 9.0 µg/g dry weight respectively (assuming a wet:dry weight ratio of 4.5, Darmono and Denton, 1990).

**Table 3**: Concentrations (mean ± S.D.) of heavy metals in shrimp (µg/g dry weight) from different sampling stations

| Location | Concentration of heavy metals (µg/g) | | | |
|---|---|---|---|---|
| | **Cd** | **Cu** | **Pb** | **Zn** |
| S1 | $0.14 \pm 0.14^{a}$ | $26.59 \pm 5.29^{a}$ | $1.19 \pm 0.38$ | $60.11 \pm 8.54^{a}$ |
| S2 | $0.19 \pm 0.15^{a}$ | $21.55 \pm 1.22^{ab}$ | $1.15 \pm 0.24$ | $38.73 \pm 19.69^{ab}$ |
| S3 | $0.24 \pm 0.16^{a}$ | $14.16 \pm 3.47^{b}$ | $1.45 \pm 0.89$ | $32.29 \pm 6.00^{b}$ |
| S4 | $0.80 \pm 0.26^{b}$ | $18.09 \pm 1.33^{ab}$ | $1.24 \pm 0.34$ | $49.32 \pm 26.99^{ab}$ |
| S5 | $0.65 \pm 0.24^{b}$ | $21.18 \pm 9.08^{ab}$ | $1.57 \pm 0.80$ | $53.49 \pm 24.20^{ab}$ |
| S6 | $0.11 \pm 0.12^{a}$ | $15.75 \pm 7.03^{ab}$ | $0.93 \pm 0.25$ | $38.50 \pm 19.27^{ab}$ |

Stations with different superscript are significantly different ($p<0.05$)

The high levels of Cd and Pb at these locations may be due to high levels of these metals in the environment. The same pattern was detected in sediment and water in this study. Cd and Pd are non-essential metals that are found to have unknown metabolic role in the organisms. No crustacean appears to regulate the body concentration of non-essentials metals (Rainbow and White, 1989). A number of studies show that concentrations of Cd and Pb in aquatic organisms depend mainly on their environmental levels (Bryan and Langston, 1992).

Cu and Zn were the most abundance metals in shrimp tissues with mean concentrations (µg/g dry weight) of 19.55 and 45.41 respectively. However, these concentrations were lower than the maximum permissible levels set by Malaysian Food Regulations (1985). The maximum permissible levels for Cu and Zn are 30 µg/g and 100 µg/g respectively. In this study, copper was present at relatively high concentration in shrimp tissues. The high levels of this metal may be related with essential role of this metal in the production of the respiratory protein, haemocyanin (Bryan, 1968; Frenet and Alliot, 1985; Paez-Osuna and Ruiz-Fernandez, 1995). Zinc was also present in large quantities in shrimp tissues. This metal plays a role in the enzymatic and respiratory process (Bryan, 1968). The littoral prawn *Palaemon elegans* regulates the total body concentration at approximately 80 µg/g (dry weight) over a wide range of dissolved metals availabilities (White and Rainbow, 1982,1984). Zinc regulation has also been demonstrated in other decapod crustaceans including *P. serratus* (Devineau and Amiard Triquet, 1985), *Pandulus montagui* (Ray *et al.*, 1980), *Carcinus maenas* (Rainbow, 1985) and other species of crab (Bryan, 1968). In addition, the high concentrations of Cu and Zn may be related to bioavailability of these metals in the environment.

*Relationship Between Heavy Metals in Environment and Shrimp*

The relationships between metals concentrations in shrimp and in the environment (pond water and sediment) were evaluated. The results were summarized in Table 4. Significant positive correlations were found between Cd concentrations in shrimp and in pond water (r = 0.610, p<0.01). However, correlations between Cd concentrations in shrimp and in sediment and also correlations between Pb concentrations in shrimp and in sediment were lower than 0.5. Othman and Razak (2001) reported similar results for *Macrobrachium* spp. The results showed that only Cd concentrations in the pond water directly influenced their concentrations in shrimp. A number of studies show that the concentrations of Cd in the aquatic organisms depend mainly on their environment levels (Heath, 1987; Bryan and Langston, 1992). No correlation was found between the Cu and Zn concentrations in shrimp and in either pond water or sediment. Cu and Zn are apparently an essential metal for crustaceans. Essential metals such as Cu and Zn are regulated in crustaceans whereas non-essential metals such as Cd and Pb appear not to be regulated (Rainbow and White, 1989).

**Table 4**: Correlation coefficients (r) between metal concentrations in shrimp and in pond water and sediment

| Relationship | Cd | Cu | Pb | Zn |
|---|---|---|---|---|
| Shrimp-pond water | 0.610<br>(p = 0.001) | -0.236<br>(p = 0.133) | 0.244<br>(p = 0.126) | 0.012<br>(p = 0.477) |
| Shrimp-sediment | 0.430<br>(p = 0.018) | 0.330<br>(p = 0.058) | 0.354<br>(p = 0.045) | 0.328<br>(p = 0.059) |

## Conclusion

The present study shows that heavy metal concentrations in water, sediments and shrimp fall within the ranges for similar environment and biota collected elsewhere. The results showed that only Cd concentrations in the environment (water) directly influenced its concentrations in shrimp. In general, the levels of heavy metals in shrimp were within the permissible levels set by Malaysian Food Regulation (1985). The low metal levels, suggested that the degree of contamination in shrimp culture areas of Johore is still low. The data represents the current situation in these areas and may acts as background levels against which future improvement or decrements in water quality may be compared.

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