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Bioaccumulation Potential of Three Toxic Heavy Metals in Shrimp, *Penaeus monodon* from Different Fractions of the Culture Environment

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Abstract

Concentrations of three toxic heavy metals *viz*. lead, cadmium and chromium in the muscle tissue of shrimp harvested from selected shrimp farms in the North Western Province of Sri Lanka were determined along with the metal levels in the source water, sediments of the water sources, the farm sediments and formulated shrimp feed used by the farms by Atomic Absorption Spectrophotometry using standard analytical procedures. Bio-transfer factors of the three metals in the muscle tissue of the shrimp were enumerated in relation to source water, sediments of the water sources, farm sediments and to formulated shrimp feed. Levels of lead, chromium and cadmium in the muscle tissue of the shrimp (Mean±SEM) were (in $\mu g\ g^{-1}$ dry weight) 0.360±0.044, 0.610±0.044 and 0.199±0.012 (n=360) respectively. Bio-transfer factors of the three metals in shrimp in relation to water were greater than 1 indicating bioaccumulation of all the metals occurs through the water phase. Of the three metals studied, bio-transfer factors of cadmium from the sediments ranged from 9 to 21 indicating high potential of bioaccumulation via the sediments. However, bio-transfer factors of chromium and lead in shrimp tissue from the sediments were lower than 1. Metal wise comparisons indicated bio-transfer factors of cadmium in shrimp in relation to sediments were significantly higher than that of the other two metals. Bio-transfer factors of the three metals in shrimp from the formulated feed ranged from 0.1 to 0.9.

Introduction

Bioaccumulation is the uptake and sequestration of contaminants by organisms from their ambient environment. In certain cases, the uptake of contaminants by biota occurs directly from the ambient medium be this, air, soil or sediments or water (Phillips 1993). Bioaccumulation of chemicals from water is known as bioconcentration and it usually occurs by passive diffusion from the ambient water across the respiratory structures, into the circulatory fluid to be deposited in lipid tissue (Connell, 1998). In certain instances, contaminants are primarily accumulated through food chains or food webs and the process is known as biomagnification where concentrations increase with trophic level. Bioconcentration or bioaccumulation can be characterized by bio-transfer factors that can be determined in relation to any fraction of the ecosystem (Phillips 1993, Connell, 1998). For the majority of animal species, uptake of metals occurs from a combination of water and food (including sediments) in varying degrees, even though the aquatic plants derive metals almost exclusively from the aqueous phase (Langston and Spence, 1995).

Farming of black tiger shrimp, *Penaeus monodon* is currently a lucrative business in the North Western Province of Sri Lanka and it has become a foreign exchange spinner and an employment generator during the last two decades. However, with the rapid industrialization and urbanization, the aquatic environment upon which the aquaculture industry entirely depends is being contaminated with anthropogenic substances such as heavy metals. In addition, anthropogenic inputs such as formulated shrimp feed, which are introduced to the culture facilities, may contain higher levels of heavy metals. Consequently, the cultured shrimp may bio-accumulate toxic metals present in their environment via food chains and / or respiratory channels. Therefore, there is a tendency that cultured shrimp may contain undesirable residue levels in their body tissues. The aim of the present study was to determine the bioaccumulation potential of three toxic heavy metals; lead, cadmium and chromium in the muscle tissue of the shrimp, *P. monodon*, cultured in the North Western Province of Sri Lanka in relation to different fractions of the culture environment viz. water, sediments and formulated feed.

Materials and Methods

Sampling of water, sediments & shrimp feed

Samples of water and sediments from the four selected water sources (Puttalum lagoon, Mundal lagoon, Gembarandiya lagoon and Dutch canal) were taken from three locations adjacent to each farm studied. Sediments were removed manually using a polyethylene scoop and the samples were stored in plastic bags at 4 °C until transported to the laboratory. In addition, three sediment samples and three samples of shrimp containing ten individuals per sample were collected from each of the twelve selected farms in the North Western Province at the end of the culture cycle. Formulated shrimp feeds used by the farms to feed the shrimp during the particular culture cycle were also obtained for the chemical analysis.

From each sampling location, six water samples were taken into pre acid washed 2L plastic bottles and kept in polystyrene boxes with ice until they were transferred to the laboratory. Water samples were filtered through 0.45 μm membrane filter immediately after taken to the laboratory and the filtrate was acidified to $pH<2$ with concentrated nitric acid (analytical grade) for dissolved metal analysis (1.5 ml conc. HNO_3 /1L sample). Samples were kept in a refrigerator at 4°C until analysis. The sediment samples were kept in a freezer at –20°C in polyethylene bags until they were processed for chemical analysis. Shrimp were cleaned with de-ionized water immediately after taken to the laboratory and their total lengths and weights were recorded. The total lengths and the weights of the shrimp collected ranged between 12.97 – 18.19 cm and 15.49 - 38.49g respectively. The cleaned samples were kept in a freezer at –20°C in polyethylene bags until analysis.

Processing of samples before digestion

Prior to digestion, each sediment sample was defrosted and put into a pre acid cleaned evaporating dish and dried in an oven at 90°C for more than 48 hours until it attained a constant weight. The dried sediments were ground using a porcelain motor and pestle and sieved through a 125 μm mesh to remove the larger particles and fine sediment was obtained for the analysis.

The shrimp were taken out from the freezer and allowed for thawing. Then, they were de-headed and their shells were removed using a plastic knife to avoid metal contamination. The flesh of each shrimp sample was homogenized using an Ultra turax homoginizer and the homogenate was weighed using an electronic balance. Then, the homogenized flesh was kept in a pre acid washed evaporating dish and dried in an oven at 70°C for >48 hours to a constant weight. The dried sample was then ground into a fine powder using a porcelain motor and pestle and kept in a clean glass container in a refrigerator at 4°C. Feed samples were oven dried at 70°C for > 48 hours until they attained a constant weight. Then the feed were ground into a fine powder using a porcelain motor and pestle and kept in cleaned plastic containers under 4°C in a refrigerator until analysis.

Digestion of sediments

For the analysis of lead, cadmium and chromium, the sediments were digested according to the method described by Cook et al. (1997). Five gram portions of the dried sediment samples were weighed accurately and digested in long glass digestion tubes with 15 ml of concentrated hydrochloric acid over night and then with 5 ml of concentrated nitric acid using a heat block at 90°C for 2 hours until the acid mixture attained a light colour or a colourless solution. After cooling, the solution was filtered through a pre-acid washed Whatman 541 filter paper and the filtrate was brought to 100 ml with de-ionized water. Two reagent blanks were similarly processed and each sample was analyzed in duplicate.

Digestion of shrimp tissue and feed

For the determination of cadmium, chromium and lead, the shrimp tissue and feed were digested using a dry ashing procedure (Jorhem 1993). A quantity of 3 g of dried sample was accurately weighed into a 100 ml Pyrex beakers and kept in the muffle furnace at 200°C.

After 2 hours, the temperature was increased at the rate of 50°C/h up to 450°C and kept over- night at the same temperature. After cooling, the ashed samples were treated with 5 ml of 6 M Hydrochloric acid and evaporated to dryness on a hot plate. Two reagent blanks were similarly processed and each sample was analyzed in duplicate.

Analysis of lead, cadmium and chromium

Analysis of lead, cadmium and chromium in the water and sediments of water sources, farm sediments, shrimp tissues and feed were carried out within two weeks after digestion of the respective samples.

The acidified filtrates of water were directly aspirated in to the Air acetylene flame of the Atomic Absorption Spectrophotometer (AAS), (GBC Aventa Ver.1.33) in triplicates and the mean concentration values were obtained. The diluted samples of sediments were run through the flame AAS for lead and chromium and the Graphite furnace was employed to measure the cadmium levels in all the sediment samples. The dry-ashed shrimp tissue and feed (in 100 ml beakers) were re-dissolved in 5 ml of 0.1N nitric acid (analytical grade) just before aspirating into the air acetylene flame of the AAS for determinations of the levels of the three elements.

Reference standard solutions of lead, cadmium and chromium were prepared using Spectro Chemical Grade, BDH pure metal standard solutions of 1000 mg l^{-1} with de-ionized water. Calibration curves for absorption of the three metals were performed with the reference metal standards, at 217.0 nm wavelength for lead, at 357.9 nm wavelength for chromium and at 228.8 nm for cadmium. Subsequent to the calibration, samples were aspirated to get the absorption at the specific wavelengths and the concentrations of the three metals in the solutions were obtained.

Triplicates were used in each analysis. To measure the cadmium levels in digested sediment samples from the Graphite Furnace, a separate set of reference standards were prepared to get the calibration curve at the same wavelength. For the validation of the methods, percentage recoveries for all three elements in the water, sediments, shrimp and formulated feed were obtained by standard additions. In reporting the results, these matrix effects were taken into consideration.

Bio-transfer factors

Degree of bioaccumulation of each of the three metals in muscle tissue of the shrimp was determined in relation to the levels recorded from source waters, sediments of the water sources, sediments of the farms and formulated feed. When the farms had used more than one feed brand, the mean value of the metal in the respective brands was used for the calculations. Dry weight basis data on the metal contents were used for the calculation of bio-transfer factors. The transfer factors of the three metals in shrimp from the water source and the culture facility, which included water, sediments and formulated feed, were calculated as follows (Rashed 2001).

$$\text{Bio-transfer factor} = \frac{\text{Concentration of the metal in organism}}{\text{Concentration of the metal in water, sediments or feed}}$$

Statistical Analysis

Data are presented as mean ± standard error of mean (SEM) of the replicates. Differences among the three metal levels (in shrimp tissues, sediments, water samples & shrimp feed) and the differences in the bio-transfer factors among the three metals were determined by one-way analysis of variance (ANOVA). Log transformed data were employed for all the statistical analysis except for the data taken for the water samples. Where differences were significant, mean values were compared by Tukey's test and $P<0.05$ was considered as statistically significant. The data were statistically analyzed by the MINITAB (version 10.2) statistical software.

Results

The concentrations of the three metals *viz.* lead, chromium and cadmium in the different fractions of the culture environment (source water, sediments of the water sources, farm sediments, and formulated shrimp feed) and in the muscle tissue of the shrimp are presented in the Table 1.

Table 1. Levels of lead, chromium and cadmium in source water [mg l^{-1}], source water sediments, farm sediments, formulated feed and in the muscle tissue of the shrimp [mg kg^{-1}] belonging to four water sources (PL = Puttalam Lagoon, ML = Mundal Lagoon, GL = Gembarandiya Lagoon, DC = Dutch Canal).

Element	Lead	Chromium	Cadmium
Water			
PL	0.290± 0.030^{c}	0.130±0.020^{b}	0.070±0.004^{a}
ML	0.090± 0.027^{ab}	0.150±0.006^{b}	0.060±0.012^{a}
GL	0.120± 0.028^{b}	0.110±0.010^{b}	0.050±0.007^{a}
DC	0.100± 0.024^{ab}	0.150±0.007^{b}	0.050±0.009^{a}
Overall mean (n=24)	**0.149±0.027^{b}**	**0.134±0.007^{b}**	**0.059±0.006^{a}**
Source sediments			
PL	5.150±0.382^{b}	18.260±1.143^{c}	0.005±0.009^{a}
ML	8.756±0.598^{b}	31.986±1.621^{c}	0.011±0.003^{a}
GL	6.402±0.389^{b}	15.456±0.877^{c}	0.015±0.003^{a}
DC	5.398±0.434^{b}	20.983±3.222^{c}	0.013±0.050^{a}
Overall mean (n=36)	**6.426±1.835^{b}**	**21.671±0.423^{c}**	**0.010±0.001^{a}**
Farm sediments			
PL	4.720±0.180^{b}	19.850±1.190^{c}	0.024±0.002^{a}
ML	9.390±0.280^{b}	25.590±3.450^{c}	0.040±0.0034^{a}
GL	7.050±0.350^{b}	24.960±2.340^{c}	0.029±0.003^{a}
DC	12.370±1.120^{b}	41.390±8.240^{c}	0.018±0.003^{a}
Overall mean (n=72)	**8.380±0.140^{b}**	**27.950±0.400^{c}**	**0.028±0.001^{a}**
Formulated feed			
PL	0.347±0.002^{a}	1.413±0.006^{b}	1.647±0.083^{b}
ML	0.323±0.013^{a}	1.767±0.180^{b}	1.977±0.248^{b}
GL	0.420±0.019^{a}	1.263±0.044^{b}	2.153±0.199^{c}
DC	0.473±0.035^{a}	1.430±0.045^{b}	2.437±0.239^{c}
Overall mean (n=24)	**0.391±0.014^{a}**	**1.468±0.056^{b}**	**2.053±0.109^{c}**
Shrimp			
PL	0.160±0.020^{a}	0.440±0.036^{b}	0.181±0.028^{a}
ML	0.330±0.041^{ab}	0.015±0.001^{b}	0.205±0.010^{a}
GL	0.580±0.139^{b}	0.850±0.131^{c}	0.239±0.023^{a}
DC	0.370±0.032^{b}	0.670±0.044^{c}	0.171±0.023^{a}
Overall mean (n=360)	**0.360±0.044^{b}**	**0.610±0.044^{c}**	**0.199±0.012^{a}**

Results are presented as mean±standard error of the mean. For each row, means not followed by the same superscript are significantly different from each other (ANOVA, Tukey's test, $P<0.05$).

Results indicated that the overall levels of lead, chromium and cadmium in the source water used for culturing the shrimp were (in mg l^{-1}) 0.149±0.027, 0.134± 0.007 and 0.059 ± 0.006 (n=24) respectively. Metal wise comparisons showed that the mean levels of chromium and lead in the water of the four water bodies tested were significantly higher than the levels of cadmium. Even though, the concentrations of lead in Puttalam lagoon were significantly higher than those of chromium, no significant difference could be observed between the two metals in water of the other three water sources. The overall concentrations of lead, chromium and cadmium in the sediments of the four water sources were (in μg g^{-1} dry weight) 6.426±1.835, 21.671± 0.423 and 0.010± 0.001(n=36) whereas the levels in farm sediments were 8.38±0.14, 27.95±0.40 and 0.028±0.001 (n=72) respectively. The levels of the three metals in the sediments of each water source were significantly different from each other and the metal levels followed the order; Cr > Pb > Cd. The levels of three metals in the farm sediments followed the same pattern. The overall metal contents in the formulated feed used by the farms to feed the shrimp were (in μg g^{-1} dry weight) 0.391±0.014 for lead (n=24), 1.468 ± 0. 056 (n=24) for chromium and 2.053 ± 0.109 (n=24) for cadmium (Table 1). These were found to be significantly different from each other. The mean level of lead in the feed used by the farms was significantly lower than that of chromium and cadmium and the level of cadmium was significantly higher than that of chromium. The overall concentrations of lead, chromium and cadmium in the muscle tissue of the shrimp (mean±SEM) were (in μg g^{-1} dry weight) 0.360±0.044, 0.610±0.044 and 0.199±0.012 (n=360) respectively and the levels of the three elements were significantly different from each other and followed the same order which could be detected in the sediments (Cr > Pb > Cd). Even though, the levels of the three metals in the muscle tissue of the shrimp obtained from the farms fed from Gembarandiya lagoon and the Dutch Canal were significantly different from each other, that trend could not be observed in the shrimp obtained from the farms fed from the other two water sources (Table 1).

Table 2 presents the bio-transfer factors of the three metals in shrimp tissue in relation to source water, source sediments, farm sediments and formulated feed. Bio-transfer factors of lead from the water of Puttalam lagoon to shrimp was significantly lower than that of chromium and cadmium where as no significant difference could be found between the bio-transfer factors of chromium and cadmium from water in the Puttalam lagoon to shrimp. Bio-transfer factors of the three metals from the water of the other three water sources to shrimp were not significantly different from each other. The overall bio-transfer factor of chromium from source water to shrimp was significantly higher than that of lead, but was not

Table 2: Bio-transfer factors of lead, chromium and cadmium in relation to *Penaeus monodon* cultured in water from four water sources in the North Western Province of Sri Lanka (PL = Puttalam Lagoon, ML = Mundal Lagoon, GL = Gembarandiya Lagoon, DC = Dutch Canal).

Transfer	Lead	Chromium	Cadmium
Water / Shrimp			
PL	0.54 ± 0.07[a]	3.30±0.28[b]	2.58±0.40[b]
ML	3.60± 0.46[a]	3.20±0.130[a]	3.42±0.17[a]
GL	4.80 ± 1.20[a]	7.70±1.20[a]	4.78±0.450[a]
DC	3.70± 0.32[a]	4.50±0.30[a]	3.43±0.50[a]
Overall mean	**3.20±0.41[a]**	**4.70±0.43[b]**	**3.55±0.23[a,b]**
Source sediments /Shrimp			
PL	0.03±0.00[a]	0.02±0.00[a]	35.43±5.53[b]
ML	0.04±0.00[a]	0.01±0.00[a]	18.00±0.88[b]
GL	0.09±0.07[a]	0.05±0.01[a]	16.03±1.53[b]
DC	0.07±0.01[a]	0.03±0.00[a]	13.38±1.957[b]
Overall mean	**0.06±0.01[a]**	**0.03±0.00[a]**	**20.71±2.07[b]**
Farm sediments/ Shrimp			
PL	0.03±0.01[a]	0.02±0.00[a]	9.71±.2.59[b]
ML	0.04±0.00[a]	0.02±0.00[a]	5.55±0.56[b]
GL	0.08±0.02[a]	0.04±0.01[a]	8.45±1.01[b]
DC	0.04±0.01[a]	0.03±0.01[a]	12.17± 1.48[b]
Overall mean	**0.05±0.01[a]**	**0.03±0.00[a]**	**8.97±0.87[b]**
Formulated feed / Shrimp			
PL	0.45±0.06[c]	0.31±0.024[b]	0.11±0.01[a]
ML	1.00±0.12[b]	0.30±0.032[a]	0.11±0.01[a]
GL	1.40±0.32[b]	0.70±0.130[a,b]	0.11±0.01[a]
DC	0.83±0.11[c]	0.47±0.037[b]	0.08±0.03[a]
Overall mean	**0.92±0.11[c]**	**0.44±0.04[b]**	**0.10±0.01[a]**

Results are presented as mean±standard error of the mean and the ranges, n=90. For each row, means not followed by the same superscript are significantly different from each other (ANOVA, Tukey's test, $P<0.05$).

significantly different from the bio-transfer factor of cadmium. Overall mean values for bio-transfer factor of cadmium from water was also not significantly different from that of lead. Overall bio-transfer factors of Pb, Cr and Cd from the water to shrimp were greater than 1 and ranged from 3 to 5. No significant difference could be observed between bio-transfer factors of lead and chromium from the sediments of sources and the farm sediments to shrimp. Bio-transfer factors of cadmium from the sediments of all four water sources to shrimp were significantly higher than that of chromium and lead (Table 2.). This pattern of variation could also be observed in the bio-transfer factors from the sediments of the farms to the shrimp tissues. The overall bio-transfer factor of cadmium from the sediments was significantly higher than that from the other two metals. Overall bio-transfer factors of cadmium in shrimp from sediments were 9 folds from farm sediments and 21 folds from source sediments. Results indicate that of the three metals, only the bio-transfer factors of cadmium in shrimp from the sediments were several folds higher than 1.

Bio-transfer factors of the three metals in shrimp tissue in relation to formulated feed were significantly different among each other in each water source. Relatively higher transfer factors from feed to shrimp were recorded for lead in each of the four water sources studied. The bio-transfer factors of lead from feed to shrimp ranged from 0.45 to 1.4 depending on the type of water source studied. The overall bio-transfer factor of lead in relation to feed was significantly higher than that of the other two metals. The lowest overall bio-transfer factor from feed was from cadmium. The order of the overall transfer factors from feed to shrimp was Pb > Cr > Cd. Figure 1 presents the variation of bio-transfer factors of the three metals in shrimp from water, source sediments, farm sediments and formulated shrimp feed. Overall mean value for the bio-transfer factor of lead in shrimp from water, which was 3.2, was significantly higher than that from the other three fractions. Mean value for bio-transfer factor of lead in shrimp in relation to formulated feed was significantly higher than that from the both types of sediments. The mean value for bio-transfer factor of chromium in shrimp from source water was 4.7, which was significantly higher compared to the other three sources; source sediments, farm sediments and formulated shrimp feed. Transfer factors of cadmium from the source sediments (20.71) and farm sediments (8.97) were significantly higher than that from the water and the formulated shrimp feed.

Discussion

The proposed safety limits recommended by the Central Environmental Authority in Sri Lanka for shellfish fishery in the coastal waters are <25 μg l^{-1} for lead, <5 μg l^{-1} for cadmium and <25 μg l^{-1} for chromium (CEA 1999). The metal levels recorded from the water of all four water sources that fed the shrimp farms studied were found to exceed the above specified limits. Therefore, these elevated concentrations of the metals in the water sources may pose risks to the health of the aquatic life in the area especially to the shellfish in the lagoons and near by coastal waters.

The monitoring of contamination in aquatic systems usually relies on determining concentrations of pollutants in sediments and biota because they tend be of several orders of magnitude higher than in overlying waters (Bryan and Langston 1992 cited by Ruiz and Saiz-Salinas 2000). Of the three metals selected for the study, chromium was the dominant metal accumulated in the shrimp tissue. In most occasions, the magnitude of the

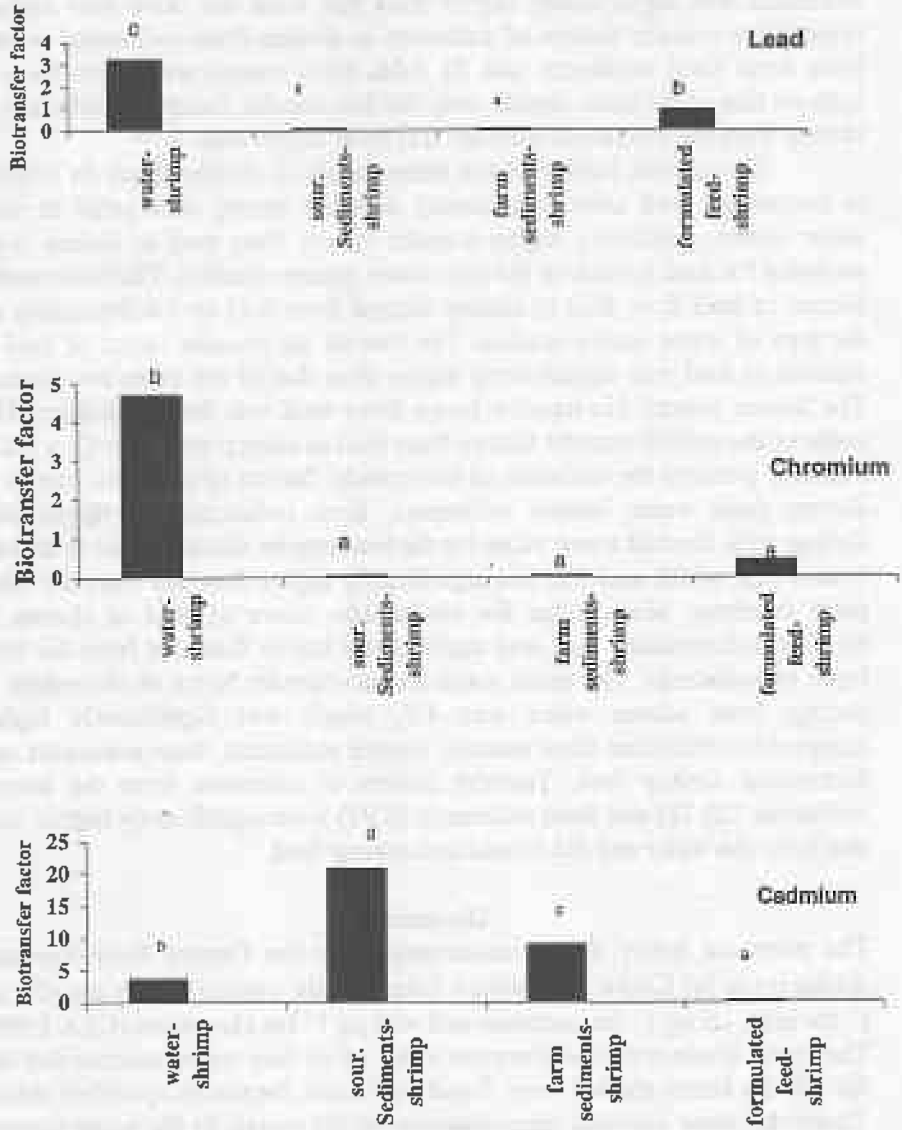

Figure 1: Comparison of the bio-transfer factors of lead, chromium and cadmium in *P. monodon* among different fractions of the culture environment. Results are presented as mean±standard error of the mean, n=360. For each metal, bars not with the same letter are significantly different from each other (ANOVA, Tukey's test, P<0.05).

metals in the shrimp tissue were in the order of Cr > Pb ≥ Cd and the overall metal contents followed the order Cr > Pb > Cd. This was in good agreement with the elevated levels of chromium and relatively low levels of cadmium in the sediments. Even though the cadmium levels in the sediments were much lower in comparison with the levels of lead and chromium during the present study, not much difference could be detected among the levels of the three metals in the shrimp tissues. This may be attributed to the different rates of uptake and elimination of different metals by the shrimp. Heiny and Tate (1997) pointed out that it is much harder to relate trace metal concentrations in fish to that in the bed sediments or to the land use pattern and concluded that the concentrations of metals in fish are related to the concentrations of metals in ingested food, water and sediments along with the rates of bioaccumulation and depuration and these factors differ for different metals and the different fish species.

In the present investigation, the bio-transfer factors of the three metals from water were greater than 1 except for one occasion, i.e., in relation to lead in the shrimp fed from Puttalum lagoon. However, the overall means of the bio-transfer factors of the three metals in shrimp from water were greater than 1 indicating the occurrence of bioaccumulation of the three metals from the water phase. Rashed (2001) had pointed out that if the bio-transfer factor for a metal is greater than 1, bioaccumulation of the metal occurs in the fish from the particular fraction of the ecosystem. As the bio-transfer factors were greater than 1 for each metal, it can be concluded that the uptake of the three metals in shrimp, *P. monodon* from water occur faster than that of the elimination. Connell (1998) pointed out that the bioaccumulation of persistent chemicals by aquatic organisms can occur from food or ambient water, but the latter source is generally the dominant in most situations. The results of the present study also indicate higher bio-transfer factors for the elements from water compared to those from the formulated feed.

The bio-transfer factors of lead and chromium from the sediments of the four water sources and from the farm sediments were lower than 1. However, the bio-transfer factors of cadmium from the sediments showed significantly higher values and they all were greater than 1 ranging from 9 to 21. The results of the present study revealed that the bioaccumulation of cadmium occur not only from the water phase but also from the bottom sediments. Rao et al. (1998) pointed out that the shrimp being benthic animals could accumulate higher concentrations of heavy metals while eating the way through sediments if the metal concentrations in water and the sediments are considerably high.

The bio-transfer factors of lead, chromium and cadmium in shrimp from the formulated feed were lower than 1 in the shrimp cultured in water from all four water sources. This indicates that the possibility of bioaccumulation from the formulated feed was very low compared to that from water and the sediments.

The present study found that the metal levels in the water of the four water sources that fed the shrimp farms studied were several folds higher than the recommended safety limits for shellfish fishery in the coastal waters. Therefore, quality of the water used for culturing the shrimp has to be improved and the effluents discharged to the water sources should strictly comply with the effluent quality standards.

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