
# Determination of Heavy Metals in *Metapenaeus dobsoni* and *Penaeus indicus* Shrimp Species Captured from Negombo and Puttalam Lagoons, Sri Lanka

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**Abstract**
This study was conducted to determine the levels of selected heavy metals in the muscle tissue of *Metapenaeus dobsoni* and *Penaeus indicus* shrimp species from Negombo and Puttalam lagoons in Sri Lanka to compare heavy metal levels in shrimp species between two lagoons and evaluate potential risks to human health associated with shrimp consumption. Samples were collected monthly from October 2022 to March 2023 and quantified for Cu and Zn using FAAS, while Cr using GFAAS. The mean heavy metal levels in µg/g wet weight in the *Metapenaeus dobsoni* captured from Negombo lagoon were found in order of Cu (4.1±1.7) > Zn (4.0±3.3) > Cr (0.061±0.031), while captured from Puttalam lagoon were found in order of Zn (6.6±4.3) > Cu (6.6±1.4) > Cr (0.076±0.005). The mean metal levels in µg/g wet weight found in *Penaeus indicus* were as follows, Cu (4.4±1.8) > Zn (2.7±1.3) > Cr (0.048±0.019) in Negombo lagoon and Cu (5.9±0.8) > Zn (4.4±2.3) > Cr (0.073±0.030) in Puttalam lagoon. The findings show that the heavy metals levels detected in shrimp samples were generally higher in Puttalam Lagoon than in Negombo Lagoon. In addition, the results demonstrated that shrimp from both lagoons and species were safe for human consumption during the study period since none of the heavy metal levels in the shrimp samples exceeded the maximum permissible level allowed for shrimp consumption according to FAO/WHO. Furthermore, THQ values indicated that there was no non-carcinogenic risk of the determined metals via the consumption of these shrimp species captured from both lagoons.

**Keywords:** heavy metals, Negombo Lagoon, potential risks, Puttalam Lagoon, shrimp consumption

## 1. Introduction

The aquatic habitats within the lagoon area are home to various marine and brackish water species of fish, shrimps, and crabs. These aquatic resources play a pivotal role in the livelihoods of the local community, as fishing is their primary source of income [1]. There is clear evidence to accept that shrimp consumption has also been increasing over the years in Sri Lanka. In addition, compared to cultured shrimp production, wild-captured shrimp from lagoons and estuaries are significantly higher in numbers [2].

On the other hand, the presence of heavy metals, such as copper, chromium, and zinc, in aquatic ecosystems has become a growing concern worldwide due to their potential impact on human health and environmental well-being. While metals are elements that naturally occur in the environment, anthropogenic activities, such as urbanization, rapid industrialization, changes in land use, and increased soil runoff, contribute to elevated metal concentrations. Moreover, the direct discharge of industrial effluents into rivers, results in the contamination of coastal ecosystems as these toxins are transported by river water [3]. Consequently, these water sources may have hazardous metal concentrations above WHO and national standard recommendations. Because of the contamination in coastal waters, fish, crustaceans, and other aquatic organisms living in these polluted environments become contaminated as well [4]. Therefore, the consumption of shrimp from these water bodies threatens human health in the long run.

Based on the accumulative behavior, persistent nature, and potential toxicity of heavy metals besides significant shrimp consumption, there is a need to analyze shrimp to ensure that contaminant heavy metal levels meet international food safety standards. To date, no study has been conducted to determine the heavy metal concentrations in shrimp from Negombo and Puttalam lagoons, which might become a potential health risk to the general public.

## 2. Materials and Methods

### 2.1. Apparatus

#### 2.1.1. Atomic Absorption Spectrometer

Thermo Scientific iCE 3000 AA05121002 v1.30 AAS equipped with a flame sample introduction system was used for the analysis of Cu and Zn in shrimp samples. Thermo Scientific iCE 3000 series GFAAS, hollow cathode lamp at 357.9 nm wavelength was used for Cr analysis.

#### 2.1.2. Muffle Furnace

A Muffle furnace from Nabertherm, Germany which has a temperature control unit was used for dry ash preparation

### 2.2. Samples and reagents preparation

#### 2.2.1. Glass-ware and containers used

50 mL volumetric flasks, 100 mL volumetric flasks, 250 mL volumetric flasks, 100 mL beakers, 250 mL beakers, glass rods, funnels, watch glasses, 10.00 mL pipette, 2.00 mL pipette, glass dropper, conical flasks, porcelain crucibles, and evaporating dishes.

#### 2.2.2. Analytical Balance

To measure the masses an analytical balance (Precisa XT 220A model), which is highly accurate and precise with four decimal point measurements was used.

#### 2.2.3. Mixer Grinder

A grinder made of stainless steel material was used to homogenize shrimp samples.

#### 2.2.4. Hot Plate

A hot plate stirrer (MSH-20D model - made in South Korea) with a temperature control unit was used in the drying process.

#### 2.2.5. Chemicals and reagents used

1. 69.71% nitric acid ($HNO_3$) analytical grade (MW - 63.01 g $mol^{-1}$ )
2. 35.0% hydrochloric acid (HCl) analytical grade (MW - 36.46 g $mol^{-1}$ )
3. Commercially available AAS grade Zn stock solution (1000 ppm)
4. Commercially available AAS grade Cu stock solution (1000 ppm)
5. Commercially available AAS grade Cr stock solution (1000 ppm)

### 2.3. Methodology

A slightly modified version of the method used by S.P.S.D. Senadheera *et al* for their study was used [5].

#### 2.3.1. Sample collection for the analysis

In this study, *Penaeus indicus* (Kiri Issa) and *Metapenaeus dobsoni* (Kadal Issa) were selected for the analysis which are the most common shrimp species captured from the Negombo and Puttalam lagoons. A total of twenty-four shrimp samples of selected species were collected from local fishermen operating within Negombo and Puttalam lagoons, consisting of five randomly selected individuals for one sample of each species from October 2022 to March 2023. Samples were placed into polyethylene bags by species and location, kept on ice in an insulated box to maintain approximately 4-5 ºC, and delivered to the laboratory. Samples were then washed with deionized water and their total lengths and weights were recorded. After that, cleaned shrimps were kept in a freezer at -20 ºC in polythene bags until analysis.

#### 2.3.2. Processing of samples before digestion

Frozen shrimp samples were thawed at room temperature and then de-headed and the shells were removed using a plastic scalpel to avoid metal contamination. Edible tissues were collected from each sample and were homogenized using a mixer grinder. The homogenate was weighed using an analytical balance and was expressed as fresh weights. Then the pre-weighed sample was kept in a pre-acid-washed evaporating dish and dried using a hot plate at 90 ºC for >24 h to a constant weight. The dried sample was powdered using a porcelain mortar and pestle and kept in a plastic bottle in a refrigerator until digestion.

#### 2.3.3. Digestion of shrimp tissue

Dry ash preparation was preferably carried out as the digestion technique for the samples. A quantity of 3.00 g of the dried sample was accurately weighed into a porcelain crucible and kept in the muffle furnace at 600 ºC for 4 hours. Then, the samples were cooled to room temperature and treated with 5 mL of 6M HCl acid and evaporated to dryness on a hot plate. After the sample cooled, it was re-dissolved in 5 mL of 0.1 M nitric acid and transferred to a 10.00 $cm^3$ volumetric flask after gravity filtering followed by micro-filtering using 0.22 µm nylon filters. Then, solutions were topped up to mark using 0.1M $HNO_3$. Prepared solutions were then transferred into pre-cleaned labeled Teflon bottles until taken for analysis.

### 2.4. Analysis of Cu and Zn by FAAS

The total amount of Cu and Zn were determined with the aid of Thermo Scientific iCE 3000 AA05121002 v1.30 scientific model FAAS.

**Table 1:** Spectrometer parameters for analysis of Zinc and Copper in shrimp by FAAS

| Spectrometer Parameter | Copper | Zinc |
|---|---|---|
| Flame type | Air/Acetylene | Air/Acetylene |
| Fuel flow Rate / l $min^{-1}$ | 0.8 to 1.1 | 0.9 to 1.2 |
| Bandpass/nm | 0.5 | 0.2 |
| Wavelength/nm | 324.8 | 213.9 |

A calibration curve ranging from 1.00 mg/L to 7.00 mg/L was used for the determination of copper while from 0.50 mg/L to 2.00 mg/L ranged calibration curve was used for the determination of Zinc.

### 2.5. Analysis of Cr by GFAAS

Total amounts of Cr were analyzed by using Thermo Scientific iCE 3000 series GFAAS, hollow cathode lamp at 357.9 nm wavelength. The calibration curve of chromium was graphed from 5 µg/L to 10 µg/L.

### 2.7. Calculation of Metal Levels

#### 2.7.1. Calculation of Cu and Zn levels

$$\text{Amount } (\mu g/g) = \frac{\text{Conc. of the sample (mg/L)} \times \text{sample volume (mL)} \times 1000(\mu g/mg) \times \text{dilution factor}}{\text{Sample weight (g)} \times 1000(\text{mL/L})}$$

#### 2.7.2. Calculation of Cr levels

$$\text{Amount } (\mu g/g) = \frac{\text{Conc. of the sample } (\mu g/L) \times \text{sample volume (mL)}}{\text{Sample weight (g)} \times 1{,}000(\text{mL/L})}$$

### 2.8. Statistical data analysis

Differences among the three metal levels in the muscle tissue of each species and the differences in the levels of each metal in each shrimp species among the two lagoons were determined by Minitab nonparametric Kruskal-Walli's test, one-way analysis of variance (ANOVA) test, and Tukey's test using the data of the present study, while considering $P<0.05$ is statistically significant.

## 3. Results and Discussion

### 3.1. Heavy Metal Levels in Shrimp

The mean levels of heavy metals in the muscle tissues of *Penaeus indicus* and *Metapenaeus dobsoni* shrimp species are shown in Table 2.

**Table 2:** Mean metal levels of selected heavy metals in *Penaeus indicus* and *Metapenaeus dobsoni* on wet weight basis

| Mean metal level (µg/g wet weight) | | | | |
|---|---|---|---|---|
| | *Penaeus indicus* (Kiri Issa) | | *Metapenaeus dobsoni* (Kadal Issa) | |
| **Heavy Metal** | **Negombo Lagoon** | **Puttalam Lagoon** | **Negombo Lagoon** | **Puttalam Lagoon** |
| Cu | 4.4±1.8 | 5.9±0.8 | 4.1±1.7 | 6.6±1.4 |
| Zn | 2.7±1.3 | 4.4±2.3 | 4.0±3.3 | 6.6±4.3 |
| Cr | 0.048±0.019 | 0.073±0.030 | 0.061±0.031 | 0.076±0.005 |

Codex Alimentarius Commission which is a combination of FAO/WHO has set maximum permissible levels for toxic heavy metals regarding shrimp [6].

**Table 3:** International standards to regulate heavy metals in shrimp.

| Heavy Metal | Maximum Permissible Level FAO/WHO (µg/g wet weight) in shrimp tissues |
|---|---|
| Copper | 30 |
| Zinc | 100 |
| Chromium | 1 |

All the obtained mean metal levels from both locations during the study period have shown significantly lower values than the Maximum Permissible Level of each metal allowed for shrimp according to FAO/WHO recommended levels. In addition, the findings show that the heavy metals levels detected in shrimp samples were generally higher in Puttalam Lagoon than in Negombo Lagoon.

### 3.2. Human Health Risk Assessment

#### 3.2.1. The Estimated Daily Intake (EDI) (µg/kg/day) of shrimp

EDI indicates the amount of metal which is in shrimp that an adult takes for one day. To calculate EDI, the following equation was used [7].

$$\text{EDI } (\mu g/kg/day) = \frac{\text{Metal conc. } (\mu g/g \text{ wet weight}) \times \text{consumption rate (g/person/day)}}{\text{Body weight (kg)}}$$

The average human body weight for adults in Sri Lanka is 57.7 kg; [8] and the consumption rate of shrimp in Sri Lanka is 0.750 g/person/day [2].

**Table 4:** Values of EDI of the heavy metals of shrimp

| EDI (µg/kg/day) | | | | |
|---|---|---|---|---|
| | *Penaeus indicus* | | *Metapenaeus dobsoni* | |
| **Heavy Metal** | | | | |
| | **Negombo Lagoon** | **Puttalam Lagoon** | **Negombo Lagoon** | **Puttalam Lagoon** |
| Cu | 0.0574 | 0.0773 | 0.0534 | 0.0854 |
| Zn | 0.0354 | 0.0566 | 0.0526 | 0.0858 |
| Cr | 0.0006 | 0.0009 | 0.0008 | 0.0010 |

Here, it is apparent that daily intakes of tested heavy metals were comparatively lower by consumption of shrimp captured from Negombo lagoon than those captured from Puttalam lagoon. Acceptable daily intake limits for the interested metals are as follows; Cu - 900 µg/kg/day; [9] Zn - 11000 µg/kg/day; [10] Cr - 35 µg/kg/day; [10] Hence, none of the metals from both locations exceeded the acceptable daily intake limit the authorized organizations provided. Hence, the consumption of the shrimp which captured from Negombo and Puttalam lagoons does not pose a risk to human health.

### 3.2.2. Target Hazard Quotient (THQ)

The health risk from the shrimp consumption also can be assessed by THQ, which represents the ratio of the estimated dose of a pollutant to the reference dose level. The reference dose level is the highest level at which no adverse health effects are expected.

When the THQ value is greater than 1.0, it indicates that the daily consumption of shrimp in the long term would likely result in adverse effects in an exposed human population.

To calculate THQ, the following equation was used [7].
(RfD - Oral References Dose)

$$THQ = \frac{EDI}{RfD}$$

The values of THQ of Cu, Zn, and Cr of both shrimp species were found below 1.0. This indicates that there was no non-carcinogenic risk of the three metals via the consumption of *Penaeus indicus* and *Metapenaeus dobsoni* shrimp species captured from Negombo and Puttalam lagoons.

**Table 5:** Values of THQ of the heavy metals of shrimp

| Heavy Metal | Oral References Dose[65] (µg/kg/day) | THQ | | | |
|---|---|---|---|---|---|
| | | *Penaeus indicus* | | *Metapenaeus dobsoni* | |
| | | Negombo Lagoon | Puttalam Lagoon | Negombo Lagoon | Puttalam Lagoon |
| Cu | 40 | $1.43\times10^{-3}$ | $1.93\times10^{-3}$ | $1.33\times10^{-3}$ | $2.14\times10^{-3}$ |
| Zn | 300 | $1.18\times10^{-4}$ | $1.89\times10^{-4}$ | $1.75\times10^{-4}$ | $2.86\times10^{-4}$ |
| Cr | 50 | $1.20\times10^{-5}$ | $1.80\times10^{-5}$ | $1.60\times10^{-5}$ | $2.00\times10^{-5}$ |

## Conclusions

The results of the present study indicated that *Penaeus indicus* and *Metapenaeus dobsoni* from Negombo and Puttalam lagoons, Sri Lanka were safe to consume since the concentrations of heavy metals (Cu, Zn, and Cr) were below the maximum permissible level (MPL) allowed for shrimp according to FAO/WHO for human consumption. The estimated daily intake (EDI) and target hazard quotient (THQ) values further support the conclusion of the low risk of adverse health effects associated with shrimp consumption from the mentioned two lagoons. The findings of statistical analysis demonstrated that among the heavy metals analyzed, only Cu and Cr exhibited a significant difference in concentration between the two lagoons.

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## Conflicts of Interest

The authors declare no conflict of interest.

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