Potential health risk assessment of selected metal concentrations of Indian backwater oyster, (Crassostrea madrasensis) from Puttalam lagoon, Sri Lanka

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Research Article

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Potential health risk assessment of selected metal concentrations of Indian backwater oyster, *(Crassostrea madrasensis)* from Puttalam lagoon, Sri Lanka

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Abstract

Oysters are considered as a nutritious seafood, but they can adversely effect on human health due to bioaccumulation potential. The current study was conducted to determine the health risks of mercury(Hg), lead(Pb), cadmium(Cd), arsenic(As), magnesium(Mg), zinc(Zn), iron(Fe) and copper(Cu) in cultured and wild *Crassostrea madrasensis* collected from Sri Lanka. The samples were collected over 13 months from July 2014-July 2015 and monthly variations of metal concentrations and influence of environment were studied between cultured and wild *C.madrasensis*. Metal concentrations were analyzed using Atomic Absorption Spectrophotometer and compared with published international standards. The accumulation order of metal concentrations was Zn>Mg>Fe>Cu>Pb>Cd>As>Hg. The mean Hg, Cd, Pb, As, Mg and Fe concentrations showed monthly variations, but not varied within cultured and wild oysters (p>0.05). A significant variation was observed in both month and source of oysters for Zn and Cu concentrations. A positive correlation was observed for mean Cd and Fe concentrations with temperature while other metals were not. The mean Hg concentrations were well below the maximum levels given by Australia New Zealand Food Standards Code (FSANZ2003) and Commission Regulation (EC1881/2006) and China (GB 2762-2017) while the mean Pb concentration was exceeded standards. The Cd level exceeded the EC (1881/2006) while not other two standards. The weekly intakes of Hg, Cu, Fe, and Zn from *C.madrasensis* were well below the Provisional Tolerable Weekly Intake (PTWI) while, the intake of Cd exceeded PTWI. Hence, the investigation indicated the health risk of consuming *C.madrasensis* emphasizing continuous monitoring to assure consumer’s health and export-oriented market.

Keywords: *Crassostrea madrasensis*, Heavy metals, Health risk, Oyster, Puttalam lagoon, Sri Lanka
1. Introduction

In recent years, the demand for the sea food such as oysters increased concerning benefits rather than the risks of consuming sea food. Oysters are recognized as a healthy sea food choice due to high contents of essential nutrients, including omega 3 fatty acids (Prato et al. 2019). However, oysters are often contaminated by toxic compounds and widely used as bio-indicators due to their ability to accumulate high levels of metals in polluted environments (Fang et al. 2003; Shaari et al. 2016). Since their specific life traits, such as a sessile and filter-feeding behavior, they have a great capacity to accumulate dissolved inorganic metal ions and organometallic ion complexes in sea water, and metal ions pre concentrated on phytoplankton (Cheung and Wong 1992; Aanand et al. 2003). The accumulated metals enter to the human system through consumption of contaminated edible oysters leading to harmful consequences (Biswas et al. 2013). If the concentrations exceed the permitted concentration, they can be considered as “potentially” hazardous for consumers (Liao and Ling 2003; Amiard et al. 2008). Hence, oysters used for food are harvested or cultured generally close to the shore, they are subject to any materials contained in drainage from land, whether it is from rivers or streams, runoff after rains or direct discharges (Suryawanshi 2011).

Metals such as Pb, Cd, As and Hg are toxic even at low concentrations while, Zn, Cu, Mg and Fe metals become toxic to living organisms when subject to high concentrations (Shaari et al. 2016). According to European Union (EU) for hazardous metals, the three non-essential metals such as Hg, Cd and Pb were proven hazardous nature and essential to keep contaminants at levels which do not cause health concerns to protect public health (EC 2006; Chitrarasu 2013). The demand for the oysters in Sri Lanka due to tourist industry and export market has created a small-scale oyster fishery in the Puttalam lagoon for both cultures based and wild collecting fisheries. Thus, this study was focused on the spatial and temporal variations of selected metals in cultured and wild oysters (Crassostrea madrasensis) in Puttalam lagoon, Sri Lanka to find the suitability for the consumption in case of metal toxicity over benefits.
2. Methodology

2.1. Sample collection and preparation

The study area was located in the North-Western Province in Sri Lanka as shown in Figure 1 and wild oysters (*Crassostrea madrasensis*) were collected from natural oyster beds at Gangewadiya area in Kala Oya estuary and cultured oysters were collected from culture racks in both Kandakuliya and Gangewadiya area.

![Fig 1: The study site at Puttalam lagoon and Kala Oya estuary in North-Western Province of Sri Lanka](image)

Approximately 15-20 market size oysters (shell length 5-7 cm) were collected from each site with monthly intervals over a 13 month period from July 2014 to July 2015. Afterward, they were transported to the laboratory in a cooler chilled with ice (4 °C). In the laboratory, the shell was removed, and the surface water was blotted; edible meat was separated from the shells, blotted and weighed (AAA 300 L, Bradford, UK). Then body tissue was homogenized and dried at 105 °C for overnight in the hot air oven (Memmert, usb/500, Germany), afterwards they were pulverized, using mortar and pestle and stored in sealed bag in freezer 117 (-20 °C) until analysis.
Surface water samples influencing oysters at Gangewadiya wild oyster beds, Gangewadiya cultured oyster racks and Kandakuliya cultured oyster racks were collected during the study period for temperature and salinity determination. The water temperature was measured in situ with the help of a mercury thermometer calibrated to 0.1 °C and the values were expressed as °C. Salinity was measured in situ using hand refractometer (YIERYI, RHS-10ATC, China) that had a range between 0-100‰.

2.2. Analysis of metal (Hg, Cd, Pb, As, Mg, Zn, Fe and Cu) concentrations

Wet digestion was performed in duplicate by weighing approximately 0.5 g homogenized dried oyster tissues with 10 mL of 65% (v/v) HNO₃ acid in a microwave digestion system (MARS-6, CEM, Matthews, USA) with the condition of 15 min ramp to 200 °C and 10 min holding time. Prior to microwave decomposition samples with added reagents were allowed to digest at room temperature in Teflon tubes for 15 minutes. The digested samples were diluted to 50.00 mL with deionized water (>18 MΩ Cm⁻¹).

Analysis of Hg was performed using an Atomic Absorption Spectrophotometer (AAS) (Varian 240 FS, Varian Inc., Mulgrave, Victoria, Australia) equipped with Cold Vapor Generation Accessory (Varian VGA 77) with closed-end cell. The Pb, Cd and As were assessed using AAS with Varian graphite tube atomizer (GTA-120). The Zn, Cu, Fe and Mg were assessed using Flame AAS (VARIAN FS 240). The standard solutions of Hg, Pb, Cd, As, Mg, Zn, Fe and Cu at 1000 mg/L were obtained from Sigma-Aldrich (Dorset, United Kingdom) which were used to prepare calibration solutions. All standards and reagents were prepared using deionized water. All results were compared with international standards Commission Regulation (EC) No 1881/2006 by European Communities, Food Standards Australia New Zealand (FASNZ, 2003) and Food Safety Standard for Maximum Levels of Contaminants in Foods, China (GB 2762-2017).
2.3. Quality control procedure

Two blanks and two spiked samples were carried out on each digestion batch and spiked sample recovery was used as a quality control of the study (80–120%). The certified quality control material (canned fish muscle, T/07194) from Food Analysis Performance Assessment Scheme (FAPAS, Sand Hutton, York, UK) for Hg and Cd was used to assure the accuracy of the analytical procedure throughout the analysis. The average field blank, derived from sample field blanks, and three times its standard deviation were used to evaluate the limit of detection (LOD). The limit of quantification (LOQ) was 3×LOD.

2.4. Statistical analysis

All statistical analysis was conducted using Microsoft Excel 2007 version and Minitab 18. Monthly variations in metal concentrations between cultured and wild oysters were analyzed using the General Linear Model and one-way ANOVA. Influence of the environment on the metal content of *C. madrasensis* was analyzed using Stepwise regression.

3. Results and discussion

The suitability of the method was evaluated by calculating the Limit of Quantification (LOQ) and the recovery of certified quality control measures for Hg and Cd. The LOQ for Hg, Cd, Pb, As, Zn, Cu, Fe and Mg was 0.07, 0.006, 0.52, 0.34, 0.43, 0.01, 0.15 and 0.32 mg kg\(^{-1}\) respectively. The assigned value of quality control of samples (n=10) for Hg (T-7194QC) was 141 µg kg\(^{-1}\) and the recovery was 106.7 % (±7.90) and the assigned value for Cd for the same QC sample was 4.99 µg/kg and the recovery was 95.10 % (±13.34). Due to the unavailability of quality control materials in the laboratory for all trace metals (except, Hg and Cd), the spiked recovery limits were found to be between (80-120) % for other metals. The results indicate the validity of this method for determining the Hg, Cd, Pb, As, Zn, Cu, Fe and Mg concentrations in the samples investigated.
3.1. Monthly variation of trace metals concentrations

The monthly variation of mean Hg, Pb, Cd and As concentrations of wild oysters in Gangewadiya (GW_W), cultured oysters in Gangewadiya (GW_C) and cultured oysters Kandakuliya (KC_C) is shown in Table 1 and the monthly variation of mean Mg, Zn, Fe and Cu concentrations of wild and cultured oysters in same locations are shown in Table 2. The results are presented in dry weight basis.

The Hg concentration ranged between <0.07-0.28 mg kg$^{-1}$ in wild oysters in Gangewadiya, <0.07 to 0.28 mg kg$^{-1}$ and <0.07 to 0.29 mg kg$^{-1}$ in cultured oysters in Gangewadiya and Kandakulutiya respectively. The Pb concentration ranged between 1.18-68.83 mg kg$^{-1}$, <0.52 to 78.88 mg kg$^{-1}$ and <0.52-78.67 mg kg$^{-1}$ respectively in wild oysters in Gangewadiya, in cultured oysters in Gangewadiya and Kandakuliya. The Cd concentration ranged between 1.34-13.41 mg kg$^{-1}$ in wild oysters in Gangewadiya, 1.53-12.98 mg kg$^{-1}$ in cultured oysters in Gangewadiya and 1.26-12.99 mg kg$^{-1}$ in cultured oysters in Kandakuliya. The mean As concentration ranged between <0.34-8.57 mg kg$^{-1}$, <0.34 to 9.12 mg kg$^{-1}$ and <0.34 -9.74 mg kg$^{-1}$ respectively in wild oysters in Gangewadiya, in cultured oysters in Gangewadiya and Kandakuliya.

The Mg concentration ranged between 83.57-57.29 mg kg$^{-1}$, 33.90 to 562.40 mg kg$^{-1}$ and 421.01 to 569.33 mg kg$^{-1}$ wild oysters in Gangewadiya, cultured oysters in Gangewadiya and Kandakuliya respectively. The Zn concentration ranged between 1080.49 to 1500.40 mg kg$^{-1}$ in wild oysters in Gangewadiya, 1057.10 to 1749.80 mg kg$^{-1}$ and 897.10-1589.60 mg kg$^{-1}$ in cultured oysters in Gangewadiya and Kandakuliya respectively. The mean Fe concentrations ranged between 168.00 to 552.40 mg kg$^{-1}$, 150.90-695.10 mg kg$^{-1}$ and 135.70-621.80 mg kg$^{-1}$ respectively in wild oysters in Gangewadiya, cultured oysters in Gangewadiya and cultured oysters in Kandakuliya. The mean Cu concentration ranged between 62.91-197.88 mg kg$^{-1}$, 75.52 to 192.10 mg kg$^{-1}$ and 40.10-185.77 mg kg$^{-1}$ respectively in wild oysters in Gangewadiya, cultured oysters in Gangewadiya and Kandakuliya.
Table 1: Monthly variations of heavy metals of Hg, Pb, Cd and As in wild oysters in Gangewadiya (GW_W), cultured oysters in Gangewadiya (GW_C) and cultured oysters Kandakuliya (KC_C)

| Metal | Location | Jul   | Aug   | Sep   | Oct   | Nov   | Dec   | Jan   | Feb   | Mar   | Apr   | May   | Jun   | Jul   |
|-------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Hg    | GW_W     | 0.22±0.03 | 0.25±0.04 | 0.11±0.00 | 0.14±0.01 | <0.07 | 0.15±0.01 | 0.09±0.03 | 0.13±0.06 | 0.13±0.02 | 0.18±0.00 | 0.20±0.02 | 0.08±0.00 | 0.10±0.00 |
|       | GW_C     | 0.22±0.03 | 0.10±0.01 | 0.09±0.03 | 0.12±0.03 | <0.07 | 0.13±0.05 | 0.12±0.06 | 0.13±0.02 | 0.15±0.02 | 0.13±0.01 | 0.11±0.00 | 0.10±0.00 | <0.07 |
|       | KC_C     | 0.25±0.07 | 0.15±0.01 | 0.10±0.05 | 0.07±0.02 | 0.14±0.00 | 0.09±0.05 | 0.12±0.03 | 0.13±0.02 | 0.14±0.00 | 0.12±0.00 | 0.09±0.01 | 0.09±0.01 | <0.07 |
| Pb    | GW_W     | 4.19±0.02 | 20.44±3.35 | 8.40±1.38 | 6.63±5.14 | 1.73±0.38 | 1.19±0.01 | 3.90±0.25 | 30.39±8.78 | 65.50±4.71 | 16.60±4.28 | 15.55±0.32 | 6.73±0.04 |
|       | GW_C     | 4.19±0.02 | 6.00±3.15 | 13.63±1.51 | 1.83±0.28 | 8.37±0.43 | 22.32±2.85 | 68.81±4.12 | 77.99±1.26 | 73.88±3.17 | 11.94±1.23 | 47.02±5.92 | 3.13±4.42 |
|       | KC_C     | 4.44±0.27 | 13.84±0.69 | 2.67±0.45 | <0.52 | <0.52 | 1.71±0.78 | 73.30±5.84 | 73.44±5.12 | 47.99±7.50 | 7.52±0.19 | 9.89±2.29 | <0.52 |
| Cd    | GW_W     | 9.86±1.20 | 1.71±0.11 | 1.47±0.18 | 1.76±0.12 | 1.58±0.05 | 1.55±0.06 | 1.61±0.05 | 7.45±1.39 | 11.85±2.20 | 10.55±1.30 | 11.08±0.72 | 10.85±0.09 | 9.40±0.82 |
|       | GW_C     | 9.86±1.20 | 2.70±1.32 | 1.75±0.07 | 1.75±0.01 | 1.57±0.05 | 1.56±0.03 | 9.81±0.69 | 11.47±0.05 | 10.72±1.04 | 11.64±0.06 | 7.99±0.74 | 12.10±1.26 | 1.66±0.00 |
|       | KC_C     | 8.32±2.18 | 1.71±0.02 | 1.73±0.05 | 1.64±0.05 | 1.66±0.05 | 1.44±0.25 | 8.35±0.47 | 12.16±1.18 | 11.43±1.82 | 9.13±0.63 | 8.50±0.24 | 6.85±2.05 | 1.54±0.07 |
| As    | GW_W     | 8.06±0.72 | 5.67±1.20 | 5.92±1.31 | 6.04±0.84 | 3.56±0.82 | 4.29±0.33 | 1.96±0.04 | <0.34 | <0.34 | <0.34 | <0.34 | <0.34 | <0.34 |
|       | GW_C     | 8.06±0.72 | 8.60±0.08 | 9.08±0.06 | 5.39±2.75 | 2.57±0.09 | 3.29±0.01 | 0.25±0.36 | <0.34 | <0.34 | <0.34 | <0.34 | <0.34 | <0.34 |
|       | KC_C     | 8.84±1.22 | 8.21±0.59 | 7.49±1.23 | 4.18±0.87 | 5.02±0.36 | 5.27±1.01 | <0.34 | <0.34 | <0.34 | <0.34 | 0.59±0.52 | 0.68±0.40 | 3.55±0.00 |

* Results are presented as mean±S.E in dry weight
Table 2: Monthly variations of trace metals of Mg, Zn, Fe and Cu in wild oysters in Gangewadiya (GW_W), cultured oysters in Gangewadiya (GW_C) and cultured oysters Kandakuliya (KC_C)

| Metal | Location | Jul     | Aug     | Sep     | Oct     | Nov     | Dec     | Jan     | Feb     | Mar     | Apr     | May     | Jun     | Jul     |
|-------|----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Mg    | GW_W     | 476.90±11.30 | 508.47±8.45 | 533.20±23.00 | 521.20±16.70 | 488.4±33.50 | 528.10±26.30 | 497.00±19.90 | 516.20±11.80 | 548.15±7.14 | 516.03±5.9 |
|       | GW_C     | 476.90±11.30 | 452.60±55.00 | 525.46±5.85 | 505.80±12.40 | 534.16±7.49 | 555.30±6.19 | 500.70±11.40 | 451.00±16.70 | 538.70±23.70 | 400.80±1.1 |
|       | KC_C     | 449.97±6.36  | 521.20±18.50 | 489.60±16.30 | 488.34±2.14 | 464.80±35.10 | 540.00±29.30 | 509.90±25.70 | 424.42±3.41  | 501.39±8.50  | 515.4±21.6 |
| Zn    | GW_W     | 1479.90±20.50 | 1296.20±26.30 | 1185.10±69.00 | 1164.50±78.00 | 1241.10±8.90 | 1172.2±10 | 1239.00±122.0 | 1162.40±82  | 1180.80±33.90 | 1276.80±48.00 |
|       | GW_C     | 1479.90±20.50 | 1495.00±255.0 | 1296.5±0.229 | 1206.40±2.89 | 1242.60±55.60 | 1166.00±12.70 | 1140.8±25.80 | 1140.8±25.80 | 1221.60±28.90 | 1268.70±43.70 |
|       | KC_C     | 1459.40±21.30 | 1585.40±4.18  | 1095.00±35.50 | 1042.00±3.48 | 1255.60±11.60 | 1010.60±72.60 | 964.7±67.6  | 1257.20±5.51 | 1032.5±86.5 | 1089.90±48.00 |
| Fe    | GW_W     | 414.20±22.40  | 306.50±19.00  | 356.70±11.70  | 458.90±14.9  | 201.28±9.97  | 237.80±6.27  | 503.95±0.82  | 178.50±10.50 | 184.04±3.22  | 542.82±9.54 |
|       | GW_C     | 414.20±22.40  | 610.70±29.40  | 669.90±25.10  | 544.35±3.85  | 164.57±7.07  | 225.40±29.20 | 300.40±0.25  | 363.50±21.20 | 153.65±2.74  | 347.90±43.4 |
|       | KC_C     | 390.60±25.60  | 583.40±38.40  | 224.7±1.89    | 319.66±2.17  | 173.17±9.63  | 362.40±14.80 | 433.36±2.51 | 405.91±0.19 | 152.15±3.15 | 450.80±24.80 |
| Cu    | GW_W     | 156.20±11.50  | 162.26±7.67   | 168.99±7.66   | 194.42±3.46  | 130.51±1.55  | 89.38±0.66  | 68.94±4.61  | 107.60±1.79  | 70.68±2.06  | 145.15±7.63 |
|       | GW_C     | 156.20±11.50  | 162.50±4.73   | 130.00±15.90  | 134.16±6.37  | 116.38±3.45  | 187.41±4.69 | 96.05±7.61  | 83.75±0.73  | 76.20±0.68  | 113.46±5.43 |
|       | KC_C     | 137.00±30.90  | 115.80±14.40  | 182.17±3.60   | 158.5±9.64   | 143.70±4.10  | 50.95±1.84  | 43.45±3.35  | 86.42±3.26  | 63.8±0.27   | 60.0±10.50 |

* Results are presented as mean±S.E in dry weight
In the present study, the mean Hg, Cd, Pb, As, Mg and Fe concentrations showed monthly variations while they did not show the significant variation between cultured and wild oysters (p>0.05). The mean Zn and Cu concentrations showed significant variations in both monthly and with the cultured and wild oysters in selected two sites. The mean Hg concentration of July in 2014 (0.23±0.02 mg kg\(^{-1}\)) was significantly higher than the mean Hg concentration in July in 2015 (<0.07 mg kg\(^{-1}\)) (ANOVA, df=12, 39, Tukey’s mean separation, p<0.001). The significantly higher concentration of Pb and Cd were recorded in March 2015 (72.31±2.65 mg kg\(^{-1}\), 11.33±0.59 mg kg\(^{-1}\) respectively). The mean As concentrations showed significantly higher concentration in July (8.32± 0.81 mg kg\(^{-1}\)), August (7.56±1.5 mg kg\(^{-1}\)) and September (7.49±1.53 mg kg\(^{-1}\)) of 2014 compared with other months. The recorded high Hg and As concentrations in July to August season and high Pb and Cd concentrations in March may due to dry season.

The significantly higher mean Mg concentrations were recorded during November 2014 (511.92±5.79 mg kg\(^{-1}\)), February, March (528.50±16.3 mg kg\(^{-1}\)) and June (534.10±10.5 mg kg\(^{-1}\)) in 2015. The significantly higher mean Zn concentrations were recorded during July 2014 (1473.00±25.10 mg kg\(^{-1}\)). However, significantly higher mean Fe concentrations were recorded during July (406.40±28.50 mg kg\(^{-1}\)), August (500.20±15.04 mg kg\(^{-1}\)), September (417.10±19.79 mg kg\(^{-1}\)), and October (441.00±19.79 mg kg\(^{-1}\)) of 2014 and February (424.50±65.80 mg kg\(^{-1}\)), May (396.00±19.75 mg kg\(^{-1}\)) and June (397.60±15.69 mg kg\(^{-1}\)) in 2015. The significantly higher mean Cu concentrations were recorded during October 2014 (162.40± 28.2 mg kg\(^{-1}\)). Several studies have shown the low concentrations of trace metals content in the body tissues in rainy seasons due to fresh water influx during this period on the ground and higher concentrations of metals in dry season. During this period, salinity is reduced and it causes to reduce of the metal uptake (Sankara-Narayan et al., 1978; Biswas et al., 2013). This may be the reason for the high Cu, Mg and Fe concentrations in September to November period which is rainy season for the North western Part of the country.

The mean concentrations of Zn and Cu showed a significant difference between cultured and wild oysters. The mean Zn concentration in both cultured and wild oysters in Gangewadiya (1258.90±28.00 mg kg\(^{-1}\) and 1250.17±21.7 mg kg\(^{-1}\) in dry weight respectively) was
significantly higher than the mean Zn concentration in cultured oysters in Kandakuliya (1152.70±37.30 mg kg\(^{-1}\) in dry weight) (ANOVA, df12,39, Tukey’s mean separation, p<0.05). However, the mean Cu concentration of wild oysters in Gangewadiya (126.05±8.33 mg kg\(^{-1}\) in dry weight) was significantly higher than the mean Cu concentration in cultured oysters in Kandakuliya (96.37±9.37 mg kg\(^{-1}\)) (ANOVA, df2,72, Tukey’s mean separation, p<0.05). As an essential metal, Zn usually accumulates at a higher concentration than other metals because the presence of this metal (Zn\(^{2+}\)) is required as an ionic cofactor for certain enzymes of metabolic activities in their tissue. Oysters can concentrate up to 50 times more Zn than bottom sediments using biota-sediment accumulation factor technique (Rebelo et al., 2003). Similarly, several studies (Pan and Wang, 2009; Shaari et al., 2016) revealed the uptake rate of Cu by bivalves are correlated with the surrounding Cu concentration, the uptake rate decrease when the concentration is low. Therefore, the significantly higher Zn and Cu concentrations in oysters in Gangewadiya may be due to high concentration of Cu and Zn in surrounding waters and sediments than Kandakuliya.

3.2. Influence of environment on metal concentration of \textit{C. madrasensis}

Stepwise regression of mean Hg concentration with water temperature, salinity, location, and culture type, revealed that variations in mean Hg percentage were not significantly explained either by temperature or salinity. No significant relationship was also observed for the mean Pb, Mg, Zn, As and Cu concentrations. The mean Cd (Adj-R\(^2\)=80.33\%, P<0.05) and Fe concentrations (Adj-R\(^2\)=47.89\%, P<0.05) showed a positive relationship with temperature (Figure 2). Environmental factors such as temperature and salinity are known to influence the trace metal availability in bivalves (Biswas et al. 2013). Seasonal fluctuation in the distribution of trace metals in a sedentary organism is controlled by an array of extrinsic and intrinsic factors, such as the extent of pollutant delivery into the estuary and the associated dilution, changes in the weight of the organism, and the direct effects of temperature, salinity and other water quality parameters which show seasonal variations. Several studies have reported significant positive correlation Cd Concentration with temperature and the results of this study are in accordance with the studies conducted by Biswas et al. (2013) and Garcia et al. (2009).
Fig 2: Regression plot of variation in (a) mean Cd concentration with temperature (b) mean Fe concentration with temperature. Red line depicts the fitted line of the model.

3.3. Comparison of metal concentrations with standard guidelines

The metal concentrations accumulation order of the present study was Zn>Mg>Fe>Cu>Pb>Cd>As>Hg. Several studies have revealed Zn as the dominant metal in oysters (Garcia-Rico et al., 2001; Suryawanshi et al. 2011, Mirzaei et al., 2016, Shaari et al. 2016, Burioli, 2017). The comparison of metal concentrations in the wild and cultures oysters with the standard guidelines is shown in Table 3.

The metal concentrations (Hg, Pb, Cd and As) were compared with maximum levels given by European Communities (EC, 1881/2006), Australia New Zealand Food Standards Code (FSANZ, 2003) and National Food Safety Standard Maximum Levels of Contaminants in Foods by China (GB 2762-2017). The mean Hg concentrations of wild oysters in Gangewadiya, cultured oysters in Gangewadiya and Kandakuliya were well below the maximum levels given by FSANZ (2003) and EC (1881/2006) and China (GB 2762-2017) while the mean Pb concentration was exceeded the maximum levels given by the FSANZ (2003) and EC (1881/2006) and China (GB 2762-2017). The mean Cd concentrations were well below the FSANZ (2003) and China (GB 2762-2017), while it was higher than the EC
The mean As concentrations were higher than the established limits for inorganic As by China (GB 2762-2017) and FSANZ (2003) while, the mean As concentration of wild oysters in Gangewadiya was below the FSANZ (2003). These elevated values indirectly indicate the conditions of water and sediment of the study sites and the exceeded limit of mean Pb, Cd and As concentrations indicate the higher content of Pb in the surrounding aquatic area. Therefore, including a depuration system before oyster consumption may help to reduce this mean value by assuring the safety of oyster consumers (Quayle and Newkirk, 1989). There were no recommended maximum permissible levels given for the Zn, Cu and Fe concentrations in aquatic animals. Hence, the concentrations of Zn, Cu and Fe were not compared to assure the oyster suitability for the consumption.

Table 3: Comparison of trace metal concentrations with the standard guidelines (mg kg\(^{-1}\) in w/w)

| Metal | EC 2006 (mg kg\(^{-1}\)) | FSANZ 2003 (mg kg\(^{-1}\)) | China 2003 (GB 2762-2017) (mg kg\(^{-1}\)) | Maximum metal concentrations in present study (mg kg\(^{-1}\)) (w/w) |
|-------|---------------------------|-----------------------------|---------------------------------|--------------------------------------------------|
|       | GW_W                      | GW_C                        | KC_C                           |
| Hg    | 0.5                       | 0.5                         | 0.5                             | 0.03                                            |
| As    | -                         | 1                           | 0.5                             | 0.97                                            |
| Cd    | 1.0                       | 2                           | 2                               | 1.43                                            |
| Pb    | 1.5                       | 2                           | 1.5                             | 2.22                                            |
| Zn    | -                         | -                           | -                               | 178.89                                          |
| Fe    | -                         | -                           | -                               | 65.62                                           |
| Cu    | -                         | -                           | -                               | 20.43                                           |
| Mg    | -                         | -                           | -                               | 67.84                                           |

*EC-: Commission Regulation (EC) No 1881/2006 (Cd and Pb limits for Bivalve mollusk and Hg limits for muscle meat of fish and crustacean), FASNZ-: Australia New Zealand Food Standards Code (Maximum levels of the metal contaminant in food, As (inorganic), Cd, Pb, Hg limits for molluscs), China (GB 2762-2017) -: Food Safety Standard for Maximum Levels of Contaminants in Foods, China(GB 2762-2017) (As-inorganic).
Data in dry weight was converting into wet weight by multiplying metal concentration in dry weight with dry weight percentage and dividing from 100.

3.4. Provisional Tolerable Weekly Intake (PTWI) of metals and health risk assessment

The JECFA established PTWI values for Hg and Cd as 4 µg kg\(^{-1}\) body weight week\(^{-1}\) and 7 µg kg\(^{-1}\) body weight week\(^{-1}\) respectively (JECFA 2011a). The Committee withdrew the PTWI of 7 µg/kg body weight for Cd and established a Provisional Tolerable Monthly Intake (PTMI) of 25 µg kg\(^{-1}\) of body weight month\(^{-1}\) (JECFA 2011b). However, the European Food Safety Authority gives the Tolerable Weekly Intake (TWI) value of 2.5 µg kg\(^{-1}\) body weight for Cd (EFSA 2009). In this study, the potential health risk was calculated only for the Hg and Cd due to the tolerable intakes for As and Pb established by JECFA was withdrawn at the Seventy second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2011c). Provisional Maximum Tolerable Daily Intake (PMTDI) for Cu, Fe and Zn are 0.5 mg kg\(^{-1}\) bw per day (JECFA 1982), 0.8 mg kg\(^{-1}\) bw per day (JECFA 1983) and 1 mg kg\(^{-1}\) bw per day (JECFA 1982) respectively and the JECFA has not given PTWI values for Mg.

The PTWI value was calculated assuming that the average body weight of a person is 60 kg. Accordingly, the estimated PTWI values for 60 kg person for Hg and Cd were 240 µg and 350 µg respectively. The estimated PTWI for 60 kg person for Cu, Fe and Zn are 210 mg, 336 mg and 420 mg respectively. As recommended by the WHO and FAO (2007), the value accepted for the safe level of intake of protein is 0.83 g/kg per day was established for a healthy life. According to that, the amount of oyster meat should consume to fulfill the daily requirement of an average weight person (60 kg) is 50 g/kg per day (WHO 2002) and the weekly protein requirement is 350 g. The mean protein percentage of *C. madrasensis* in Sri Lanka is 6.69% (Subasinghe et al. 2021). Hence, if the weekly protein requirement of an adult person is fulfilled by oysters, it should be consumed 5231.7 g (5.2 kg for a week) oyster meat. But the estimated per capita fish consumption of Sri Lankan’s in 2016 was 16.8 kg/year (Fisheries Statistics 2020). Then, the calculated weekly fish consumption of Sri Lankan’s is 322.19 g and the amount was well below considered with the calculated oyster meat value to fulfill weekly protein requirement. Hence, the contribution from oysters for PTWI was calculated considering the weekly fish consumption of Sri Lankan’s and accordingly calculated weekly intake of Hg was 12.89 µg and Cd was 547.72 µg through cultured oysters Kandakuliya which
was recorded maximum values for Hg and Cd. Therefore, the weekly consumption of oysters to meet will not exceed the recommended PTWI level of Hg but, the intake of Cd through oysters exceeded the limit of PTWI. As well as the contribution from oysters for PTWI of Cu, Fe, and Zn are 8.23 mg, 26.35 mg and 71.60 mg respectively. Therefore, the consumption of oysters to meet the weekly protein requirement will not exceed the recommended maximum level of Cu, Fe and Zn metals.

4. Conclusion
In the present study, the mean Hg, Cd, Pb, As, Fe and Mg concentrations did not show the significant variation between cultured and wild oysters while they showed the monthly variations. The mean Zn and Cu concentrations showed significant variations in both monthly and, within the cultured and wild oysters. Although, the Hg levels in oysters were well below the maximum permissible levels given by EU/EC (1881/2006), FSANZ and China (GB 2762-2017) the elevated levels of Pb and Cd and weekly intake of Cd reveals the health risk of consumption of oyster, *Crassostrea madrasensis* growing in both culture sites in Sri Lanka. Although the health risk assessment exposes the consumption of oysters to meet the weekly protein requirement will not exceed the recommended maximum level of Hg, Cu, Zn and Fe metals the comparisons of heavy metals with standard guidelines hinders the export-oriented market due to exceeded level of lead and cadmium. Hence, the continuous monitoring of contaminations and periodical assessment of possible health risks must be done for the assurance of consumer’s health and export-oriented market.

5. Acknowledgment

5. Declarations

5.1 Ethics approval and consent to participate
Approval for collection of wild oysters was obtained from the Ethics Review Committee of Sri Lanka and project approving committee of Postgraduate Institute of Science, University of Peradeniya, which consists of a member of Ethics Review Committee of Sri Lanka. Collection of *C. madrasensis* from the wild was done with the permission from the Department of Wildlife Conservation, Sri Lanka.
5.2 Consent for publication
Not applicable

5.3 Availability of data and materials
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

5.4 Competing interests
The authors declare that they have no competing interests

5.5 Funding
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5.6 Authors' contributions
Madhusha Mihirani Subasinghe conceived and designed the experiments, performed the experiments, analyzed and interpreted the data and was the major contributor in writing the manuscript. B. K. Kolitha Kamal Jinadasa contributed to the conceive and designs the experiments and analyzes and interprets the data. Ayanthi N. Navarathne contributed to analyze and interpret the data. Sevvandi Jayakody contributed to write the paper. All authors read and approved the final manuscript.

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