Baseline health risk assessment of trace metals in bivalve shellfish from commercial growing areas in the estuaries of Ashtamudi and Vembanad (Kerala, India)

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Received: 8 March 2021 / Accepted: 29 June 2021 / Published online: 16 July 2021
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Abstract
Trace metal concentrations were monitored in the yellow clam (Paphia malabarica), green mussel (Perna viridis) and edible oyster (Crassostrea madrasensis) from growing areas in the Ashtamudi and Vembanad estuaries, Kerala. Samples of shellfish (clams n=26, mussels n=18, oysters n=36) and environmental parameters (salinity, temperature, pH and rainfall) were measured in these growing areas from July 2012 to December 2014. Ranges of mean annual concentrations (mg/kg) were Ni (0.46–0.65); Co (2.87–3.49); Fe (80.0–119.4); Mn (3.88–9.38); Zn (40.8–76.2); Pb (1.28–2.00); and Cu (1.59–4.38). In Ashtamudi, clams had higher mean concentrations of Ni, Co, Fe, Mn and Pb than oysters. Mean concentrations of Ni, Pb (in all species), Zn (in clams and mussels) and Cu (in mussels) did not exceed maximum permissible limits mandated by the Food Safety and Standards Authority of India. Mean Mn concentrations exceeded the World Health Organization guideline (1 mg/kg) in the three species while mean Fe concentrations in clams and oysters did not exceed the guideline (100 mg/kg). Target hazard quotients were generally ≤1, except for a few Pb results in clams and mussels. Although results suggest no health risk to consumers for the reference doses, daily intakes and elements considered, regular monitoring of trace metals is recommended to maintain consumer protection given increasing anthropogenic and climatic pressures on the shellfish growing areas.

Keywords Kerala · Bivalves · Metals · Monitoring · Risk assessment · Target hazard quotient

Introduction
Coastal pollution is a global environmental problem, including accumulation of trace metals in water, sediments and living organisms. While trace metals occur naturally in the environment, growing human activity on the coast is increasing the concentrations to levels higher than background in many parts of the world. Anthropogenic sources of trace metals include industrial and municipal wastewater discharges, urban and agricultural runoff, leaching from landfills and harbour and shipping activities (Fürstner and Wittmann 1983). Bioaccumulation of trace metals by filter-feeding bivalve shellfish above certain levels can pose a health risk to consumers (Abdallah 2013; Jović and Stanković 2014). Elevated concentrations may lead to decreased fertility, cellular and tissue damage, cell death and dysfunction of a variety of organs in consumers (Ribeiro et al. 2002; Damek-Poprawa and Sawicka-Kapusta 2003). The most toxic metals to shellfish and other marine animals are mercury, cadmium and lead (Pb) (OSPAR 2015).

India has a long coastline with an estimated total estuarine area of 3.9 million ha, of which 1.24 million ha are considered potentially suitable for shellfish farming (Ayyappan and Diwan 2006). Currently, about 13% of this area is used for aquaculture (MPEDA 2018). In 2018, the total harvest of bivalve shellfish in
the country was ca. 133,000 tonnes (CMFRI 2019). Production is dominated by clams (76.3% of the 2018 production) followed by mussels (15.3%) and oysters (8.4%) (CMFRI 2019). Estuaries in the State of Kerala together contributed > 80,000 tonnes of shellfish in 2018. The main cultivation areas are concentrated in the Vembanad and Ashtamudi, two estuarine systems that open to the central Arabian Sea. The main species farmed in these estuaries are the green mussel (*Perna viridis*) and the edible oyster (*Crassostrea madrasensis*) (Chinnadurai et al. 2014; Mohamed et al. 2016).

The subject of trace metal concentrations in commercially harvested shellfish is very relevant but insufficiently studied in Kerala (George et al. 2013; ShibiniMol et al. 2015; Ragi et al. 2017). The evidence base is still insufficient to assess consumer exposure to trace metals linked to shellfish consumption. The Central Marine Fisheries Research Institute recognises that trace metals in fishery resources pose unique risk assessment challenges and identified the need to undertake a surveillance study and health risk assessment to address information gaps. Guidance for good shellfish farming practices requires local authorities to assess the sanitary status of the growing areas and implement short-term control measures (e.g. harvest prohibition) if contamination is elevated and areas are deemed unsuitable for health reasons (Mohamed et al. 2019). In this paper, we present new monitoring data on trace metals to support these risk assessments, namely concentrations of nickel (*Ni*), cobalt (*Co*), iron (*Fe*), manganese (*Mn*), zinc (*Zn*), lead (*Pb*) and copper (*Cu*) in three species of shellfish monitored in growing areas in the Vembanad and Ashtamudi estuaries. We compare the observed results with those previously reported in the literature, including concentration ranges and seasonality. We then discuss whether the observed concentrations exceed nationally and internationally set limits for these substances. Finally, we present data on the daily intake, reference dose and target hazard quotient (THQ) to indicate health risks posed by the trace metals. Baseline risk assessments for mercury and cadmium in these coastal ecosystems have been carried out by other researchers (Ramasamy et al. 2017; Sruthi et al. 2018; Seethal Lal et al. in press) and therefore these elements were not included in our monitoring programme.

**Methods**

**Sample collection**

Samples of the yellow clam (*Paphia malabarica*), green mussel (*Perna viridis*) and edible oyster (*Crassostrea madrasensis*) were collected monthly from growing areas in the Ashtamudi and Vembanad estuaries between June 2012 and December 2014 (Fig. 1). Upon collection, shellfish samples were washed with seawater, placed in cool boxes containing ice packs to keep temperature at < 4°C and transported to ICAR - Central Marine Fisheries Research Institute, Kochi for trace metal analyses. All samples were kept in storage for a maximum of 4 h prior to analyses.

**Sample preparation and analytical procedures**

In the laboratory, the shellfish were cleaned with Mill-Q water to avoid contamination with sediment. Shellfish flesh was removed from the shells with a clean shucking knife and the tissues were homogenised and digested using a microwave digestion system. The shucked soft tissues were then weighed and taken in a vessel for sample digestion.

A concentration of nitric acid:perchloric acid (9:4) was prepared and 7 ml of combined acid was added to each vessel for pre-digestion of samples and left overnight for soaking. After pre-digestion, the samples were digested using a high-performance microwave labstation (ETHOSPLUS) for 40 min at 200 °C. After cooling, the samples were diluted and made up to 50 ml using distilled water. The samples were filtered and stored under cool temperature until taken for analysis. The trace metals were analysed by inductively coupled plasma–optical emission spectrometry (ICP-OES) (Optima 4000 DV, Perkin Elmer). Standards of Ni, Co, Fe, Mn, Zn, Pb and Cu were prepared from the respective stock solutions (Merck). 1% HNO3 was used as a blank which was prepared in deionised water. The limit of detection of each element determined by using absorbance of the blank and the standard solution of 1 ppm is presented together with the recovery efficiency in Table 1. This ICP-OES method provides quantitative results with high accuracy, is relatively easy to operate (Sneddon and Vincent 2008) and is routinely used at ICAR.

**Health risk calculations**

Food consumption data were collected using a quantitative food frequency questionnaire to estimate the shellfish consumption rate for local communities. Fifty people with an average body weight of 65 kg were selected in the study area and asked to complete a questionnaire about their daily shellfish consumption. This information was collected through in-person (face-to-face) interviews. Based on the questionnaire results, the estimated mean consumptions of clams, mussels and oysters were 120, 150 and 58 g/person/day, respectively. It should be noted that shellfish consumption patterns differ considerably between regions and even between populations within the same region and therefore these values represent local consumption levels.

The health risk assessment comprised the estimated daily intake (EDI) and oral reference dose (RfD) as established by the US Environmental Protection Agency and the Joint FAO/World Health Organization (WHO) Expert Committee on Food Additives (USEPA 2011; FAO and WHO 2013). The RfD was used to evaluate the EDIs of metals in shellfish. The
EDI (μg/kg/day) was calculated using the following equation:

$$\text{EDI} = C_{\text{shellfish}} \times \left( \frac{d_{\text{shellfish}}}{\text{bw}} \right)$$

where

- $C_{\text{shellfish}}$ = average trace element concentration in shellfish (mg/kg wet weight)
- $d_{\text{shellfish}}$ = daily shellfish consumption (g/day) and
- $\text{bw}$ = the average body weight (kg) of the target population.

The oral RfDs were 0.04 (Cu); 0.004 (Pb); 0.3 (Zn); 0.14 (Mn); 0.02 (Ni); 0.03 (Co); and 0.7 (Fe) mg/kg/day (USEPA 2015). These oral RfDs are based on the safe upper level of trace metal oral intake for an adult with an average body weight of 65 kg. The target hazard quotient (THQ) was calculated using the following equation: $\text{THQs} = \text{EDI/RfD}$.

A THQ ≤ 1.0 was considered to represent no obvious adverse health effects (and therefore a negligible hazard) as a result of exposure to trace metals from shellfish consumption. A THQ > 1.0 was considered to represent an increasing risk of adverse health effects (Anandkumar et al. 2018).

**Statistical analyses**

Descriptive statistics (annual and seasonal means and standard deviations) were determined for trace metal concentrations.
Analyses of variance followed by post hoc Tukey HSD tests were carried out to determine if there were any significant differences in mean concentrations between seasons. The seasonal periods considered in the analyses were as follows: pre-monsoon (February–May), monsoon (June–September) and post-monsoon (October–January). Statistical tests were assessed at 95% and 99% confidence levels. The statistical analyses were carried out using R software.

**Results and discussion**

The yellow-foot clam, the green mussel and the edible oyster are widely harvested for human consumption in Kerala estuaries (Chinnadurai et al. 2016, 2020). These shellfish provide high-quality protein with essential amino acids for maintenance and growth of the human body and are therefore good complements of a healthy diet for local communities (Chakraborty et al. 2016a, 2016b; Joy and Chakraborty 2017; Krishnan et al. 2019). Annual and seasonal mean concentrations of Ni, Co, Fe, Mn, Zn, Pb and Cu in shellfish tissues are presented in Table 2. Health risk threshold concentrations used internationally are summarised in Table 3.

Considering the three shellfish species together, the ranges of mean annual concentrations for individual trace metals were as follows: Ni (0.46–0.65); Co (2.87–3.49); Fe (80.0–119.4); Mn (3.88–9.38); Zn (40.8–76.2); Pb (1.28–2.00); Cu (1.59–4.38) (all in mg/kg).

Many studies worldwide have reported substantial differences in mean concentrations of trace metals between species (e.g. Maanan 2008; Sakellari et al. 2013). These differences could be due to differences in trophic level (Chen et al. 2000), size (Páez-Osuna and Marmolejo-Rivas 1990), seasonal factors (Swaileh 1996; Othere 2003; El-Moselhy and Yassien 2005) and productivity levels in the growing waters (de Mora et al. 2004; Pinto et al. 2015; Lino et al. 2016). In Ashtamudi, clams were more contaminated with trace metals than oysters, except for Zn and Cu. This result supports the use of clams as an indicator species in the shellfish growing area monitoring programme because they occupy the broadest range of habitats and, on average, reflect greater health risk.

Statistically significant seasonal differences were found in mean concentrations for some metals (Table 2). In clams, mean concentrations of Zn and Cu were significantly higher in the monsoon and pre-monsoon periods, respectively. In mussels, Co and Mn concentrations were significantly higher in the pre-monsoon period and Pb and Cu concentrations were significantly higher in the monsoon period. In oysters, Fe and Mn concentrations were significantly higher in the pre-monsoon period.

Seasonal variations of trace metal concentrations in shellfish has been reported in many studies (Boyden and Phillips 1981; Swaileh 1996; Hung et al. 2001; El-Moselhy and Yassien 2005; KrishnaKumari et al. 2006; Maanan 2008; Belabet al. 2013; Pinto et al. 2015). Boyden (1974) reported that the metabolic rates of shellfish vary with size, season and the extent of contamination in the environment. The seasonality in metal concentrations found in the present study could be associated with changes in the weight of soft tissues of the oysters, which in turn are related to their gametogenesis-spawning cycle (Boyden and Phillips 1981). This is in agreement with many other studies which identify the reproductive cycle and food availability as drivers of seasonality in trace metal concentrations (Páez-Osuna et al. 1995; Swaileh 1996; Othere 2003; Maanan 2008; Pinto et al. 2015). Rainfall intensity is also likely to influence changes in trace metal concentrations in estuaries (Belabet al. 2013) and is certainly the cause of elevated trace metal concentrations detected during the monsoon period. Elevated pre-monsoon concentrations may be associated with lower water flows and longer resident times of the waters in the estuaries due to the closure of the bar mouths (Krishnakumar et al. 2006) and the effect of pollution sources on the shoreline near the shellfish growing areas. These sources are well characterised in State of the Environment reports (KSCSTE 2007).

**Iron**

Fe was the most abundant trace metal in the three species of shellfish tested. Concentrations of this metal ranged from 37.3 to 195.6 mg/kg. The health risk threshold for this element set out by the WHO is 100 mg/kg (Table 3). The highest mean annual concentration was detected in clams (119.4 ± 38.7 mg/kg) while the lowest was detected in mussels (80.0 ± 35.2 mg/kg). Similar results have been reported for Villorita cyprinoides, P. viridis and Perna indica from the Vembanad and Ashtamudi estuaries (George et al. 2013; Ragi et al. 2017; Yahiya et al. 2018). In oysters and clams, the gradients of seasonal Fe concentrations were as follows (from the highest to the lowest): pre-monsoon > post-monsoon > monsoon; while in mussels the gradient was as follows: pre-monsoon > monsoon > post-monsoon (Table 2).

The mean Fe concentrations in clams were higher than those reported by KrishnaKumari et al. (2006) (65.7–260 μg/g dry wt) but lower than those detected Parvez Al-Usmani et al. (2015) in Goa (1205–2506 ppm, dw). Fe concentrations in mussels were lower than those reported by Rivaneker and Parulekar (1998) (1900–4200 μg/g dry wt) and Parvez Al-Usmani et al. (2015) (1906–2802 ppm, dw) in Goa, by Krishnakumar et al. (2006) from Karwar, Karnataka (96.4–286.5 μg/g wet wt) and by Satheeswaran et al. (2019) in the Vellar Estuary, East coast of India (132 mg/kg wet wt). Fe concentrations in mussels were however similar to those found at sites along the Calicut and Mangalore coasts of India (15.7–90.6 mg/kg wet wt) (Krishnakumar et al. 2006; Sasikumar et al. 2006; Sasikumar et al. 2011). Fe
concentrations in oysters (C. madrasensis) were lower than those reported by Parvez Al-Usmani et al. (2015) in Saccostrea cucullata from Goa, west coast of India. The iron concentration recorded in C. madrasensis in the present study was similar to those of S. cucullata from Bombay coast (KrishnaKumari et al. 1992) (312±160 mg/kg dry wt) and in Ostrea edulis from east coast of India (80.49 mg/kg wet wt) (Satheeswaran et al. 2019).

Zinc

Zn was the second most abundant trace metal detected in the shellfish samples. Zn concentrations ranged widely from 10.0 to 149.8 mg/kg. The highest mean annual concentration was detected in oysters (76.2 mg/kg) and the lowest was detected in mussels (40.8 mg/kg). These concentrations are similar to

Table 3 Mean trace metal concentrations in shellfish from Ashtamudi and Vembanad estuaries and risk threshold concentrations

| Trace metal | Mean concentration (this study) (mg/kg) | Threshold concentration (mg/kg) |
|-------------|----------------------------------------|---------------------------------|
| Ni          | 0.59±0.39 0.65±0.45 0.46±0.29         | 2 0.15                          |
| Fe          | 119.4±3.0 80.0±3.0 91.7±3.0           | 100 1.5                         |
| Mn          | 6.0±1.5 9.38±3.0 3.88±1.5             | 1 0.75                         |
| Zn          | 48.1±3.0 40.8±3.0 76.2±3.0            | 50 100 50                       |
| Pb          | 2.00±0.1 1.28±0.2 1.87±0.2            | 2 1.5 2.5                       |
| Cu          | 2.58±0.2 1.59±0.2 4.38±0.2            | 3 30 30                         |

1 World Health Organization (1993)
2 European Commission (2006)
3 Malaysian Food Regulation (1985)
4 Food Safety and Standards Authority of India (2011)
those reported in other studies undertaken in India (8.7–151.2 mg/kg) (Lakshmanan and Nambisan 1983; Rivanker and Parulekar 1998; Krishnakumar et al. 2006; Sasikumar et al. 2006, 2011; Ragi et al. 2017). However, some studies have found comparatively lower concentrations of Zn in shellfish from sites on the East coast of India (Senthilnathan et al. 1998; Sarkar et al. 2008; Asha et al. 2010; Satheeswaran et al. 2019).

The maximum permissible limit for this element by both the FSSAI and WHO is 50 mg/kg (Table 3). This limit is higher than the mean annual concentrations detected in clams (48.1 mg/kg) and mussels (40.8 mg/kg) but lower than the mean detected in oysters (76.2 mg/kg). Therefore, there is no apparent Zn risk from consuming clams from Ashtamudi or mussels from Vembanad.

**Manganese**

Mn concentrations ranged from 0.32 and 14.6 mg/kg. The WHO sets out a risk threshold of 1 mg/kg for this element (Table 3). The highest concentration was found in clams (14.6 mg/kg) and the lowest in oysters (0.32 mg/kg) (Table 2). Similar concentrations have been reported in V. cyprinoides from the Vembanad Estuary (Babukutty and Chacko 1992). Mn concentrations in clams, mussels and oysters from the Ashtamudi and Vembanad estuaries were similar to those found in oysters from the Bombay coast (28 ± 21 mg/kg dry wt) (KrishnaKumari et al. 1992) and in mussels and oysters from the Karnataka coast (6.67 ± 0.24 and 6.80 ± 0.76 mg/kg wet wt) (Krishnakumar et al. 1990, 1998; Sasikumar et al. 2006), in clams from the Gulf of Mannar (27.88 ± 3.7 mg/kg dry wt) (Asha et al. 2010), in mussel from the Vellar estuary, East coast of India (13.08 mg/kg wet wt) (Satheeswaran et al. 2019), in clams from Sunderban (32.02 mg/kg dry wt) (Saha et al. 2006) and in oysters from Andaman coastal waters (3.88 ± 0.27 mg/kg wet wt) (Seetharaman et al. 2015).

**Copper**

Cu concentrations varied widely between species. In oysters, Cu concentrations ranged from 0 to 13.1 mg/kg while in clams and mussels Cu concentrations ranged from 0 to 6.95 mg/kg and from 0 to 4.67 mg/kg, respectively. The FSSAI prescribes a limit of 30 mg/kg (Table 3) which is relatively higher than the maxima detected in this study. The WHO estimate for daily Cu intakes via food is 2–30 mg/kg (WHO 1993) and therefore we can conclude that there is no risk of Cu due to consumption of shellfish from the Ashtamudi and Vembanad estuaries. Cu concentrations detected in this study were generally lower than those previously reported for sites in the Ashtamudi and Vembanad estuaries (Lakshmanan and Nambisan 1983; Babukutty and Chacko 1992; Kaladharan et al. 2005; George et al. 2013) and similar to those reported by ShibiniMol et al. (2015) and Ragi et al. (2017). A few studies have however reported higher concentrations in shellfish from the Ashtamudi and Vembanad estuaries (Lakshmanan 1988; Sivaperumal et al. 2007).

**Lead**

Pb concentrations ranged from 0 to 5.86 mg/kg in clams, from 0 to 3.67 mg/kg in mussels and from 0 to 4.27 mg/kg in oysters. The maximum permissible limit for Pb set by the WHO (1993) and MFR (1985) is 2 mg/kg (Table 3). According to EC (2006) and FSSAI (2011), Pb concentrations in shellfish should not exceed 1.5 and 2.5 mg/kg, respectively (Table 3). Mean annual concentrations found in this study were 2 mg/kg in clams from Ashtamudi, 1.28 mg/kg in mussels from Vembanad and 1.87 mg/kg in oysters from Ashtamudi and are therefore below the FSSAI limit. Concentrations were however generally higher than those reported previously for these estuaries (0.22–6.63 mg/kg) (Lakshmanan and Nambisan 1983; Babukutty and Chacko 1992; Kaladharan et al. 2005; Sivaperumal et al. 2007; George et al. 2013; ShibiniMol et al. 2015; Ragi et al. 2017). Higher concentrations than those presented here have been reported in clams from the Mandovi Estuary (Goa) (KrishaKumari et al. 2006; Patra et al. 2019).

**Nickel**

Ni concentrations ranged from 0.10 to 1.04 mg/kg in clams, from 0.10 to 1.42 mg/kg in mussels and from 0.43 to 0.95 mg/kg in oysters. The maximum permissible limits for Ni prescribed by the WHO and FSSAI are 2 and 1.5 mg/kg, respectively (Table 3). Therefore, Ni concentrations in mussels were well below these thresholds. Ni concentrations in mussels from northern Vembanad Estuary were higher than those in clams and oysters from the Ashtamudi Estuary. This result is consistent with results published by Yap et al. (2016) and supports the assumption that mostly anthropogenic sources contribute to the Ni contamination in the Vembanad estuary.

The Ni concentrations in shellfish found in this study were comparable to those reported previously for sites in the Ashtamudi and Vembanad estuaries and other sites on the east and west coasts of India (Sankar et al. 2006; Sasikumar et al. 2006, 2011; Asha et al. 2010; Satheeswaran et al. 2019). However, the mean Ni concentration in clam (V. cyprinoides) from Vembanad estuary reported by ShibiniMol et al. (2015) was five times higher than Ni concentrations recorded for this species in this study, while concentrations reported by Ragi et al. (2017) were two times lower compared to those reported here.
Cobalt

Co concentrations ranged from 0.33 to 9.42 mg/kg in clams, from 0.18 to 7.53 mg/kg in mussels and from 0.19 to 7.20 mg/kg in oysters. The WHO has estimated daily Co intakes via food of 5–45 μg/day (WHO 1993). The concentrations of Co found in shellfish from the Ashtamudi and Vembanad are well below this limit. However, Co concentrations are higher than those reported by Sivaperumal et al. (2007), George et al. (2013) and Ragi et al. (2017) for the Ashtamudi and Vembanad estuaries. Lower concentrations (6.58 mg/kg wet wt) were reported by Babukutty and Chacko (1992).

Health risk assessment

The oral RfD ranged from 0.004 (Pb in clams and oysters; Zn in mussels) to 0.700 (Fe in the three species) (Table 4). Mean annual EDIs ranged from 0.071 (Ni) to 14.330 (Fe) in clams, from 0.098 (Ni) to 12.010 (Fe) in mussels and from 0.027 (Ni) to 5.323 (Fe) in oysters (all in mg/day). The THQ in clams ranged from 0.044 (Mn) to 1.005 (Pb) in clams, from 0.081 (Ni) to 0.800 (Pb) in mussels and from 0.022 (Ni) to 0.452 (Pb) in oysters. THQs varied between seasons and were generally higher in pre-monsoon and monsoon periods.

Annual and seasonal THQs due to intake of trace metals through shellfish consumption in coastal populations are presented in Table 4. Overall, the THQs were ≤1, except for two Pb results in P. malabarica in the pre-monsoon period (1.82) and annual (1.00) (Table 2). Similarly, the THQs in P. viridis were low, characteristic of products acceptable for human consumption, except for the Pb in the monsoon season (1.4) (Table 2), indicating no health hazard in local population due to mussel consumption. In C. madrasensis, THQs were ≤1 for all metals and in all seasons, also indicating no health hazard from oyster consumption to consumers.

Comparison with previous studies

Accumulations of metals in shellfish are associated with site-specific metal bioavailability, filtering capacity, sexual

| Site (species) | Trace metal | Oral reference dose (RfD) (mg/kg/day) | Estimated daily intake (EDI, mg/day) | Target hazard quotient (THQ) |
|---------------|-------------|--------------------------------------|-------------------------------------|-----------------------------|
|               |             | Pre-monsoon (February–May) | Monsoon (June–September) | Post-monsoon (October–January) | Annual Pre-monsoon (February–May) | Monsoon (June–September) | Post-monsoon (October–January) | Annual |
| Ashtamudi Estuary (P. malabarica) | Cu | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 |
| | Pb | 0.004 | 0.300 | 0.140 | 0.020 | 0.030 | 0.700 | 0.040 | 0.040 | 0.040 |
| | Zn | 0.300 | 0.478 | 0.064 | 0.115 | 0.811 | 0.700 | 0.300 | 0.300 | 0.300 |
| | Mn | 0.140 | 8.273 | 0.084 | 0.479 | 18.81 | 0.040 | 0.003 | 0.003 | 0.003 |
| | Ni | 0.020 | 0.259 | 0.098 | 0.479 | 12.33 | 0.400 | 0.004 | 0.004 | 0.004 |
| | Co | 0.030 | 0.215 | 0.068 | 0.582 | 14.33 | 0.120 | 0.012 | 0.012 | 0.012 |
| | Fe | 0.700 | 4.100 | 0.071 | 0.043 | 0.044 | 0.044 | 0.044 | 0.044 |
| |     |    |    |    |    |    |    |    |    |    |
| Vembanad Estuary (P. viridis) | Cu | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 |
| | Pb | 0.004 | 0.062 | 1.725 | 0.020 | 0.030 | 0.700 | 0.040 | 0.040 | 0.040 |
| | Zn | 0.300 | 0.336 | 1.359 | 0.099 | 0.080 | 17.81 | 0.040 | 0.003 | 0.003 |
| | Mn | 0.140 | 11.019 | 1.212 | 0.120 | 0.119 | 12.33 | 0.400 | 0.004 | 0.004 |
| | Ni | 0.020 | 11.019 | 1.212 | 0.120 | 0.119 | 12.33 | 0.400 | 0.004 | 0.004 |
| | Co | 0.030 | 0.120 | 0.099 | 0.120 | 0.120 | 0.120 | 0.120 | 0.120 | 0.120 |
| | Fe | 0.700 | 14.330 | 12.010 | 1.407 | 1.407 | 1.407 | 1.407 | 1.407 | 1.407 |
| Vembanad Estuary (C. madrasensis) | Cu | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 | 0.040 |
| | Pb | 0.004 | 0.062 | 1.725 | 0.020 | 0.030 | 0.700 | 0.040 | 0.040 | 0.040 |
| | Zn | 0.300 | 0.336 | 1.359 | 0.099 | 0.080 | 17.81 | 0.040 | 0.003 | 0.003 |
| | Mn | 0.140 | 11.019 | 1.212 | 0.120 | 0.119 | 12.33 | 0.400 | 0.004 | 0.004 |
| | Ni | 0.020 | 11.019 | 1.212 | 0.120 | 0.119 | 12.33 | 0.400 | 0.004 | 0.004 |
| | Co | 0.030 | 0.120 | 0.099 | 0.120 | 0.120 | 0.120 | 0.120 | 0.120 | 0.120 |
| | Fe | 0.700 | 14.330 | 12.010 | 1.407 | 1.407 | 1.407 | 1.407 | 1.407 | 1.407 |
maturity and body size and their position in water column (McConchie and Lawrance 1991; Che ne et al. 2000). Table 5 compares concentrations of trace metals in shellfish detected in the present study with those reported in other recent studies performed in the same study areas. Generally, the results indicate greater concentrations of Ni and Fe in the Vembanad Estuary than in the Ashtamudi Estuary. Comparing data reported only for Ashtamudi, the present study found higher concentrations of Co, Fe, Mn and Pb than previous studies. For Vembanad Estuary, concentrations of Fe and Mn were also higher in the present study. However, on an individual trace metal level, the concentrations are not markedly higher/lower than those reported previously suggesting no substantial changes in the amounts of metal bioavailability over time in the study areas.

### Conclusions

Essential trace metals present in shellfish such as Fe, Cu and Zn play important roles in biological systems when present at low concentrations (Hogstrand and Haux 1991). However, when trace metals exceed certain concentrations, including essential and non-essential elements such as Pb and Cd, can cause toxicity. In the present study, we monitored concentrations of Ni, Co, Mn, Pb, Cu, Zn and Fe in three species of shellfish (clams *P. malabarica*; mussels *P. viridis*; oysters *C. madrasensis*) sampled from the Ashtamudi and northern Vembanad estuaries, the most important shellfish growing areas in Kerala. We estimated the daily intake of trace metals due to shellfish consumption for an adult as compared to the oral reference dose recommended by the USEPA (2015). We found that concentration ranges for most trace metals were below the health risk thresholds set out by national and international guidelines and therefore consumption of shellfish from the study areas does not pose a health risk to consumers concerning these elements. However, some of the observed concentrations of Fe and Zn were slightly higher than the permissible limits. While this study adds baseline information on the range of trace metal concentrations that may be found in commercially harvested shellfish, further studies are needed to characterise exposure pathways and identify sensitive (sub) populations.

### Abbreviations

- **THQ**: Target hazard quotient; **EDI**: Estimated daily intake; **RfD**: Oral reference dose; **FSSAI**: Food Safety and Standards Authority of India; **WHO**: World Health Organization; **EC**: European Commission; **MFR**: Malaysian Food Regulation

### Acknowledgements

S. Chinnadurai gratefully acknowledges fellowship received from the National Agricultural Innovation Project (NAIP) of the World Bank (P. Code 2000035102). The authors are grateful to the Director of the Central Marine Fisheries Research Institute (ICAR-CMFRI) for logistical and technical support and advice during this study. Thanks also due to the Director of the Central Institute of Fisheries Technology (ICAR-CIFT) and Head of the Quality Assurance and Management Division for the trace metal analyses.

### Author contributions

SC: conceptualization; methodology; validation; investigation; formal analysis; writing—original draft; writing—review

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**Table 5** Concentrations of trace metals in shellfish from the Ashtamudi and Vembanad estuaries reported in the present and previous recent studies

| Site                  | Species          | Sampling period | Ni  | Co  | Fe  | Mn  | Zn  | Pb  | Cu  | Reference                                      |
|-----------------------|------------------|-----------------|-----|-----|-----|-----|-----|-----|-----|------------------------------------------------|
| Ashtamudi Estuary     | *Perna viridis*  | 2003            | -   | -   | -   | -   | -   | -   | -   | 88.81 14.91 4.20 4.20 Varshney (2003)          |
|                       | *Villorita cyprinoides* | ns      | 0.47| 0.22| 12.76| 0.97| 13.15| 0.35| 2.40 Ragi et al. (2017)*                     |
|                       | *Perna viridis*  | ns              | 0.20| 0.25| 9.97| 0.79| 14.14| 0.31| 1.71                                         |
|                       | *Perna indica*   | 2017            | -   | -   | 12.93| -   | -   | -   | 0.87 - Yahiya et al. (2018)                   |
|                       | *Paphia malabarica* | 2012-2014 | 0.59| 3.42| 119.4| 6.0 | 48.1 | 37.2 | 1.85 Present study                           |
|                       | *Crassostrea madrasensis* | 2012-2014 | 0.46| 2.87| 91.7 | 3.88| 76.2 | 1.87 | 4.38 Present study                           |
| Vembanad Estuary      | *Sunetta scripta*| 1990-1998       | -   | -   | -   | -   | -   | -   | 3.74 0.5 0.61 Kaladharan et al. (2005)*       |
|                       | *Perna viridis*  | 2003            | 0.89| 0.17| -   | 0.43| 37.7 | 0.37| 11.7 Sivaperumal et al. (2007)                |
|                       | *Villorita cyprinoides* | 2003 | 0.76| 0.06| -   | 0.46| 18.5 | 0.32| 3.9                                          |
|                       | *Villorita cyprinoides* | 2007-2008 | -   | -   | -   | -   | -   | -   | 55.1 1.17 3.22 Raveenderan and Sujatha (2011) |
|                       | *Villorita cyprinoides* | 2010-2011 | 0.56| 1.07| 67.69| -   | 3.83 | 0.12| 0.32 George et al. (2013)*                   |
|                       | *Villorita cyprinoides* | 2013   | 3.79| -   | -   | -   | 22.13| 1.15| 2.72 ShibiniMol et al. (2015)*                |
|                       | *Perna viridis*  | 2012-2014       | 0.65| 3.49| 80.0 | 9.38| 40.8 | 1.28| 1.59 Present study                           |

*Data reported in mg/kg dry weight converted to mg/kg wet weight using a conversion factor of 0.17 (Yap et al. 2016)

*ns* not specified. Bold entries mean concentrations exceeding the FSSAI limits for Ni, Zn, Pb and Cu (see also Table 3)
and editing. CJAC: conceptualization; writing—review and editing. VG: statistical analysis; writing—review and editing. VK: conceptualization, funding acquisition. KSM: conceptualization; writing—review and editing; project administration

**Funding** This work was financially supported by the ICAR-NAIP (National Agricultural Innovation Project), Ministry of Agriculture and Farmer Welfare, Government of India, New Delhi.

**Data availability** The data that support the findings of this study are available on request from the corresponding author.

**Competing interests** The authors declare no competing interests.

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