

Full Length Research Paper

Accumulation of some potentially toxic metals and polycyclic aromatic hydrocarbons (PAHs) in marine clam *Liochoncha ornata* collected from the Omani Sea

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Clam samples of *Liochoncha ornata* were collected for a period of one year from July 2009 to June 2010 at monthly interval. The soft tissue of clams was analyzed to detect some potentially toxic metals and polycyclic aromatic hydrocarbons (PAHs). Accumulation of Hg, Cd and Pb in the clam tissues showed variability with a mean concentration of 0.011 ± 0.009 , 1.769 ± 0.942 and 0.015 ± 0.039 $\mu\text{g/g}$ wet weight for Hg, Cd and Pb, respectively. Cadmium had recorded a particularly high potential of accumulation in *L. ornata*. The bioaccumulation of PAHs in the clams appeared to be selective and ranged from 4.80 to 12.0 ng/g; wet weight. The most carcinogenic PAHs, such as benzo (a) pyrene and dibenz (a,h) anthracene, were in all cases below the limits of detection. No distinctive relationship was found between different size class and contaminant uptake by the clam. The concentrations of both of Pb and Hg in *L. ornata*, were found to be below the safe limits suggested by various authorities and thus gave no indication of pollution by such elements. However, continuous monitoring of these and other pollutants in the coastal marine ecosystem is advised.

Key words: Clam, Mercury, Cadmium, Pb, polycyclic aromatic hydrocarbons (PAHs), Oman.

INTRODUCTION

Enormous quantities of noxious pollutants have been released into marine ecosystems over the last few decades. Among these pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs) represent major pollutants of the marine environment (Onen et al., 2011; Li et al., 2013). Marine organisms tend to accumulate different dietary and waterborne contaminants including heavy metals, PAHs and others from the environment which they live in (de Mora et al., 2005; Fowler et al., 2007; Serpe et al., 2010). Heavy metals and PAHs are introduced into the environment via natural and anthropogenic processes (Connell et al., 1999; Perez-

Gregorio et al., 2010). Cd, Pb and Hg are potentially toxic metals and the well-known Minamata disease, the Itai Itai disease and Saturnism have shown the severe effects of these toxic metals on human health. PAHs are known to be toxic, genotoxic and carcinogenic, and to bioaccumulate (Siu et al., 2004; Kumosani et al., 2013). Benzo[a]pyrene, a carcinogen and teratogen that is included in the 16 PAHs designated by United States Environmental Protection Agency as priority contaminants (Akcha et al., 2000; Serpe et al., 2010). In marine environments, bivalves are common, highly visible, and ecologically and commercially important on a

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global scale as food and nonfood resources. Because of their benthic and sedentary mode of life, they are easily exposed to environmental pollution (heavy metals, persistent organic pollutants, etc.) and bioaccumulate these pollutants. Therefore, bivalves are usually used as models in the field of environmental toxicology (Rittschof and Mc Clellan-Green, 2005). The clam *L. ornata*, which is abundant and widely distributed along the coastal and estuarine areas of East Asia, is an important commercial marine bivalve in Oman. It mainly inhabits sandy or muddy substrates in the marine lower intertidal and shallow sub tidal areas, which are particularly susceptible to be impacted by PAHs from coastal oil spills and toxic metals.

In the Gulf region, the influx of tankers and offshore oil operations, intentional or accidental oil spills, ballast water discharging, dredging and infilling for coastal development, uncontrolled sewage and industrial wastewater discharges are important sources of pollution (Essa et al., 2005). Maintaining good marine environmental quality in the Gulf region is crucial for several socioeconomic reasons. The region relies heavily upon the seawater itself as a source of fresh water through desalination (Price et al., 1993). Abundant seafood, particularly fish and shrimp, are valuable for both local consumption and export revenue. Most of

Oman's population resides in coastal areas and fish is a common diet to them. However, the relatively fragile Gulf ecosystem experiences high temperatures, salinity and UV exposure and, as a result, many species function close to their physiological limits (Sheppard, 1993) and any added stress imposed by contaminants could have severe consequences. Such a problem can be enhanced given that contaminant inputs

undergo less dilution and slower dispersion than would occur in open marine systems because the semi-enclosed Gulf has poor flushing characteristics (Sheppard, 1993). To ensure a supply of clean, contaminant-free portable desalinated water and healthy and safe seafood, monitoring of contaminants in different marine matrices has recently emerged as a necessity. These new results for clams contribute importantly to the regional database for the Gulf region, especially as regards the Omani Sea.

There have been only a few studies published concerning organic and inorganic levels observed in fish, shellfish and sediments (Al-Hashami and Al-Zorba, 1991; Al-Sayed et al., 1994; Fowler et al., 1993; de Mora et al., 2005; Al-Busaidi et al., 2011) on the Gulf and Gulf of Oman environment. However, efforts have mainly focused on metal concentrations, and the bioaccumulation and temporal distribution of heavy metals and polycyclic aromatic hydrocarbons have been much less studied. The aim of this work was to investigate the real situation in terms of accumulation and temporal distribution of the contamination by selected potential toxic metals and PAHs in the clam (*L. ornata*) collected from the Omani Sea Coast.

MATERIALS AND METHODS

Sampling plan

Al Batinah is located at about 24°0'0" N 57°0'0"E. A narrow, well-populated coastal plain and it occupies an important location on the coast of the Omani Sea. Shohar in Al-Batinah region represents the major clam fishing ground. The sampling stations along the coastline in Al-Batinah regions for clams in the Omani Sea were established in Figure 1. The clams were collected monthly during July 2009 to June 2010 by fishermen. About 100 to 150 individuals of clam samples were collected with different shell size from a wide spread well sorted fine sand in shallow waters of the Sohar region. After all samples were collected, they were washed with clean seawater at the point of collection, and placed in a clean plastic bag and transported in chilled conditions to the laboratory with proper labeling. Samples were frozen at -20°C until sample preparation.

Sample preparation

For analysis, after thawing, the samples were divided into small (20 to 25 mm), medium (26 to 30 mm) and large (>30 mm) based on shell length. The total weight of each individual was measured to the nearest 0.1 g, and the shell length of each individual clam was determined to the nearest 0.1 mm using Vernier calipers. The soft body parts of individuals were removed carefully deshelling the clam with a knife and then rinsed. All samples were packed separately in polyethylene bags with proper labeling and used for analysis.

Chemicals and reagents

All reagents were analytical reagent grade and milliQ 18 mΩ pure water (MilliQ, Millipore, USA) was used for preparation of reagents and standards. Calibration standards of Hg, Cd and Pb were purchased from Merck, Germany. The standard solutions for calibration were prepared from a stock solution of 1000 mg/L by successive dilutions with 1.5% nitric acid. The standards were diluted appropriately and used to calibrate the Inductively Coupled Plasma Atomic Emission Spectrometer (ICP AES) and direct mercury analyzer (DMA). All glassware and other containers were thoroughly cleaned with 1.5% (w/v) nitric acid solution then rinsed with MilliQ water several times and air-dried prior to use. PAH standards were purchased from Supelco, USA. QuEChERS extraction salts were purchased from Restek, USA.

Toxic metals analysis

Apparatus

Samples were digested using microwave lab station (Milestone ETHOS PLUS, Italy) of 2450 MHz, frequency magnetron and 1100 W maximum power equipped with TFM (fluoroplastic) closed vessels. The power system used with the focused microwave apparatus provides continuous microwave emission at each power level. The system is a closed vessel microwave apparatus, but it is equipped with a fume extraction system.

Toxic metals extraction and analysis

For each sample, 0.4 g of homogenized clam tissue (wet weight) was accurately weighed and transferred into a 25 ml quartz vessel together with 5 ml of concentrated (65%) nitric acid (HNO₃) and 1 ml of (35%) hydrogen peroxide (H₂O₂). The extraction vessel was placed in the microwave digestion system and the digestion



Figure 1. Study site.

Table 1. ICP-AES Instrument operating conditions.

| Parameter | |
|---------------------------|----------------|
| View Direction | Axial |
| Position | Low |
| Torch | Mini |
| Gas | Argon |
| Plasma gas | 10 L/min |
| Auxiliary gas | 0.6 L/min |
| Carrier gas | 0.7 L/min |
| Source equilibration time | 15 s |
| Pump speed | 20.0 |
| Pump flow rate | 1 mL/min |
| Detector | CCD |
| RF power | 1200 W |
| Nebulizer | 0.7 L/min |
| Sample aspiration rate | 1 ml/min |
| Read | Peak intensity |
| Number of replicates | 3 |
| Exposure time | 30 s |
| Solvent rinse | 35 s |
| Sample rinse | 40 s |

program was run in two steps (step (1) 25-180°C for 30 min at 1000W; step (2) 180°C for 15 min at 1000W). All the digested samples were made up to 25 ml with milliQ water in pre acid washed standard flasks and used for analysis. Cd and Pb were analyzed by Inductively Coupled Plasma Optic Emission Spectrometer (ICP AES, ICPE-9000, Shimadzu, Japan) at 220 nm

for cadmium and 210 nm for lead. The operating conditions for the ICP AES are specified in Table 1. The calibration equations and linear relation coefficients are $y=850.81x + 0.8629$ ($r^2=0.9997$) and $y=50.187x + 0.6152$ ($r^2=0.9998$) for Cd and Pb, respectively. For mercury, 0.1 g of clam soft tissue was accurately weighed in sample boat and total mercury was determined by direct mercury analyzer (DMA-80, Milestone, Italy) without further sample preparation. The calibration equation and linear relation coefficient are $y=0.0393x + 0.0328$ and $r^2=0.99$, respectively.

PAHs extraction and analysis

Extraction and analysis of PAH were carried out according to the method by Ramalhosa et al. (2009) included naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene, dibenzo(ah)anthracene, benzo(ghi) perylene and indeno (1,23-cd) pyrene. For the analysis, approximately 0.5 g of homogenized clam tissues was transferred to the QuEChERS column and 8 ml of ACN was added and the column contents were mixed for three minutes using a vortex mixer (Sibata, Japan). After centrifugation in a centrifuge (Kokusan, H-3R, Japan) during 3 min at 3400 RPM, the extract was recovered. Prior to injection into the high performance liquid chromatography (HPLC) system, supernatant was filtered through a 0.20 micron PTFE membrane filter into an HPLC vial and used for analysis. PAHs were analyzed by HPLC with FL and UV detectors (Agilent, 1100 series). Separation of the compounds was performed in a PAH column (Supelcosil LC PAH, 250 x 4.6 mm; 5 µm particle size; Bellefonte, PA, USA) maintained at 22±1°C. The injection volume was 40 µl. Acetonitrile (solvent A) and water (solvent B) were used as a mobile phase at the flow rate of 0.8 ml/min. The initial composition of the mobile phase was 50% ACN and 50% water and a linear gradient to 100% was programmed in 17 min, with a final hold of 20 min. Initial conditions were reached in

2 min and maintained for 6 min before next run. Fluorescent detection was conducted at six different wavelengths of excitation and emission and each compound was detected at its optimum excitation/emission wavelength pair: 315/260 nm (naphthalene and fluorine), 366/260 nm (phenanthrene), 430/260 nm (anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene dibenzo(ah)anthracene, benzo(ghi) perylene and 505/290 nm indeno (1,23-cd) pyrene. Acenaphthylene and acenaphthene determinations were done with UV detector at 230 nm. PAH identification was carried out with an individual stock solution of each PAH by comparison of their retention times with those of the standards. The concentration of the studied compounds was determined by comparing peak areas in the sample with those found in mixtures of PAH standards of known concentration analyzed in the same condition. External calibrations with PAHs mixed standards, using at least 6 calibration points, were performed. Suitability of the method was checked by recovery tests of fortified clam samples (Table 2) that showed mean recovery between 80 percent and 110 percent of the individual PAHs. LODs between 0.05 ng/g wet weight (benz(a)anthracene) and 0.67 ng/g wet weight (naphthalene) were obtained. Concerning the maximum level of 5 ng/g wet weight established by the European Union for benzo (a) pyrene in bivalves, the LOD attained is sufficiently low for the method to be used for the monitoring purposes.

Quality control

All samples were taken in triplicates and all measurements were run in triplicates for standard and samples. Metal and PAH concentrations were calculated and expressed in $\mu\text{g/g}$ and ng/g wet weight, respectively. The analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Accuracy of the analytical method was monitored by analyzing certified reference material (Mussel tissue - NIST SRM 2976) and fortification. The standard reference material was analyzed during each batch and the heavy metal recovery rates were 84% for Cd, 93% for Hg and 89% for Pb. A recovery test was carried out by spiking of standard solutions of PAHs in homogenized samples. The recoveries for polycyclic aromatic hydrocarbons during these experiments were found to be between 80 to 110%, no batches were outside of these limits.

Statistical analysis

Results of heavy metal concentrations and PAHs were analyzed using SPSS 19.0 (SPSS INC., Chicago, IL) software. One-way and two-way ANOVA and Duncan multiple range were used to examine differences among, temporal and size class and relationship among metals. All statistical analyses were performed at the 5% critical level.

RESULTS AND DISCUSSION

Grand mean metal concentration

The annual mean concentrations of potentially toxic metals in the clam collected from the Al - batina governorate of the Omani Sea from July 2009 to June 2010 were $0.011 \pm 0.009 \mu\text{g/g}$ wet weight of mercury, $1.769 \pm 0.942 \mu\text{g/g}$ wet weight for cadmium and $0.015 \pm 0.039 \mu\text{g/g}$ wet weight for lead, respectively

(Table 3). The contents of these toxic metals in the clam reflected the variations and natural availability for accumulation by clam in Al-batinah governorate. The pattern of metal occurrences, in order of decreasing concentrations in *L. ornata*, were almost like $\text{Cd} > \text{Pb} > \text{Hg}$. There are several published reports that clam is capable of bioaccumulating toxic metals in its soft tissue and metal concentrations remained on average within the variations commonly explained in the literature for natural areas or areas slightly affected by metal pollution (Abaychi and Mustafa, 1988; Bilos et al., 1998; Alfonso et al., 2005; Li et al., 2006). The presence of sulfur rich metallathionein protein in the soft tissues of bivalves is responsible for the high content of metals in bivalves (Kramer, 1994; Geret and Cosson, 1999). Heavy metal uptake occurs mainly from water, food and sediment. However, Roesijiadi and Robinson (1994) reported that the effectiveness of metal uptake from their sources may differ in relation to ecological needs, metabolism of animals, concentrations of the heavy metals in water, food and sediment as well as some other factors such as salinity, temperature and interacting agents.

Temporal variations

Temporal variations in Hg, Cd and Pb concentrations in clam of Al-batinah coast of the Omani Sea were recorded (Figure 2a to c). Variations in mercury concentrations over a sampling period were not statistically significant ($p > 0.05$) in clam from the study site (Figure 2a). The lowest ($0.003 \mu\text{g/g}$) and highest ($0.034 \mu\text{g/g}$) Hg concentrations were determined in February 2010 and July 2009, respectively and the concentrations varied significantly ($p < 0.05$). Clams exhibited a gradual decrease in mercury concentration from July to October however, a declining trend or significantly no change from November 2009 to June 2010. Temporal variation in the concentration of Hg followed a non-uniform trend with November recording a peak value of $0.014 \mu\text{g/g}$ in the clam. Variations in Hg concentrations throughout the time make evident the complexity of the dynamic of Hg in the marine environment. Different biological and physiological factors can determine metal bioavailability in the environment. The present study did not observe any discrete point source of the Hg in the studied region. This could be due to natural processes as weathering, hydrological conditions, intense leaching mineralized rock or run-off, particulate matter resuspension and primary production. These processes are highly variable on a periodic basis and could possibly account for the difference in monthly Hg concentration in the clams. Between months, no hike in Hg concentration was observed. The general differences in mean concentrations between months were most likely due to the inherent toxic metal variability associated with differences in food availability and changes in metabolic rates connected with changes in geographical parameters during the study period.

Table 2. Recovery (%) of PAHs from clam.

| PAHs | Spiked concentration (ng/g) | Recovery concentration (ng/g) | Percentage of recovery (%) |
|------------------------|-----------------------------|-------------------------------|----------------------------|
| Naphthalene | 20 | 21.1 | 105.5 |
| | 100 | 107.2 | 107.2 |
| | 200 | 220 | 110 |
| Acenaphthylene | 20 | 18.36 | 91.8 |
| | 100 | 93.4 | 93.4 |
| | 200 | 172 | 86 |
| Acenaphthene | 20 | 19.3 | 96.5 |
| | 100 | 92.4 | 92.4 |
| | 200 | 181 | 90.5 |
| Fluorene | 4.0 | 3.78 | 94.7 |
| | 20 | 20.2 | 101 |
| | 40 | 39.0 | 97.5 |
| Phenanthrene | 2.0 | 1.81 | 90.5 |
| | 10 | 8.87 | 88.7 |
| | 20 | 18.46 | 92.3 |
| Anthracene | 2.0 | 1.03 | 96.5 |
| | 10 | 9.11 | 91.1 |
| | 20 | 19.64 | 98.2 |
| Fluoranthene | 4.0 | 3.51 | 87.8 |
| | 20 | 17.88 | 89.4 |
| | 40 | 36.48 | 91.2 |
| Pyrene | 2.0 | 1.83 | 91.7 |
| | 10 | 9.42 | 94.2 |
| | 20 | 17.76 | 88.8 |
| Benzo (a) anthracene | 2.0 | 1.84 | 92.0 |
| | 10 | 9.91 | 99.1 |
| | 20 | 18.48 | 92.4 |
| Chrysene | 2.0 | 1.79 | 89.5 |
| | 10 | 8.65 | 86.5 |
| | 20 | 18.05 | 90.2 |
| Benzo (b) fluoranthene | 4.0 | 3.90 | 97.7 |
| | 20 | 17.92 | 89.6 |
| | 40 | 36.2 | 90.5 |
| Benzo (k) fluoranthene | 2.0 | 1.894 | 94.7 |
| | 10 | 9.68 | 96.8 |
| | 20 | 18.3 | 91.5 |
| Benzo (a) pyrene | 2.0 | 1.65 | 82.5 |
| | 10 | 8.62 | 86.2 |
| | 20 | 18.22 | 91.1 |

Table 2. Contd.

| | | | |
|--------------------------|-----|-------|------|
| Di benz anthracene (a,h) | 4.0 | 3.47 | 86.9 |
| | 20 | 16.28 | 81.4 |
| | 40 | 35.96 | 89.9 |
| Benzo (g,h,i) perylene | 4.0 | 3.29 | 82.4 |
| | 20 | 18.1 | 90.5 |
| | 40 | 35.44 | 88.6 |
| Indeno pyrene (1,2,3-CD) | 2.0 | 1.6 | 80.0 |
| | 10 | 8.54 | 85.4 |
| | 20 | 16.56 | 82.8 |

Table 3. Grand mean and SD of Hg, Cd and Pb concentrations ($\mu\text{g/g}$; wet weight) in the soft tissues of Clam *L. ornate*.

| Mercury | | | Cadmium | | | Lead | | |
|---------|----------------|---------|---------|----------------|---------|-------|----------------|---------|
| Mean | $\pm\text{SD}$ | Maximum | Mean | $\pm\text{SD}$ | Maximum | Mean | $\pm\text{SD}$ | Maximum |
| 0.011 | 0.009 | 0.034 | 1.769 | 0.942 | 3.92 | 0.015 | 0.039 | 0.13 |

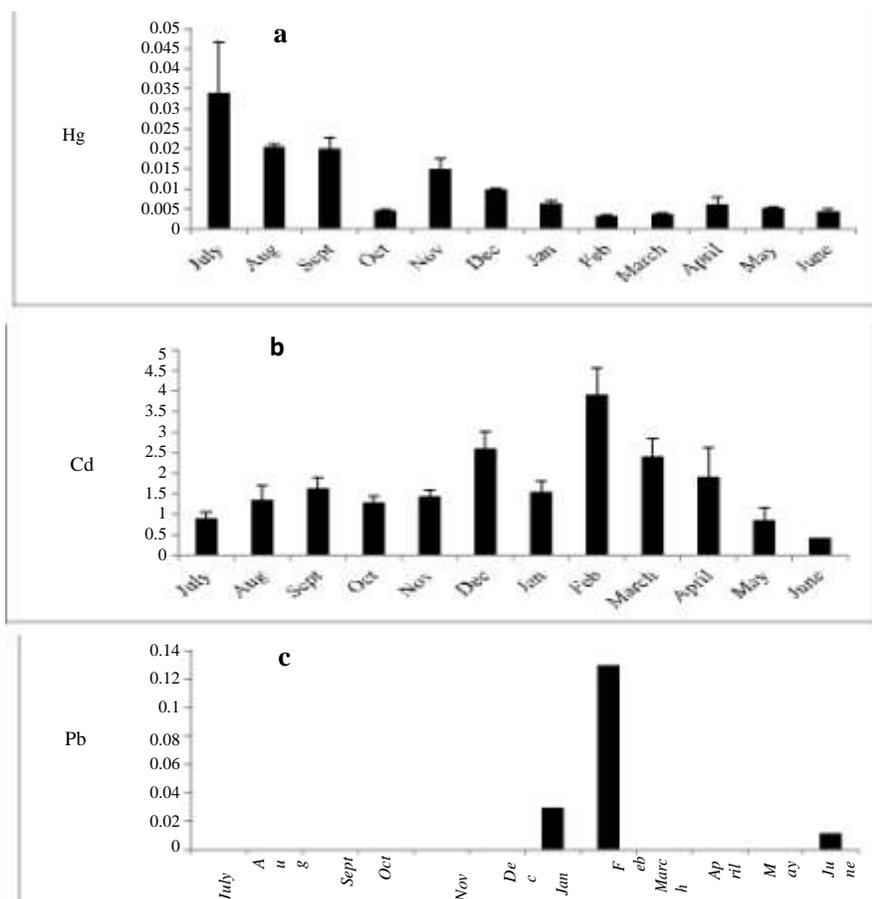


Figure 2. Temporal distribution of Hg (a), Cd (b) and Pb (c) concentrations ($\mu\text{g/g}$; wet weight) in the soft tissue of clam *L. ornate*.

For Cd, significant temporal variations were observed (Figure 2b). The concentration values obtained were varied from 0.42 µg/g in June 2010 to 3.92 µg/g in February 2010. Significant differences ($p < 0.05$) in Cd concentrations were observed between months indicates the influence of environmental factor and local input. Clams exhibited a gradual rise in Cd concentrations from July to February except in January. There was however, a declining trend or significantly change from February to June. The highest Cd concentration of 3.92 µg/g in February indicates that external input. However, the profile did not reveal any external source, assuming only natural variations. In addition to natural and anthropogenic inputs, biological variables such as size, sex or changes in tissue composition and reproductive cycle as well as the season of the sampling and the hydrodynamic of sea water have to be considered as far as variations in metal concentrations are concerned (Madkour et al., 2011). Ke and Wang (2001) reported that Cd accumulation by oysters is drastically reduced with the increase of salinity.

Pb concentrations remained less than the detection limit throughout the sampling period except in January, February and June 2010. The highest concentrations were recorded in February at 0.13 µg/g indicates that a significant time specific dependence on Pb concentrations and anthropogenic input source affected the sampling point.

There was a marked seasonality with Hg concentration being greater in summer (April to October) as compared to winter (November to March). However, the differences were not statistically significant. Overall clam Cd concentrations demonstrated a seasonality being higher during winter months versus warmer summer seasons. Observed seasonal trends in Cd concentrations may be related to changes in tissue weight due to reproductive cycle and corresponding pattern of energy storage and mobilization as stated by Dridi et al. (2007) with an increase in tissue weight associated with sexual maturation and generally occurring in spring-summer. Variability of heavy metal concentrations can also be caused by changes in physiological conditions of bivalves (Ferreira et al., 2004) and environmental parameters including temperature, pH, salinity, oxygen concentration (Phillips, 1976). According to Haynes and Tooley (1998), seasonal differences in the metal concentrations in mussels from non-impacted environments are usually related to physiochemical variations of waters which can alter metal bioavailability and /or the feeding rates of the organisms. Bryan (1993) mentioned that the temporal variations in metal concentrations in the bivalves are related to variations in local phytoplankton productivity. His observations are reinforced by the fact that an increase in phytoplankton productivity implies an increase in bivalves nutritional status, which in turn leads to an increase in metal concentrations in organism observed during winter months.

Size variations

Hg concentration in the whole tissue of the small sized clams (20 to 25 mm) varied from 0.003 to 0.005 µg/g. The medium sized clams (26 to 30 mm) recorded values between 0.004 and 0.005 µg/g of Hg. Hg concentrations in the tissues of large sized clams (above 30 mm) ranged from 0.003 to 0.005 µg/g. Cd concentration in the whole tissue of the small sized clam varied from 0.338 to 0.65 µg/g. The medium sized clams recorded Cd values between 0.45 and 0.61 µg/g. Cd concentrations in the tissues of large sized clams ranged from 0.53 to 0.73 µg/g. Pb concentrations in the whole tissue of the small sized clams varied from 0.001 to 0.008 µg/g. The medium sized clams showed Pb values between 0.001 and 0.06 µg/g. Hg was higher in medium sized clams than small and large sized clams (Figure 3a). Large sized clams showed relatively higher cadmium concentrations than small and medium sized clams but not statistically significant (Figure 3b). Pb was detected in small and medium sized clams but not in large sized clams (Figure 3c). Multiple comparison tests performed after 2 way ANOVA on the mean concentrations and size parameters in the soft tissue of clams indicated that no distinct relationship between size class and metal accumulation by the marine clam *L. ornata*. Large sized clams exhibited a trend similar to what was observed for small and medium sized clams, suggesting similar regulating mechanisms of Hg and Cd in the tissues of clams regardless of size. Pb was detected in small and medium sized clams but not in large sized clams suggests that larger size classes are sexually mature and have an efficient metabolism and detoxifying process to keep the concentrations of relatively lower (Connell et al., 1999).

PAHs

PAHs are known as prevalent contaminants in the marine environment. Their source, distribution and fate in the environment have been studied intensively (Baussant et al., 2009; Brooks et al., 2011; Li et al., 2013). PAHs were not detected in most of the months in clam samples. Hence, the data obtained for all the months were pooled to calculate the mean, standard deviation and total PAHs values. Concentrations of individual compounds of PAHs in clam samples are shown in Table 4. The concentration of the sum of 16 PAHs in clams was 53.82 ng/g wet weight. Low molecular weight two rings compounds naphthalene, acenaphthylene and acenaphthene were not detected however, fluorine was detected and the mean and maximum values were 5.05 and 11.8 ng/g, respectively. Among detected compounds, pyrene was found at maximum concentration with 12 ng/g and the lowest concentration was found at chrysene with 4.8 ng/g. The specific PAHs benzo (fluoranthene), benzo (k) fluoranthene, di benz (a,h) anthracene, benzo (g,h,i)

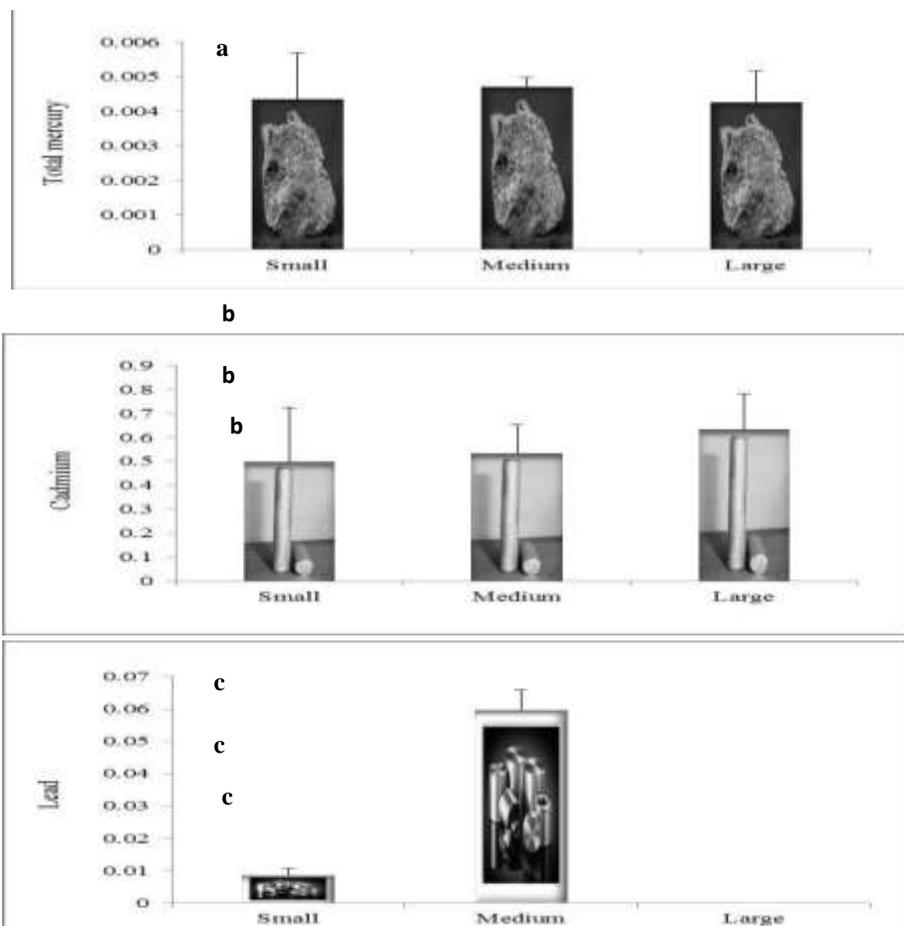


Figure 3. Toxic metals Hg (a), Cd (b) and Pb (c) in relation to clam sizes(µg/g; wet weight).

Table 4. Concentrations (ng/g; wet weight) of PAHs in *L. ornate*.

| Name | Clam | | |
|--------------------------|------|------|--------------|
| | Mean | ±SD | Max |
| Naphthalene | n.d. | n.d. | n.d. |
| Acenaphthylene | n.d. | n.d. | n.d. |
| Acenaphthene | n.d. | n.d. | n.d. |
| Fluorene | 5.05 | 5.99 | 11.80 |
| Phenanthrene | n.d. | n.d. | n.d. |
| Anthracene | n.d. | n.d. | n.d. |
| Fluoranthene | 4.37 | 5.17 | 10.10 |
| Pyrene | 3.95 | 5.65 | 12.00 |
| Benzo (a) anthracene | n.d. | n.d. | n.d. |
| Chrysene | 1.75 | 2.28 | 4.80 |
| Benzo (b) fluoranthene | n.d. | n.d. | n.d. |
| Benzo (k) fluoranthene | n.d. | n.d. | n.d. |
| Benzo (a) pyrene | n.d. | n.d. | n.d. |
| Di benz (a,h) anthracene | n.d. | n.d. | n.d. |
| Benzo (g,h,i) perylene | n.d. | n.d. | n.d. |
| Indeno (1,2,3-CD) pyrene | n.d. | n.d. | n.d. |
| Total PAHs | | | 53.82 |

n.d. : not detected.

perylene and indeno (1,2,3 - CD) pyrene were not detected. Bivalves can directly absorb low molecular weight PAHs, while heavier molecular weight hydrocarbons are mainly ingested through the digestive system. Phenanthrene and pyrene are estimated to be absorbed at 88 and 74% respectively when the less soluble Benzo (a) Pyrene is estimated to be absorbed through particle ingestion (Baumard et al., 1998; Piccardo et al., 2001). Therefore, besides the variety of sources, the strong difference in pattern of PAHs within fish species could be explained by the turbidity, the presence of organic matter but also by the differences of decontamination kinetics for each individual PAH (Rantamaki, 2007).

Most individuals PAHs were present at low levels and were demonstrated to be affected by just, one, two or three different individuals, mainly of low molecular weights fluorene, fluoranthene and high molecular weights pyrene and chrysene. In these cases, contamination can be linked to more than one type of pollution. Sources of PAHs are multiple in the environment (Piccardo et al., 2001; Tiwari et al., 2013). The low molecular weight PAHs (< 3 aromatic ring) and their alkylated homologues are the principal constituents of crude oil as mentioned by Gundlach et al. (1983) and Readman et al. (1986). PAHs generated during high temperature combustion such as occurred during the oil well fires are mainly higher molecular weight (> 4 aromatic ring) non-alkylated compounds, many of which are carcinogenic. One such PAH, pyrene, is normally produced through combustion (Perez-Gregorio et al., 2010) and was present in the clams at concentrations ranging between 3.95 and 12 µg/kg. These concentrations are not exceptional and are typical of those which have been measured in other marine fish and shellfish of the world.

Conclusion

The present study showed that, the order of decreasing concentration is Cd>Pb>Hg. Cd was the most and Hg was the least accumulated toxic metal in the studied clam tissue. There was a marked seasonality in metal accumulation pattern and no distinctive relationship was found between metal uptake and different size class. In a comparison of metal levels in clams sampled at the Omani Sea with the maximum permissible levels set by the Omani regulation and the European Union, the clams collected from the Omani sea were within the legal standard for lead and mercury. The cadmium concentration in *L. ornata* exceeded the maximum limit allowed for human consumption by the EU and Omani regulation, which warrants a detailed investigation of the origin of this metal into the marine environment. Clam showed detectable amount of some PAHs compounds. In general, most individuals PAHs were present at low levels and the composition pattern of PAHs in clam is

mostly dominated by four ring PAHs. However, none of the clam samples contained detectable level of the most carcinogenic compound benzo (a) pyrene. The results suggest that environmental impacts attributable to metals and PAHs associated with clam are likely to be minimal however, continuous monitoring of organic and inorganic pollutants in Omani fish is recommended with more number of species in view of the health of consumers.

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