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Reference:

Kumar Vikas, Sinha Amit Kumar, Rodrigues Paulo Pinheiro, Mubiana Kayawe Valentine, Blust Ronny, De Boeck Gudrun.-
Linking environmental heavy metal concentrations and salinity gradients with metal accumulation and their effects : a case study in 3 mussel species of Vitória estuary and Espírito Santo bay, Southeast Brazil
The science of the total environment - ISSN 0048-9697 - 523(2015), p. 1-15
DOI: <http://dx.doi.org/doi:10.1016/j.scitotenv.2015.03.139>
Handle: <http://hdl.handle.net/10067/1248180151162165141>

**Linking environmental heavy metal concentrations and salinity
gradients with metal accumulation and their effects:
a case study in 3 mussel species of Vitória estuary and Espírito Santo
bay, Southeast Brazil**

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Abstract

The present study was conducted to link the heavy metal load in three species of mussels (*Perna perna*, *Mytella falcata* and *M. guyanensis*) from the estuaries and bays around Vitória island, south-east of Brazil, with the salinity gradient and the heavy metal levels in the abiotic environment (including water, suspended particulate matter (SPM) and sediment). Primarily based on the salinity gradient, a total of 26 sites around Vitória Island were selected for sampling of water, SPM, sediments and organisms. Besides tissue metal levels, the condition index and energy stores (glycogen, lipid and protein) were quantified as an indicator of fitness in response to metal pollution. Dissolved metals in water indicate that Cd and Mn content was higher along Espírito Santo Bay, while Al, Co, Cu, Cr and Fe were elevated in the sites with low salinity such as river mouths, estuarine and sewage canals. Likewise, suspended matter sampled from low salinity sites showed a higher heavy metal load compared to moderate and high salinity sites. Though mussels were sampled from different sites, the contamination for Cd, Cu, Fe and Mn was higher in mussels inhabiting low salinity sites (*M. guyanensis* and *M. falcata*) compared to *P. perna*, a high saline water inhabitant. However, a higher Zn body burden was observed for *P. perna* compared to *Mytella* species. Tissue Fe accumulation (but not Mn and Zn) correlated with heavy metal levels in suspended material for all three species, and for *M. falcata* this correlation also existed for Cd and Cu. Energy store and condition index in all mussels varied depending on the sampling sites and correlated with salinity gradient rather than tissue metal concentration. Overall, metal concentration in mussels did not exceed the safe levels as per the international standards for metals, and would be of no risk for human consumption.

Key words: *Biomonitoring, Mussels, Perna perna, Mytella falcata, Mytella guyanensis, Suspended Particulate Matter, Energy store, Condition index.*

1. Introduction

Heavy metals (or trace elements) are naturally found in the hydrosphere, with many of them essential in minimal quantities for the well-functioning of living organisms. Nowadays, much more attention is drawn to the wide occurrence of metal pollution in aquatic systems. Heavy metals are deemed serious pollutants because of their toxicity, persistence and non-degradability in the environment. Some heavy metals (Hg, Cr, Cd, Ni, Cu, Pb and Zn) may transform into persistent metallic compounds with high toxicity (Hyun et al., 2006; Maanan, 2007). Even though some metallic compounds can be absorbed onto the suspended particles and sediments, they can be released into the water under favorable conditions depending on salinity, Eh, and pH (Xu and Yang, 1996; Zhou et al., 2008). Furthermore, these metals can bioaccumulate in aquatic organisms and magnify in the food chain, and thus can threaten human health (Fergusson, 1990; Jin, 1992; Zhou et al., 2008; Li et al., 2010). Elevated concentrations of heavy metals in tissue can cause toxicity, and adversely affect the physiological, metabolic, ion-regulatory and ecological performance (Walker et al., 2012). In the coastal region of Vitória (State of Espírito Santo capital) Southeast Brazil more than 400 industries (typically for steel and iron ore processing) were established by late 2000 and they contribute to the emission of pollution and particulate matter (Ovalle et al., 2013). In general, mussels are consumed all over the area (nearby Vitória, Brazil) without any quality control for public health, underpinning the importance of biomonitoring programs not only for environmental protection but also for health security.

Biomonitoring is a scientific technique assessing environmental exposure, including human exposure to natural and synthetic chemicals, based on sampling and analysis of organism's tissues and fluids. The use of particular organisms as biomonitors of heavy metal bioavailability in coastal water allows comparisons to be made over space and time, as biomonitors provide integrated measures of the ecotoxicologically significant fraction of ambient metal in water, suspended matter and sediment (Phillips and Rainbow, 1993; Rainbow, 1995; Sarkar et al., 2008; Besse et al., 2012). The well known 'mussel watch' monitoring program is used to assess the spatial and temporal trends in chemical contamination in estuarine and coastal areas. Mussels are commonly preferred for biomonitoring of

1 aquatic metal pollution because of their advantages over other organisms such as wide geographical
2 distributing, abundancy, sedentary, tolerance to environmental alterations, tolerance to various
3 environmental contaminants, high bioconcentration factors of pollutants, very low-level metabolizing
4 enzyme activities of organic contaminants, wide and stable populations, reasonably long-lived,
5 reasonable size and sturdy enough to survive in field and laboratory studies (Boening, 1999; Tanabe
6 and Subramanian, 2003; Sarkar et al., 2008; Zhou et al., 2008).

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14 In general, sediments act as sinks for trace metals in estuaries and other coastal waters as they
15 can readily bind with metal, and are also the source of metals for animals, particularly for burrowing
16 infauna. It is important to distinguish two routes of metal uptake from the sediment – firstly dietary
17 uptake of metals associated with sediment ingested by deposit feeders, and secondly uptake from
18 solution in interstitial pore water released in equilibrium from the sediment particles (Rainbow, 2006;
19 Sarkar et al., 2008). On the other hand, pollutants associated with suspended particulate matter might
20 be more important for filter feeders. When confronted with polluted environments, biota utilizes
21 different physiological and metabolic strategies to maintain homeostasis. Energy translocation which is
22 reflected as the tissue energy stores is the most common compensatory response to cope to adverse
23 situations, and also assist to estimate fitness and/or condition status (Capuzzo, 1988; Smolders et al.,
24 2002, 2004). Persistent organic pollutants (POPs), widely occurring contaminants in coastal areas, can
25 cause potentially harmful effects on ecosystems including human health. POPs are resistant to
26 biological degradation, and are characterized by low water and high lipid solubility, leading to their
27 bioaccumulation in the food chain (Iwata et al., 1993). POPs such as Polychlorinated Biphenyls
28 (PCBs) and Dichloro-Diphenyl- Trichloroethane (DDT) and its metabolite, Polybrominated Diphenyl
29 Ethers (PBDE) Hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB) are of global concern
30 due to their intensive use for agricultural and industrial purposes particularly in developing countries.
31 (Mao, 1995; Miyamoto and Klein, 1998).

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58 Considering the potential threat of aquatic pollution to mankind, the main aim of the present
59 study was to perform biomonitoring for pollutants in the coastal region of Vitória, Espírito Santo,
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1 Southeast Brazil through the assessment of heavy metal contamination in mussels (*Perna perna*,
2 *Mytella falcata* and *M. guyanensis*) and other environmental compartments (water, suspended matter
3 and sediment) and to link these with the occurring salinity gradient and the condition of the mussels
4 expressed as the energy status (glycogen, lipid and protein) and the condition index (dry tissue
5 weight/shell weight).
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11 **2. Material and Methods**

12 **2.1. Description of the study sites**

13 The study area is situated in a metropolitan vicinity i.e., Vitória, Espírito Santo State, which is located
14 in the Southeast of Brazil (geographic coordinates of 20° 19' of South Latitude and 40° 20' West
15 Longitude). The Grande Vitória (metropolitan area) comprehends the Vitória Capital and its main
16 cities are Vila Velha, Cariacica and Serra. However, this coastal area is composed of river systems, an
17 estuarine region and bays. Most of the human activity and environmental impacts are concentrated on
18 the central zone i.e. Vitória Island (Fig. 1). The estuarine region around the Vitória Island is boarded
19 by continental areas and has a main freshwater influx from the median size Santa Maria River, smaller
20 rivers, Bubu, Itanguá, Marinho and Aribiri. Additionally, there is also the output from natural creeks,
21 transformed in urbanized fluvial drainage channels, and from effluent sewage from the cities. Tides
22 from the Atlantic Ocean influence the Santa Maria River Delta Northwest of the island encompassing
23 18 km² of mangrove ecosystem.
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48 Comparable to the study by Jesus et al. (2004), the total study area was divided in four zones
49 including: 1) Passagem Canal (sampling sites: MS, MO, GO, UA, UF); 2) upper Vitória Bay, which
50 comprises the estuarine area northwest face of Vitória Island with a high influence of rivers (sampling
51 sites: SP, IC, RB, NP, RS, IL); 3) Port Canal comprising the south side of the Vitória Island (sampling
52 sites: FU, SL, RA, RM, RI, SA); 4) and Espírito Santo Bay which is oriented toward the Atlantic
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Ocean (sampling sites: CS, TP, FS, HA, IB, IF, PA, PC, CP). For abbreviations and detailed information of each station see table 1.

2.2. Collection of samples

Water, suspended matter, sediment and bivalve (*Perna perna*, *Mytella falcata* and *M. guyanensis*) samples were collected from the selected sites in single time frame during February – March (summer season) 2007. For 3 stations, 2 different mussel species were collected from the same location. The first step was the identification of relevant sampling spots, based on each ecosystem specificity (estuary, mangrove, island, urbanized channel and ocean), previous literature (Furley et al., 1997; Jesus et al., 2004) and possible pollution sources (industrial and urban sewage) and influence of its discharge. The 26 stations formed a sampling area which partially covered the previous sampling spots for sediment studied by Furley et al. (1997) and Jesus et al. (2004) and other points identified as having an important environmental impact, including a control sample from area (CS, Setiba, 50 km from Vitória) that was considered to be unpolluted. Other sampling points were identified during the field campaign, such as near the mussels harvest areas from local fisherman.

The samples were collected in the water line during low tide. For each site, a total of 20 mussels, 50 ml of water, 3 samples of suspended matter and 3 sediment samples were collected. Water physical-chemical parameters were measured for each sampling site. Salinity was measured by salinometer (Extech Instruments, USA) and pH was monitored by a handy pH electrode (Hanna Instruments, USA). The samples were collected and subsequently stored in a freezer at -20°C, and brought to the Laboratory of Systemic Physiological and Ecotoxicological Research (SPHERE), Department of Biology, University of Antwerp, Belgium for further analysis.

Sediment was collected in plastic petri dishes, water in plastic tubes, suspended particles in portable filter (composed of a filter holder, syringe, and membrane micro-filters (0.45µm), which are subsequently kept in 10 ml plastic tubes). Mussels were kept in plastic bags.

2.3. Heavy metal analysis

Sediments were collected from each station; three replicate samples were collected from top first centimeters around the biological sampling site. The samples were further split into two aliquots for the assessment of heavy metal concentration and for organo-compounds. The digestion of the sediment for metal concentration analysis was made after freeze-drying in closed bombs using an industrial digestion microwave system (Ethos 900, Milestone, USA). The digestion of 3 g (whole) sediment was done in a mixture of 1.5 mL highly purified concentrated (69 %) HNO₃ and 4.5 mL HCl (37%). Samples were processed in batches of 12 samples including a blank sample and a standard reference sample. The digested samples were made up to 50 mL with highly purified Milli-Q water. Metal concentrations in the final solutions were determined by Inductively Coupled Plasma -Atomic Emission Spectrometer Liberty series II (ICP-AES) for Cu, Fe, Mn, Zn and Al while Inductively Coupled Plasma - Mass Spectrometer (ICP-MS; UltraMass 700, Varian, Australia) was used for the typically ultra-trace elements including Cd, Co, Cr, Ni, Pb and Ag. Yttrium was used as an internal standard to correct for interference from high-dissolved solids arising from high salt content and tissue matrix effects.

Suspended particles were sampled in 3 replicates by filtering water from each site using membrane filters (0.45µm). The filtration was always stopped and the volume of the filtrate was recorded when the flow rate decreased to about half. Total clogging of the filters was always avoided. The filters were carefully collected and placed in polypropylene tubes and then freeze dried. The filters were then digested in 250 µL HNO₃ (69%) acid by heating in a microwave oven using the same procedure as the sediments. After digestion, the solutions were diluted to 2% of acid and metal concentrations in the resulting solutions were then determined using ICP-AES and ICP-MS.

For determining concentrations of dissolved metals in the field water samples, 1.5 mL of high purity HNO₃ was added to each sample which was previously filtered (0.45 µm pore size) and for more saline samples, dilution with Milli-Q water was performed to achieve about 3 ppt dissolved

solids. Metal analyses in all the samples were then performed with ICP-MS instrument with yttrium as internal standard.

Ten individual mussels were dissected, biomass of soft tissue separated from the shell. The tissues were rinsed with Milli-Q water during the dissection to avoid interference of other particles with the analysis of the biomass metal concentration. The byssus was removed and the tissues were freeze-dried. The digestion of the tissue was made in the microwave in a mixture of HNO₃ (5 mL or 10 mL depending of the biomass size), and hydrogen peroxide (0.4 ml of 27 % H₂O₂). Digestion was carried-out by heating the digest in four consecutive steps with increasing microwave power i.e. 80, 160, 240 and 320 W, each step lasting 5 minutes. The digested solutions were then made up to 50 mL volume with Milli-Q water and metal determinations were performed with ICP-AES and ICP-MS.

The non-linear estimation modeling for the bioaccumulation for each metal was done to assess to what extend the different metal pools i.e. sediment, water and suspended particles maybe relevant to the observed metal accumulation in the biota. The following equation was used

$$Y = a + b \cdot V(\text{SPM}) + c \cdot V(\text{Water}) + d \cdot V(\text{SED})$$

in which: 'a' represents another distinct factor not explained by the present data, 'b' is the multiplication factor for the variable suspended matter V(SPM) metal concentration, 'c' is the multiplication factor for the variable water V(Water) metal concentration, 'd' is the multiplication factor for the variable sediment V(SED) concentration.

2.4. Estimation of organo-compounds

In a total 10 sites (CS, TP, PA, PC, UA, MS, RS, RB, RM and SL) were chosen to be tested for the sediment contamination of organo-compounds. One g of sediment was fractioned and the concentration of organo compound contamination (PCBs, pesticides organochloride (DDT, HCHs, chlordanes) and PBDEs) in the samples were analyzed by Hewlett Packard (Palo Alto, CA, USA) 6890

GC connected via direct interface with a HP 5973 MS at the Toxicological Centre of the University of Antwerp, Belgium.

2.5. Quality control

In this study, quality control measures involved several steps starting with the inclusion of preparation blanks to take into account the background concentrations of metals in chemical reagents used and any other form of unintended contamination during the preparation of the samples. Background concentrations given by the blank samples were always been taken into account in calculating metal concentrations in the actual samples. Another form of quality control was the inclusion of reference certified materials during the preparation stage and were carried along through the whole procedure. Mussel tissue (CRM 278R) and calcareous loam soil (BCR 141R) purchased from Institute for Reference Materials and Measurements (IRMM), Geel, Belgium, were used for mussel and sediment samples, respectively. In the case of organic contaminants, quality control measures involved regular analyses of procedural blanks, blind duplicate samples and inclusion of certified reference material of PCBs in cod liver oil (CRM 349). In all cases and for all chemical contaminants studied, the recoveries were consistently within the traditionally accepted 10% range of the certified values.

2.6. Determination of energy reserve

For each station, 10 individual mussel's tissue were homogenized in 4 mL of Milli-Q water and aliquots were taken for analysis of glycogen, protein and lipid, and the total energy reserve was estimated (KJ/g). Samples of mussels were analyzed for protein content by Bradford's method (Bradford, 1976), glycogen content by using the anthrone reagent (Roe and Dailey, 1966) and lipid content were measured following Bligh and Dyer (1959).

2.7. Statistical analysis

All data have been presented as mean values $\pm$ standard deviation (S.D). For comparisons between different stations for the same species a one-way analysis of variance (ANOVA) was performed followed by the least significant difference (LSD) test. Student's unpaired two-tailed t-test was used for single comparisons. Pearson correlation was performed to show the relationship among various variables. The data were analyzed by Statistical Package for the Social Sciences (SPSS) version 20.0. A probability level of 0.05 was used for rejection of the null hypothesis.

3. Results

3.1. Heavy metals in suspended matter and water

The concentration of heavy metals in the suspended matter (SPM) is presented in the table 2. The two stations with low salinity (RM and RI) showed the highest concentration for most of the metals compared to the other stations and the reference site (CS). As and Ag level were highest in the CP and FU stations respectively. The Cr concentration was detected only for the stations RM, FU and RS (2.90, 0.32 and 0.11 $\mu\text{g/L}$ respectively). Highest concentrations were for Mn 63.38 $\mu\text{g/L}$ (RM), for Fe 6792.59 $\mu\text{g/L}$ (RM), for Co 0.639 $\mu\text{g/L}$ (RM), for Zn 134.77 $\mu\text{g/L}$ (RM), for As 4.75 $\mu\text{g/L}$ (CP), for Ag 0.3508 $\mu\text{g/L}$ (FU) and for Pb 27.2615 $\mu\text{g/L}$ (RM) respectively whereas lowest concentration value were for Mn 0.36 $\mu\text{g/L}$ (FS), for Fe 23.80 $\mu\text{g/L}$ (FS), for Co 0.002 $\mu\text{g/L}$ (FS), for Zn 0.15 $\mu\text{g/L}$ (FS), for As 0.06 $\mu\text{g/L}$ (FS), for Ag 0.0001 $\mu\text{g/L}$ (FS), and for Pb 0.0218 $\mu\text{g/L}$ in FS respectively. Concentration of Ni was only detected in location RM (2.75 $\mu\text{g/L}$). The concentration of Cd in suspended matter ranged from 0.0002 $\mu\text{g/L}$ in the control site CS (also in FS) to 0.3961 $\mu\text{g/L}$ in RM, followed by 0.1309 $\mu\text{g/L}$ in RI. Cd levels in other stations were 100 times lower than CS, FS, RM and RI.

Heavy metals concentration in water is given in the table 3. Analogous to the findings in suspended matter, concentrations of most of the heavy metals were also relatively higher in stations

with low salinity (particularly in RM and RI) compared to the high salinity and reference (CS) stations. Typically Al, Cd, Cr, Mn, Pb and Zn concentrations in the water were highest at the river mouth and upper stream areas such as RM and RI. However, Ag concentration showed the highest value (0.030 µg/L) in PA, while most of estuarine locations and river mouths in upper Vitória Bay were below detectable limit. Likewise, concentrations of Ni, Co and Fe in water were higher along Espírito Santo Bay ranging for Ni from 1.04 µg/L in RS to 4.53 µg/L in FS, for Co from 0.30 µg/L in RS to 1.10 µg/L in IF, and for Fe from 301.58 µg/L in RS to 874.42 µg/L in CP respectively. Cu concentration in water ranged from 3.11 µg/L in RI to 12.25 µg/L in FS, and were similar to As levels, which ranged from 2.46 µg/L in RI to 10.34 µg/L in CP, followed by 10.28 µg/L in FS.

3.2. Heavy metals in sediment

A mixed response of heavy metals concentration in sediment was observed along the sampling sites attributed to the salinity gradient (Table 4). Ag concentration ranged from 13.83 µg/g in UF, followed by higher values for FU, PC, UA, TP and RI to the lowest value of 0.45 µg/g in SL. Al concentration exhibited wide ranges and were highest in the small rivers sites RA (58763.70 µg/g), RI, RB and RS, followed by some estuarine locations around upper Vitória Bay (IL, MS, SA), while the lowest value (485.67 µg/g) was detected in the control site CS. The Cd concentration in sediment ranged from 0.39 µg/g in IB to 7.23 µg/g in the UF station. The Co concentration in sediment ranged from 1.18 µg/g in the HA station to 13.96 µg/g in the PC sampling site, followed by RA (11.06 µg/g), IL (8.39 µg/g), RI SL and RB. The Cr concentration in sediment ranged from 3.92 µg/g in the TP station to higher levels of 80.03 µg/g in the PC, followed by RA (68.51 µg/g), RB (50.65 µg/g) and RI (50.45 µg/g).

The highest Cu concentration in sediment was found at PC (94.70 µg/g) and the lowest at SP with 0.32 µg/g. The Fe concentration showed the lowest value in the control station CS with 2243.42 µg/g and the highest amount (65395.08 µg/g) in the station IL. The Mn concentrations for the study area showed the lowest value for NP with 21.08 µg/g and the highest in PC with 742.98 µg/g, whereas Pb concentrations displayed the lowest value for the control site CS of 12.45 µg/g and the highest in

SL of 257.30 µg/g, followed by the UF station with 182.4 µg/g. The Zn concentrations in sediment values ranged from a minimum 2.65 µg/g in FU to a maximum value 34.04 µg/g in UF.

3.3. Heavy metal accumulation in mussels

The three species of mussels that were analyzed in the study show different salinity preferences: *Perna perna* (Mexilhão), a marine species, *Mytella falcata* (Sururu) more common in estuarine environments and *Mytella guyanensis* (Bacucu), also an estuarine species but more associated with fresh water.

Figure 2 represents the mean values for Cd, Cu, Fe, Mn and Zn concentration inside the mussels' soft tissues. In station PC both *P. perna* and *M. falcata* were present, and at RI and RB both *M. falcata* and *M. guyanensis* were observed. These are indicated in the figures by connecting arrows and consequently are used to analyze the difference between species exposed to the same environmental conditions.

Concentration of Cd in *P. perna* ranged from 0.39 µg/g at PA to 2.33 µg/g at CS in Espírito Santo Bay samples (Fig. 2). *M. falcata* exhibited higher averages than *P. perna* and varied from a minimum of 1.39 µg/g in NP to a maximum of 11.45 µg/g in the RI. A significant difference was noted for Cd level in *M. falcata* sampled from NP and RI. The highest value for the *M. guyanensis* was found in the RI (6.33µg/g) and lowest in IL (2.41 µg/l). At PC station, *P. perna* showed a significant lower Cd concentration than *M. falcata*. However, for the RB station, *M. guyanensis* accumulated higher ($P > 0.05$) Cd than *M. falcata*. Cu concentrations in tissue (Fig. 2) of *P. perna* ranged from 6.24 µg/g in TP to 13.55 µg/g in the PC. Similar to Cd, higher averages of Cu concentrations were found in tissue of *M. falcata*, which ranged of 6.02 µg/g in MS to a maximum of 44.5 µg/g in RI. The highest value in *M. falcata* was noted in RI site which was higher ($P < 0.05$) than MS. For *M. guyanensis* the Cu concentrations ranged from 19.90 µg/g in RA to 25.46 µg/g in RB. At station PC, Cu concentration in *P. perna* was numerically lower (but insignificant, $P=0.053$) than in *M. falcata*. In the station RI, the value for *M. falcata* was significantly higher than in *M. guyanensis*;

whereas, for the RB station, the internal load of Cu in *M. guyanensis* was considerably higher ($P < 0.01$) than in *M. falcata*.

P. perna and *M. falcata* sampled from various sites showed a wide range of accumulated Fe and Mn concentrations, indicating considerable variability in the bioavailability of these metals among the sampling sites (Fig. 2). The Fe concentrations in *P. perna* ranged from 150.77 µg/g (PA) to 1964.34 µg/g (CP station). In *M. falcata* Fe values ranged from 561.02 µg/g (at PC station) to 1926.22 µg/g (at RI). *M. guyanensis* showed Fe concentrations between 940.59 µg/g (RA) and 1540.27 µg/g (RI). *P. perna* in the PC station showed lower but insignificant Fe concentrations than *M. falcata* for the same location. In station RI, Fe in *M. falcata* was higher ($P > 0.05$) than in *M. guyanensis*, conversely to the RB station, where values for *M. guyanensis* were higher ($P > 0.05$) than for *M. falcata*.

Mn concentration in *P. perna* ranged from 8.06 µg/g to 37.53 µg/g (PA and HA stations, respectively), and in comparison to site PA, the Mn concentration was significantly elevated in CS, HA and CP. In *M. falcata* the minimum value was 14.33 µg/g (RS) and maximum 50.15 µg/g at the PC collection point. The values for *M. guyanensis* were between 24.0 µg/g in the RI and 56.63 µg/g in the RB station. At PC station *P. perna* showed lower ($P < 0.05$) Mn concentrations than *M. falcata*. For RB station, values for *M. guyanensis* were higher ($P < 0.01$) than for *M. falcata*.

Contrary to Fe and Mn, Zn concentration for all the three mussel species along the collection sites did not alter significantly (Fig. 2). However, for the RB site, the Zn concentration in *M. guyanensis* was considerably higher ($P < 0.05$) than in *M. falcata*.

The correlation between tissue metal concentrations with different environmental compartments can be found in table 5. The results for the nonlinear regression attributed to the bioaccumulation are presented in table 6. The estimation was done for *P. perna* and *M. falcata* which had enough data, with some significant results. For Cd accumulation in *M. falcata*, the significant factors were the suspended matter ($P = 0.0085$, $b = 14.092$) and water ($P = 0.033$, $c = -0.020$). The Cu bioaccumulation model showed significant results for sediment ($P = 0.018$, $d = 0.006$) and other factors

($P=0.046$, $a=12.027$) for *P. perna*, while for *M. falcata*, the important factor was the suspended matter ($P=0.007$, $b=1.710$). Fe showed only a significant value for the suspended matter ($P=0.026$, $b=0.097$) for *M. falcata*. The last two metals, Mn and Zn showed only significant values for other factors ($P=3.64^{-6}$ and 4.77^{-6} , and $a=29.17$ and 103.07 , respectively) for *M. falcata* bioaccumulation.

3.4. Energy content in mussel

The energy content of whole body was analyzed for all the three species of mussels and is presented in figure 3. At Espírito Santo Bay, the *P. perna* showed high similarity for the IB, IF and PA stations (no significant difference), which were significantly different from the other stations FS, PC, CP and CS. *M. falcata* showed no difference between most of the stations, except for MO, SP and UA. The first two were significantly higher than other sites while collection from UA showed the lowest energy reserve. *M. guyanensis* did not show any significant difference among the four different sampling stations. Relationship between energy reserve and metal accumulation in mussels is presented in table 7. Interestingly, a significant negative correlation between the Cd concentration in the tissue and energy reserve was evident only in the *P. perna* species.

Average value of condition index (dry-tissue weight/shell weight) for *P. perna*, *M. falcata* and *M. guyanensis* were 0.125, 0.080 and 0.063 respectively (data not shown). Correlations between condition index and tissue metals accumulation is described in table 7. We found that the condition index for *P. perna* was negatively correlated with Fe ($P < 0.01$, $r = -0.308$) and Zn ($P < 0.001$, $r = -0.513$). For *M. falcata* the negative relations were for Cd ($P < 0.05$, $r = -0.196$), Fe ($P < 0.001$, $r = -0.3946$) and positive for Mn ($P < 0.001$, $r = 0.4033$). In case of *M. guyanensis*, the relation was negative with Fe ($P < 0.01$, $r = -0.468$).

3.5. Organocompounds content in sediment

The assessment of persistent organic pollutants (PCB, DDT, HCH and PBDE) was done for 9 sites (TP, PA, PC, UA, RS, RM, SL, RB and MS) of sediment that covered the study area in a

representative way and the control station CS (suppl. Table S1). HCH (HCB) and PBDEs (BDE 47, BDE 99, BDE 100, BDE 153) were undetected by chromatography. CS and TP did not exhibit any detectable value in the present study, as expected for CS for being the control site. For the other stations, low values were found for distinct PCBs and DDT derivatives. The weight used in the analysis is indicated as limit of quantification (LOQ) in mg for each xenobiotic measured.

4. Discussion

4.1. Heavy metal burden in environmental compartments

4.1.1. Sediment

Many aquatic pollutants are strongly allied with the fine deposits that are rich in organic matter, and the way of interaction with these deposits determines the bioavailability and toxicity of these metals (Chen and White, 2004). Besides the natural input of organic matter, sewage and urban discharges are released directly in the estuarine system especially in the Passagem canal. This area is at higher risk from human discharge (industrial and urban) leading to higher concentrations of heavy metal in the sediment, which could be increased by dredging and port activities. The sea side in Espírito Santo Bay is characterized by more coarse oceanic sediment with diverse physio-chemical characteristics because of the tidal perturbation and higher circulation.

Differences of heavy metal concentrations in sediment were expected since a previous sediment sampling campaign in 2000-2001 (Jesus et al., 2004) done at the same stations indicated Espírito Santo Bay with the lowest metal concentrations, while the Vitória harbor canal showed the highest. Their sampling did not show detectable levels of Cd, while the Cd range was 0.39 to 7.23 µg/g in our study without any clear trend between sites. Jesus et al. (2004) did not mention detection limits, but it is likely that discharge from nearby industries, urban growth and increase of sewage production added to the sediment concentrations over the years. Also Co concentration was under the detection limit in previous study, and ranged from 1.80 to 13.96 µg/g in our study showing lower values for the

marine environment (Espírito Santo Bay) with the exception of sampling site PC which is located at the outlet of Passagem canal. Cu, Fe and Al concentrations followed the similar trend with the previous study where Espírito Santo Bay samples were lower compared to the other stations. Concentration of Mn (27.20 to 742.98 µg/g range) in sediment showed to be higher for Espírito Santo Bay stations, in tune with the trend reported by Jesus et al., 2004. Pb and Zn did not show any comparable patterns. In general, the Espírito Santo Bay's sediment were characterized by lower contaminated values of Al, Co, Cr, Fe and Cu which can be related to the marine influence: a lower mean percentage of organic matter (6%) and clay in the sediment, high water circulation and renewal with cleaner water (Jesus et al., 2004).

Temporal changes in the estuary dynamics could be one of the causes of the high difference in ranges compared to the previous study by Jesus et al. (2004). Also, the previous study covered sampling during the whole year, however, in the present study samples of the sub-tidal layer of sediment were collected only at one time point. More importantly, in the previous study, samples were collected deeper by dragging and only the silt-clay fractions (<63 µm) was analyzed. Therefore, discrepancy in the metals concentrations may in part due to the sediment's fractions retention capacity for pollutants as we used whole sediment samples for the present study. It is obvious that the environmental conditions greatly influence metal concentration patterns and accumulation capacity with lower levels at Espírito Santo Bay; but clearly also local contamination in or nearby small river mouths with high human sewage, for example PC and RA exhibiting high values for majority of metals analyzed, play an important role.

4.1.2. Water and suspended matter

In the present study, we found higher concentrations of dissolved metals in water and suspended matter in the sites with low salinity such as river mouths, the upper Vitória Bay estuarine area, and sewage canals. Direct discharge from house hold, sewage disposal and effluents from the nearby

industries into the riverine system or the enclosed bay might have contributed to a higher level of heavy metal load among freshwater and estuarine stations.

The high values of dissolved metals in water coincided with high sediment concentration for Cr, Co, Zn and Ni, but low levels in the dissolved form were related with high sediment metal concentrations for Ag, Al, Cd, Cu, Fe, Mn and Pb. The presence of extremely high concentration of Ag in FU in suspended matter was paralleled with relatively high sediment and water value. FU is localized near to a sewage treatment plant, the discharge from this plant might cause a high Ag load in all aquatic compartments.

The correlations between compartments (except for Fe and Al) showed significant correlations for water-suspended matter for Mn ($P=0.001$), Co ($P=0.0001$), As ($P=0.0001$), Cu ($P=0.0001$), Zn ($P=0.0001$), Pb ($P=0.0001$); suspended matter-sediment for Zn ($P=0.001$), Ag ($P=0.001$), Cd ($P=0.0001$); and water-sediment for Co ($P=0.023$), Cr ($P=0.018$), Zn ($P=0.020$) and Ni ($P=0.001$) (Table 8). The observed correlations are justifiable since there is transport from water to suspended matter, with subsequent sedimentation, and also release of the sediment contamination into suspended matter or dissolved concentrations due to perturbation and mixing.

4.2. Bioaccumulation in mussel species

Mussels are good accumulators of heavy metals and generally body concentrations tend to reflect environmental exposures (Goldberg et al., 1983; Luten et al., 1986; Stronkhorst, 1992; De Kock and Kramer, 1994). In the present biomonitoring programme, one of the prime goals was to relate metal body contents of mussels to bioavailable metal levels found in the surrounding environment. Although the experimental species are located in different environments (*P. perna* is usually present in higher salinities, while the *Mytella* genus are found in lower salinity), the heavy metal contamination for Mn, Cu, Fe, and Cd was highest in *Mytella guyanensis*, followed by *M. falcata* and *P. perna*. In general, these correspond with the pattern of heavy metal concentrations measured in water and suspended matter; marked by a relative higher metal load in low salinity sites compared to the high salinity sites.

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2 Additionally, studies have shown that the chemical speciation of heavy metals is strongly influenced
3 by salinity (Van Eck and De Rooij, 1993; Paucot and Wollast, 1997; Zwolsman et al., 1997; Gerringa
4 et al., 1998). Therefore, in addition to the lower total concentrations, it is very likely that in the marine
5 environment of Espírito Santo Bay, heavy metals formed complexes with chloride which reduced the
6 bioavailability of heavy metals even further. The higher bioavailability of metal in low salinity
7 environment will enhance the internal accumulation by organisms, as was registered in dwelling
8 mussel *M. guyanensis*.
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11 Comparison between the concentrations of metal in tissue of *P. perna* showed a small decrease of Cu
12 (also observed in sediment), Mn, and Zn (130.2 to 101.5 µg/g) and an increase in Fe contamination,
13 compared to 10 years earlier based on general means of similar stations (Furley et al. 1997). The Cu
14 concentrations in the sediment had a significant relationship with *P. perna* ($P=0.006$ and $r=0.82$)
15 (Table 5). Nonlinear regression showed that Cu concentration in the sediment was the only factor that
16 significantly influenced Cu bioaccumulation in *P. perna* (Table 6). The Mn concentrations decreased
17 compared with a decade ago. The concentrations in sediment showed the highest concentration in
18 Espírito Santo Bay, which was different for the tissue, water and suspended matter. Furley et al. (1997)
19 related the possible sources to detergent input in the area, and also indicated potential sources from
20 coal deposit piles from melting plants industries. The Fe concentrations in suspended matter and water
21 showed a positive relationship ($P=0.008$, $r=0.812$ and $P=0.016$ and $r=0.77$ respectively) with Fe
22 concentrations in *P. perna* tissue. From 2000 to 2005 the mean concentrations for soluble Fe for the
23 Espírito Santo Bay (where this species is found) were in general lower considering comparable stations
24 described by Oliveira (2006) (with maximum values approximately 420 µg/g and means less than 100
25 µg/L), and sediment concentrations showed a decrease. So, Fe contamination in the mussel seems to
26 reflect relatively recent increases in suspended matter Fe. Although Cd concentrations in water and
27 suspended matter ($P=0.0001$ and $r=0.9734$) were correlated, there was no significant relation between
28 Cd concentrations in the aquatic compartments with the concentrations in *P. perna*, and it did not add
29 any significance to the model (Table 6). Presumably, the increased concentration in sediment could
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1 cause an increase in the other compartments, including the tissue. Interestingly for tissue, Cd showed
2 significant negative relationship with body composition (energy content) (Table 7). Possibly, the
3 decreased condition index leads to a slower growth and thus a larger concentration of toxicants over a
4 longer period of time in a smaller tissue volume (Lucas and Beninger, 1985; Stevenson and Woods,
5 2006).

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7 For *M. guyanensis*, Fe concentration in suspended matter was the only factor with significant
8 ($P= 0.034$ and $r=0.9661$) relationship with its concentration in the tissue (Table 5). Regrettably, a
9 regression model could not be provided for *M. guyanensis* because of the small number of samples.

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11 No previous comparable metal accumulation data is available for *M. falcata*. Tissue
12 accumulation for Cd, Cu and Fe reveals significant relationships with suspended matter concentrations
13 (Table 5). Interestingly, when modelling regressions of the same heavy metals (Cd, Cu, Fe), suspended
14 matter correlated strongly and significantly for *M. falcata* only, not for *P. perna* (Table 6); suggesting
15 that these metals in suspension are the key route contributing to the metal body burden of the *M.*
16 *falcata*. The present data and former information reveal that such discrepancies may also be explained
17 by differential species response related to feeding habit, substrate preference or genetic differentiation
18 (Yap et al., 2002; Li et al., 2012; Brahim Errahmani et al., 2014).

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20 Furley (1997) had confirmed that the values of heavy metal concentrations in the mussels'
21 tissue (sampled from same area as for the present study) were lower than the allowed maximum
22 limited for human consumption as per the Brazilian legislation at that time. However, Saraiva (2000)
23 analyzed the concentration of heavy metals Cd, Cr, Cu, Fe, Ni, Pb and Zn in the Santa Maria River
24 estuary (Vitória-ES) through the assessment in *Crassostrea rhizophorae* and *Mytella guyanensis*, and
25 reported high levels of contamination present in these species particularly for Zn and Cr, suggesting
26 increasing risks of other species being contaminated as well. The current study revealed that heavy
27 metals concentration in the mussels (*P. perna*, *M. falcata*, *M. guyanensis*, from all stations) were lower
28 than international standards for human consumption (data compiled by the Food and Agricultural
29 Organization of the United Nations, Wagner and Boman, 2004; and Health Ministry of World Health
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Organization) (suppl. Table S2), recommending the inclusion of more diverse species of bivalves for the biomonitoring programme.

4.3. Effect of salinity gradient on the metal bioaccumulation pattern

Usually with a decrease in salinity, it is expected that there is an increase of the uptake of heavy metals by marine species (Nugegoda and Rainbow, 1989; Jesus et al., 2003), although there are exceptions (Phillips, 1977). It has been reported that Cd and Zn uptake by *M. edulis* was maximum at low salinity (Phillips, 1976; Jackim et al., 1977; Ali and Taylor, 2010) as also observed in *Mya arenaria*, *Mulinia lateralis* and *Nucula proxima* (Jackim et al., 1977) and *Perna viridis* (Yap et al., 2003), suggesting an inverse relationship of metal uptake with salinity gradient. In the present work, we noted considerable high metal accumulation in mussels collected from freshwater sources relative to high salinity stations. Moreover, we found negative correlations between body burden of Cd ($P < 0.01$), Cu ($P < 0.01$), Fe ($P < 0.05$) with salinity (Fig. 4). Thus, our findings are in tune with the various hypotheses proposing that environmental parameters such as salinity are very influential on the rate of uptake and accumulation of different trace metals by aquatic organisms including mussels (Mubiana and Blust, 2007). However, it is hard to separate the effects of salinity gradients from metal gradients since they showed opposite directions; the more marine environments were also less polluted.

4.4. Energy budget and condition index

The difference between available energy reserves and energy consumption has been proposed to be indicative of an organism's overall condition (De Coen and Janssen, 2003), and a decrease in the available energy reserves can be used as a biomarker of metal stress (Scott- Fordsmand and Weeks, 2000). In this context, the energy status of mussels was studied to gain insight into the costs of living in an environment with metal pollution. We noted a negative significant correlation between the Cd concentration in the tissue and the body composition reserve in *P. perna* (Table 7). Moreover, the lowest energy reserve for the same species at sites PC, CP and CS corresponds with highest tissue Cd

load. The elevated level of internal Cd causes harmful biochemical shifts such as diseases, emphysemas, hypertension, chronic and acute effects on the metabolism, and usually disruption at all functional levels (Fergusson, 1990). Moreover, metal resistance often involves an increased production of ligands, proteins, and enzymes involved in detoxification, which are energy demanding biological process. Therefore, a decline in an energy reserves or augmentations in the metabolic rates have been associated with metal toxicity in animals (Moolman et al., 2007). Furthermore, corresponding to the finding of metal accumulation in mussels, energy reserve was also dependent ($r = 0.3860$, $P < 0.05$) on the salinity (Fig. 5A). Low energy content was noted in mussels collected from fresh water site and exactly at the same sites a reverse pattern (i.e. elevation) was revealed for body burden for many metals (Cd, Cu, Fe and Mn); suggesting that the salinity gradient not only influences the metal uptake, but to certain extent is also associated with the dynamics of the energy budget and the osmoregulatory costs at low salinity.

In general, Cd is a non essential element, exhibiting high toxicity and is widely considered as a representative element for metal pollution. Therefore, it is advisable to monitor the Cd levels for these stations regularly over the time. Bioaccumulation of Cu, Fe, Zn and Mn did not reveal a significant correlation with energy reserve (Table 7) for any of the studied species. These are the essential metals for the function of most living organism, apparently, and are probably handled better than non-essential elements.

Total body energy content of *M. falcata* showed a similarity between most of the stations, except for MO and SP with high glycogen levels, which coincides with the high input of nutrients from human settlements. The condition index are often used as measures for the well being of mussels (Mersch et al., 1996; Soto et al., 2000; Martel et al., 2003) and showed spatial variation as well as interspecific differences however with a high variability. Similar to the bioaccumulation of metals and energy budget pattern in molluscs, condition index also seems to be influenced by salinity gradient ($r = 0.8412$; $P < 0.01$) (Fig. 5B). When the metal load of the mussels were related to their respective condition indices considering pooled data of all the stations, significant relationship was evident for

many of the metals (Table 7), but again, it is difficult to separate effects of salinity and metal accumulation. In general, the processes responsible for metal uptake and accumulation in mussels are actively controlled by physiological and biochemical processes. The condition index (i.e. dry tissue weight/shell weight) reflects the changes in the nutrient state of mussel such as stored energy reserves and the animal's metabolic response to environmental stress. As such, condition index can be a useful tool for estimating the effect of metal contamination on the health condition of the mussels, provided the variation in other environmental factors such as tidal exposure, body size, sex, reproductive status or food availability are limited or are considered in the monitoring program.

4.5. Organocompounds

PCBs are persistent pollutant in the environment and can cause toxicity by increasing risks of cancer. Another major constrain for these chemicals is attributed to the accumulation in the fatty tissues and provoke bio-magnification through the food chain (Binelli and Provini, 2003; Burreau et al., 2006). The PCBs and DDTs concentrations found in Vitória Island area in sediment were very low (suppl. Table S1), with a total range from 0.4 to 11.0 and 0.4 to 2.0 ng/g, respectively. It is comparable to the isolated areas reported by Klánová et al. (2008) from James Ross Island in southeast coast of the Antarctic Peninsula, where the persistent organic pollutants were determined in sediments (and soils) ranging from 0.32 to 0.83 ng/g for PCBs, from 0.19 to 1.15 ng/g for DDTs. HCHs and HCB were below the detection limit for Vitória sediment, while Antarctica's sediment resulted in ranges from 0.14 to 0.76 ng/g or HCHs, from 0.95 to 4 ng/g for HCB. The sources for these compounds in Antarctica were identified as atmospheric and small local contamination (Klánová et al., 2008). Nevertheless, the samples in the study area, showed no alarming concentrations, and much lower than other contaminated or polluted regions for PCBs, DDTs, HCB and PBDEs.

5. Conclusion

The results of the present study suggest that the sampling sites located along the riverine and estuarine side at upper Vitória Bay had high load of the majority of metals investigated (compared to the Espírito Santo Bay area) in the abiotic compartments, particularly for the dissolved matter and suspended particles. These could be attributed to waste disposal from the nearby mining industries and the organic waste from house hold. Many correlations were found between metal concentrations between different compartments: water-suspended matter (Mn, Co, As, Cu, Zn, Pb), suspended matter-sediment (Zn, Ag, Cd) and water-sediment (Cr, Co, Ni, Zn) suggesting interdependency of compartments in determining the bioavailability of metals. Correspondingly, there was an interaction between salinity on the Cd, Cu and Fe uptake by all three mussels (*M. guyanensis*, *M. falcata* and *P. perna*) and bioaccumulation of these metals were at its maximum at low salinity sites. This was also reflected by a parallel reduction in energy stores as well as condition index. Nevertheless, metal concentrations analyzed in mussels were lower than the maximum limits permitted in the legislation, even in the upper Vitória Bay area. However, temporal trends were noted (with reference to Jesus et al., 2004) and further rise of the contaminants at biotic and abiotic ecological compartments due to increasing human activities can be well expected in future, therefore, a long term and bi-seasonal temporal biomonitoring program for pollution is advised. Furthermore, present information on the contaminant levels of PCBs, DDTs and metabolites in sediment samples is potentially useful in considering toxicological and public health implications, however, the levels were much lower than the alarming concentrations.

Acknowledgments

The technical assistance of Adrian Covaci, Steven Joosen, Karin Van den Bergh and Nemo Maes is gratefully acknowledged. This research was funded by Fonds Wetenschappelijk Onderzoek - Vlaanderen (FWO)- Project G.0123.07N . Amit Kumar Sinha is a research fellow supported by the Fonds Wetenschappelijk Onderzoek – Vlaanderen [FWO Grant12A8814N].

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Figure Captions

Figure 1. Study area, marine and estuarine region of Vitória, Espírito Santo, south-east of Brazil. Sampling places indicated on the map are listed in the table 1.

Figure 2: Cadmium (Cd), Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) concentration ($\mu\text{g/g}$) in different mussel species- *P. perna*, *M. falcata* and *M. guyanensis* sampled from different locations. The connectors indicate the same station with two species. Values are represented in mean $\pm$ standard deviation. Different superscript letters denote significant differences ($P < 0.05$) among different stations for same species. Asterisk (*) indicate significant differences between two similar stations for two different mussel species (* $P < 0.05$; ** $P < 0.01$)

Figure 3. Energy reserve (KJ) in *P. perna*, *M. falcata* and *M. guyanensis* at different stations. Values are represented in mean $\pm$ standard deviations. Different superscript letters denote significant differences ($P < 0.05$) among different stations for same species.

Figure 4. Relationship between metal accumulation (pooled value of *P. perna*, *M. falcata* and *M. guyanensis*) and salinity gradient.

Figure 5. Relationship between (A) energy content in mussels (pooled value of *P. perna*, *M. falcata* and *M. guyanensis*) and salinity gradient, (B) condition index of mussels (pooled value of *P. perna*, *M. falcata* and *M. guyanensis*) and salinity gradient.

Tables

Table 1

Brief description of stations identified in the study area, Vitória region

Code	Sample Place	Brief description	City	pH	Salinity (ppt)	Mussels
Passagem canal						
MS	Mangue Serra	Mangrove areas sited upper stream, receiving more urban discharge from Serra City and Vitória respectively.	Serra	7	10	<i>Mytella falcata</i>
MO	Maria Ortiz		Vitória	8	18	<i>Mytella falcata</i>
UF	UFES Ed.Física	A median upper canal of mangrove area where a lot of the city sewage discharge goes into. Presence of the <i>Mytella</i> genus. The order from upper stream is GO, UA and UF.	Vitória	7	18	<i>Mytella falcata</i>
UA	UFES Antena		Vitória	7	15	<i>Mytella falcata</i>
GO	Goiabeiras		Vitória	8	16	<i>Mytella falcata</i>
Upper Vitória Bay						
SP	FAESA São Pedro	These stations are localized in the upper part from Vitória bay before the Port complex, and with more influence of mangrove characteristics, however the mangrove ecosystem was seriously impacted at the land reclamation process. Receives urban discharge by many sources.	Vitória	7	13	<i>Mytella falcata</i>
IC	Ilha das Caieiras		Vitória	8	20	<i>Mytella falcata</i>
RB	Rio Bubu	The small and median rivers mouth, with mangrove, where the RS has a larger flow and a wider drainage basin. RB receives discharge from Cariacica City and both are upper in the estuarine region in the continental side. Sited upper stream near to the river mouths however in the Vitória island side. Mangrove area more influenced by rivers.	Cariacica	9	10	<i>Mytella falcata</i> and <i>Mytella guyanensis</i>
RS	Rio Santa Maria		Cariacica	7	0	<i>Mytella falcata</i>
NP	Nova Palestina		Vitória	7	10	<i>Mytella guyanensis</i>
IL	Ilha do Lameirão		Vitória	7	5	<i>Mytella falcata</i>
Port Canal						
FU	Ilha da Fumaça	Another island connected to the main island, it was a mangrove area, and nowadays it is the end point of a sewage treatment plant.	Vitória	8	17	<i>Mytella falcata</i>
SL	Saldanha	Localized at the vicinity of a port complex, it is an urbanized coast, in the old village center.	Vitória	8	18	<i>Mytella falcata</i>
RA	Rio Aribiri	Small Rivers from Vila Velha drainage area, receiving the sewage of the city. Usually had a mangrove area associate with it.	Vila Velha	8	27	<i>Mytella guyanensis</i>
RM	Rio Marinho		Vila Velha	7	4	<i>Mytella falcata</i>
RI	Rio Itangua		Vila Velha	7	0	<i>Mytella falcata</i> and <i>Mytella</i>

SA	Santo Antônio	It is localized in the upper part from Vitória bay before the Port complex, and with more influence of mangrove characteristics. Receives urban discharge by many sources.	Vitória	7	10	<i>guyanensis</i> <i>Mytella falcata</i>
Espírito Santo Bay						
CS	Setiba	The control point is sited 50 km from Vitória, much less polluted area.	Guarapari	9	35	<i>Perna perna</i>
TP	Terceira Ponte	A bridge pillar in the proximity of the in the endpoint of Vila Velha City's sewage discharge, it was a mangrove canal, with high influence of the ocean.	Vila Velha	8	33	<i>Perna perna</i>
FS	Farol de Santa Luzia	Sited in Vila Velha city in a rock coast with open ocean water influence.	Vila Velha	8	34	<i>Perna perna</i>
HA	Hawaizinho	A rock island connected to the city, receive the waters of the Vitória bay Canal, from the local settlement, and medical clinics from the Enseada do Suá, however it has more influence of the ocean.	Vitória	8	32	<i>Perna perna</i>
IF	Ilha do Frade	Medians urbanized islands, formed by granite formation, having more water circulation than the previous point, sited more distant from the main sewage discharge.	Vitória	9	35	<i>Perna perna</i>
IB	Ilha do Boi	A small rock island in the middle of an enclosed bay, the bay (Curva da Jurema beach) was built by the land reclamation over the shallow marine environment and nowadays receives direct urban discharge mainly by residences, and highly concentrated apartments settlement.	Vitória	8	34	<i>Perna perna</i>
PA	Pedra da Andorinha		Vitória	8	35	<i>Perna perna</i>
PC	Ponte de Camburi	A bridge at the end point of the Passage Canal to the Espírito Santo Bay, where a lot of the city sewage discharge goes into. It has muddy bottom characteristics with presence of mangrove trees, and presence of the <i>Mytella</i> genus. The <i>Perna perna</i> is sampled in this area as in the protection pier to the canal mouth.	Vitória	8	33	<i>Perna perna</i> and <i>Mytella falcata</i>
CP	Pier de Camburi	Sited in the middle of the Camburi beach, Espírito Santo Bay, a marine area, with diminished hydrodynamic (construction of the smelting plant and port complex in one of the bay's extremity end) compared with the past.	Vitória	9	31	<i>Perna perna</i>

Table 2

Concentration ($\mu\text{g/L}$) of heavy metals in the suspended matter. Mean concentration with standard deviation among three replicates ranged from 0.5- 5% (not shown).

Stations	Salinity	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Passagem canal												
MS	10	BDL	0.22	0.0042	0.002	BDL	0.04	356.60	0.64	BDL	0.1252	1.22
MO	18	0.0003	0.19	0.0020	BDL	BDL	0.47	295.30	0.71	BDL	0.1991	1.29
GO	16	0.0049	0.61	0.0151	0.065	BDL	0.52	981.13	2.74	BDL	0.7155	3.83
UA	15	0.0100	0.20	0.0050	0.006	BDL	0.17	219.18	1.20	BDL	0.1530	1.01
UF	18	0.0155	0.19	0.0220	0.017	BDL	0.20	294.16	1.15	BDL	0.1934	1.06
Upper Vitória Bay												
SP	13	0.0017	0.21	0.0016	0.008	BDL	0.13	382.88	1.40	BDL	0.1950	0.75
IC	20	0.0007	0.19	0.0014	0.007	BDL	0.11	253.64	1.87	BDL	0.1542	4.19
RB	10	0.0011	0.25	0.0105	0.004	BDL	0.02	190.87	13.25	BDL	0.2334	2.43
NP	10	0.0026	0.72	0.0073	0.082	BDL	0.30	1362.68	5.55	BDL	0.7005	2.27
RS	0	0.0005	0.10	0.0056	0.022	0.11	0.07	768.35	3.14	BDL	0.3624	1.50
IL	5	0.0011	0.35	0.0042	0.023	BDL	0.13	915.53	1.89	BDL	0.3721	2.18
Port Canal												
FU	17	0.3508	0.23	0.0192	0.023	0.32	1.07	369.20	5.08	BDL	0.4549	4.89
SL	18	0.0046	0.14	0.0025	0.006	BDL	0.49	136.96	4.85	BDL	0.5042	0.69
RA	27	0.0025	0.21	0.0031	0.006	BDL	0.22	177.59	1.16	BDL	0.1958	3.40
RM	4	0.1282	1.43	0.3961	0.639	2.90	18.28	6792.59	63.38	2.75	27.2615	134.77
RI	5	0.0680	0.77	0.1309	0.466	BDL	14.12	6565.67	36.24	BDL	8.0387	33.72
SA	10	0.0037	0.30	0.0032	0.028	BDL	0.25	448.56	2.77	BDL	0.3960	1.62
Espírito Santo Bay												
CS	35	0.0002	0.63	0.0002	0.037	BDL	0.04	260.53	4.46	BDL	0.1973	0.55
TP	33	0.0127	0.11	0.0046	BDL	BDL	0.21	89.62	1.65	BDL	0.1332	2.10
FS	34	0.0001	0.06	0.0002	0.002	BDL	BDL	23.80	0.36	BDL	0.0218	0.15
HA	32	0.0026	0.17	0.0020	0.004	BDL	0.01	76.87	3.43	BDL	0.0843	0.63
IB	34	0.0029	0.17	0.0032	0.008	BDL	0.06	88.44	2.63	BDL	0.0923	0.60
IF	35	0.0012	0.17	0.0009	0.007	BDL	0.01	60.77	1.72	BDL	0.0562	0.25
PA	35	0.0002	0.11	0.0046	0.003	BDL	0.01	30.61	0.75	BDL	0.0510	0.41
PC	33	0.0121	0.27	0.0032	0.006	BDL	0.56	197.26	2.31	BDL	0.1878	1.37
CP	31	0.0013	4.75	0.0006	0.083	BDL	0.06	1223.42	15.29	BDL	0.5139	1.55

BDL – Below Detectable Limit ($< 0.01 \mu\text{g/L}$).

Table 3

Concentrations ($\mu\text{g/L}$) of heavy metal in water. Mean concentration with standard deviation among three replicates ranged from 0.5- 5% (not shown).

Station code	Salinity	Al	Ag	As	Cd	Cr	Co	Cu	Fe	Mn	Ni	Pb	Zn
Passagem canal													
MS	10	30.06	BDL	7.19	BDL	0.73	0.66	6.78	432.13	35.25	2.71	0.04	17.60
MO	18	30.49	BDL	8.08	0.02	BDL	0.81	9.66	606.13	30.21	3.64	0.05	18.55
GO	16	44.05	BDL	8.19	0.01	BDL	0.76	8.46	484.05	25.29	3.12	0.05	17.70
UA	15	33.88	0.02	7.22	BDL	0.12	0.79	10.41	503.75	30.60	3.07	0.13	17.08
UF	18	31.10	0.01	7.55	0.01	BDL	0.87	9.95	533.78	22.73	3.45	0.04	17.24
Upper Vitória Bay													
SP	13	64.88	BDL	7.20	BDL	0.43	0.71	8.15	477.22	33.89	2.81	0.07	16.71
IC	20	21.43	BDL	8.37	BDL	BDL	0.82	9.90	527.10	23.28	3.35	0.05	19.53
RB	10	31.78	BDL	7.33	BDL	0.04	0.64	7.65	383.38	23.27	2.46	0.06	17.58
NP	10	28.40	BDL	6.39	0.01	0.21	0.58	6.09	340.42	28.62	2.13	0.06	18.28
RS	0	84.08	BDL	3.17	0.01	1.25	0.30	3.43	301.58	36.61	1.04	0.09	13.11
IL	5	78.37	BDL	5.57	0.29	0.55	0.53	5.29	478.95	43.48	3.08	0.07	15.51
Port Canal													
FU	17	20.01	0.02	7.44	BDL	BDL	0.81	10.00	540.11	17.52	3.44	0.04	17.35
SL	18	20.85	0.01	7.95	0.01	BDL	0.85	10.60	560.53	9.98	3.57	0.03	18.60
RA	27	32.72	BDL	9.07	BDL	BDL	0.87	10.90	586.56	14.33	3.90	0.02	19.74
RM	4	104.73	BDL	5.04	BDL	1.47	0.64	5.50	568.45	189.72	2.03	0.38	40.85
RI	5	141.79	BDL	2.46	0.03	2.63	0.72	3.11	546.87	341.54	1.87	0.42	14.53
SA	10	29.45	0.01	8.13	0.01	0.11	0.80	9.85	522.39	27.17	3.42	0.09	21.27
Espírito Santo Bay													
CS	35	6.99	0.01	9.59	BDL	BDL	0.95	12.15	658.72	0.51	4.46	0.02	22.66
TP	33	28.13	0.01	9.59	BDL	BDL	0.91	11.90	649.45	8.73	4.15	0.05	19.70
FS	34	57.54	0.01	10.28	0.01	BDL	0.92	12.25	682.92	1.19	4.53	0.02	21.51
HA	32	14.45	0.01	9.15	0.01	BDL	0.96	12.11	658.18	3.04	4.41	0.02	19.72
IB	34	10.78	0.01	9.57	BDL	BDL	0.92	12.21	652.50	2.77	4.38	0.04	20.49
IF	35	8.72	0.01	8.49	0.01	BDL	1.10	9.80	510.33	1.66	4.28	0.03	18.01
PA	35	5.86	0.03	8.21	0.01	BDL	0.96	8.61	521.45	2.09	4.51	0.04	16.98
PC	33	14.94	0.01	8.23	BDL	BDL	0.95	11.24	562.67	13.79	4.04	0.06	17.65
CP	31	122.80	0.01	10.34	0.01	BDL	0.95	11.96	874.42	3.29	4.30	0.03	21.08

BDL - Below Detectable Limit ($< 0.01 \mu\text{g/L}$).

Table 4

Concentration ($\mu\text{g/g}$) of heavy metals in sediment samples. Mean concentration with standard deviation among three replicates ranged from 0.5- 5% (not shown).

Station code	Salinity	Ag	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Passagem canal												
MS	10	1.79	26681.12	1.26	5.38	35.53	6.34	38418.92	95.85	BDL	32.13	3.76
MO	18	1.95	3447.75	1.40	2.83	14.07	11.82	10764.05	379.99	BDL	28.07	4.07
GO	16	1.53	14635.65	0.85	3.26	20.62	8.85	20919.73	52.10	BDL	29.17	3.70
UA	15	3.42	17170.68	1.64	5.11	27.42	23.49	19438.04	60.48	BDL	22.95	3.18
UF	18	13.83	9357.95	7.23	4.78	24.75	43.28	13751.41	45.76	BDL	182.46	34.04
Upper Vitória Bay												
SP	13	1.16	5776.72	0.51	3.26	11.33	0.32	14133.92	62.40	BDL	25.28	3.64
IC	20	1.26	3107.07	1.25	3.16	6.42	14.98	4689.62	49.01	BDL	27.86	4.08
RB	10	1.33	37912.10	0.94	6.21	50.65	13.70	56462.61	189.50	BDL	46.04	3.14
NP	10	1.13	1348.55	0.86	2.04	4.23	2.83	4604.10	21.08	BDL	22.98	3.82
RS	0	0.97	36362.07	1.15	3.33	36.16	10.29	35098.67	88.45	BDL	38.59	3.50
IL	5	1.94	28758.42	0.55	8.39	34.99	7.43	65395.08	185.36	BDL	38.60	3.75
Port Canal												
FU	17	5.80	15483.25	1.07	4.75	18.15	31.01	18905.70	75.77	BDL	36.31	2.65
SL	18	0.45	19113.38	1.54	6.73	25.78	13.63	31463.58	242.56	BDL	257.30	2.67
RA	27	1.41	58763.70	1.93	11.06	68.51	37.53	55842.15	192.58	BDL	65.91	2.84
RM	4	1.44	5590.76	0.75	2.18	16.19	15.92	10901.58	60.40	BDL	29.70	4.38
RI	5	2.67	42263.08	1.31	7.81	50.45	35.38	39508.91	178.54	BDL	49.22	3.07
SA	10	1.29	24162.37	0.86	5.15	37.32	31.80	29462.24	106.41	BDL	28.16	2.93
Espírito Santo Bay												
CS	35	1.35	485.67	1.13	2.05	4.36	3.69	2243.42	37.54	BDL	12.45	3.67
TP	33	2.92	2460.30	1.29	1.80	3.92	2.88	5679.74	27.20	BDL	23.58	3.72
FS	34	1.11	8806.33	0.65	2.48	16.06	2.02	11796.10	172.30	BDL	120.10	3.74
HA	32	1.60	2476.89	0.46	1.18	7.50	3.49	9906.73	476.98	BDL	21.43	3.84
IB	34	1.68	13013.14	0.39	3.13	21.17	4.31	17339.25	272.36	BDL	31.39	3.55
IF	35	1.49	976.54	1.71	2.84	4.63	2.51	2878.91	40.76	BDL	31.43	3.90
PA	35	1.04	18436.80	1.38	2.11	31.64	11.61	23166.15	172.24	BDL	32.48	3.48
PC	33	5.14	41687.14	3.28	13.96	80.03	94.70	71979.90	742.98	BDL	132.34	9.69
CP	31	1.30	540.95	1.60	3.45	4.31	3.83	3085.62	105.37	BDL	16.40	3.98

BDL - Below Detectable Limit ($< 0.01 \mu\text{g/g}$).

Table 5

Correlation between tissue accumulation and metal concentration in different environmental compartments (suspended matter, water and sediment)

Suspended Matter		Cd	Cu	Fe	Mn	Zn
<i>P. perna</i>	r	-0.3466	-0.3466	0.8115	0.5369	-0.3890
	P-value	0.361	0.361	0.008	0.136	0.301
<i>M. falcata</i>	r	0.4885	0.6244	0.5218	-0.0708	0.2495
	P-value	0.047	0.007	0.032	0.787	0.334
<i>M.guyanensis</i>	r	0.8063	-0.4703	0.9661	-0.0101	-0.1106
	P-value	0.194	0.530	0.034	0.990	0.889
Water						
<i>P. perna</i>	r	0.4627	-0.3200	0.7683	0.0758	0.1008
	P-value	0.210	0.401	0.016	0.846	0.796
<i>M. falcata</i>	r	-0.4306	-0.1411	-0.1560	-0.3121	-0.1623
	P-value	0.084	0.589	0.550	0.223	0.534
<i>M.guyanensis</i>	r	0.0278	0.0414	0.1527	-0.3661	0.2778
	P-value	0.972	0.959	0.847	0.634	0.722
Sediment						
<i>P. perna</i>	r	-0.1180	0.8231	-0.6049	0.4308	0.2851
	P-value	0.762	0.006	0.084	0.247	0.457
<i>M. falcata</i>	r	0.2138	0.1730	0.2921	0.0514	-0.1852
	P-value	0.410	0.507	0.255	0.845	0.477
<i>M.guyanensis</i>	r	0.4268	-0.7458	-0.7401	0.4386	-0.4220
	P-value	0.573	0.254	0.260	0.561	0.578

(r) is the correlation factor and *P* values significant when < 0.05.

Table 6

Nonlinear regression for the bioaccumulation model based on the equation
 $Y = a + b \cdot V(\text{SPM}) + c \cdot (\text{Water}) + d \cdot (\text{SED})$

Metal	Specie	Estimate	Standard	t-value	p-level	Lo. Conf	Up. Conf
Cd	<i>P. perna</i>	a	1.273	0.5296	2.40474	0.061261	2.63482
		b	-183.815	107.5228	-1.70955	0.148044	92.58085
		c	0.007	0.0037	1.91765	0.113269	0.01661
		d	0.002	0.0387	0.03917	0.970268	0.10108
	<i>M. falcata</i>	a	1.88852	1.839863	1.02645	0.323401	5.86331
		b	14.09243	4.552101	3.09581	0.008515	23.92664
		c	-0.02009	0.008439	-2.38116	0.033236	-0.03833
		d	0.22139	0.150742	1.46869	0.165695	0.54705
Cu	<i>P. perna</i>	a	12.02727	4.540919	2.648642	0.045501	23.70008
		b	-0.00815	0.015145	-0.538273	0.613477	0.03078
		c	-0.35684	0.399132	-0.894038	0.412264	0.66916
		d	0.00574	0.001666	3.447720	0.018283	0.01003
	<i>M. falcata</i>	a	2.001755	11.30799	0.177021	0.862220	26.43119
		b	1.709719	0.53781	3.179042	0.007254	2.87159
		c	1.758235	1.33351	1.318500	0.210094	4.63911
		d	-0.000240	0.00877	-0.027392	0.978563	0.01871
Fe	<i>P. perna</i>	a	261.3350	1223.550	0.21359	0.839305	3406.571
		b	0.8157	0.531	1.53484	0.185413	2.182
		c	0.9751	1.942	0.50212	0.636908	5.967
		d	-0.0021	0.002	-1.36300	0.231053	0.002
	<i>M. falcata</i>	a	1277.720	628.8041	2.031983	0.063112	2636.168
		b	0.097	0.0385	2.503982	0.026390	0.180
		c	-1.067	1.1615	-0.918234	0.375219	1.443
		d	0.001	0.0006	1.121429	0.282393	0.002
Mn	<i>P. perna</i>	a	12.87575	5.756696	2.236656	0.075531	27.67381
		b	1.22227	0.620298	1.970452	0.105863	2.81679
		c	0.30809	0.649233	0.474551	0.655109	1.97700
		d	0.00323	0.001972	1.639182	0.162101	0.00830
	<i>M. falcata</i>	a	29.17112	3.814255	7.64792	0.000004	37.41131
		b	0.15605	0.188184	0.82925	0.421926	0.56260
		c	-0.04942	0.034083	-1.44990	0.170780	0.02421
		d	0.00062	0.002323	0.26845	0.792567	0.00564
Zn	<i>P. perna</i>	a	81.70238	59.69511	1.368661	0.229393	235.1535
		b	-6.55338	7.63002	-0.858894	0.429642	13.0602
		c	-0.07094	2.94431	-0.024095	0.981709	7.4977
		d	0.73982	1.46841	0.503826	0.635793	4.5145
	<i>M. falcata</i>	a	103.0700	13.81820	7.45900	0.000005	132.9224
		b	0.1817	0.08846	2.05400	0.060657	0.3728
		c	-0.5770	0.36668	-1.57352	0.139611	0.2152
		d	-0.3804	0.37317	-1.01948	0.326573	0.4257

Coefficients: (a) for other variables; (b) for suspended matter (SPM); (c) for water; and (d) for sediment.

Level of confidence: 95.0%.

Table 7

Correlation between energy reserve, condition index and metal accumulation.

Species	Cd		Cu		Fe		Mn		Zn	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Energy reserve										
<i>P. perna</i>	-0.69	0.041	-0.21	0.582	-0.61	0.080	-0.40	0.282	0.18	0.641
<i>M. falcata</i>	0.12	0.647	0.22	0.405	-0.15	0.577	0.10	0.711	-0.10	0.694
<i>M. guyanensis</i>	-0.19	0.805	0.37	0.626	0.52	0.482	-0.14	0.856	-0.15	0.847
Condition index										
<i>P. perna</i>	-0.183	0.103	-0.089	0.428	-0.308	0.005	-0.015	0.989	-0.513	0.000
<i>M. falcata</i>	-0.195	0.011	-0.113	0.143	-0.394	0.000	0.4033	0.000	-0.032	0.676
<i>M. guyanensis</i>	-0.174	0.283	-0.162	0.318	-0.468	0.002	0.0493	0.763	0.185	0.253

(r) is the correlation factor and *P* values significant when < 0.05.

Table 8

Correlation between the metal concentrations for each compartment

Metal	Compartiments	Suspended matter	Water	Metal	Compartiments	Suspended matter	Water
Cr	Water	r=0.115 P=0.325		Cu	Water	r=0.6788 P=0.0001	
	Sediment	r=0.1133 P=0.932	r=-0.3934 P=0.018		Sediment	r=-1.293 P=0.488	r=-0.0567 P=0.762
		Suspended matter	Water			Suspended matter	Water
Fe	Water	r=0.0531 P=0.777		Zn	Water	r=0.6894 P=0.0001	
	Sediment	r=0.1292 P=0.488	r=-0.1823 P=0.326		Sediment	r=0.5872 P=0.001	r=0.8885 P=0.02
		Suspended matter	Water			Suspended matter	Water
Mn	Water	r=0.5429 P=0.001		Ag	Water	r=0.2578 P=0.161	
	Sediment	r=-0.02023 P=0.277	r=-0.0428 P=0.818		Sediment	r=0.5791 P=0.001	r=0.0647 P=0.730
		Suspended matter	Water			Suspended matter	Water
Co	Water	r=0.6923 P=0.000		Cd	Water	r=0.1421 P=0.446	
	Sediment	r=0.1547 P=0.406	r=0.4072 P=0.023		Sediment	r=0.9734 P=0.0001	r=0.491 P=0.491
		Suspended matter	Water			Suspended matter	Water
Ni	Water	r=-0.0692 P=0.734		Pb	Water	r=0.9622 P=0.0001	
	Sediment	r=-0.0692 P=0.712	r=0.9988 P=0.00		Sediment	r=-0.1397 P=0.453	r=-0.1352 P=0.468
		Suspended matter	Water			Suspended matter	Water
As	Water	r=0.993 P=0.0001		Al	Sediment		r=0.2588 P=0.160

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