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# *Meretrix meretrix* as an Indicator of Heavy Metal Contamination in Maputo Bay

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## Abstract

Heavy metal contamination is sometimes described as a ticking environmental bomb. The east African region has a rapid population growth and anthropogenic influence is believed to increase, probably leading to higher loads into the biosphere of heavy metals. The object of this study is to map today's levels of Cd, Cu, Pb, Cr, Fe, Al, and a few more trace metals around Maputo city. Matola River runs through industrial areas and discharges into Maputo Bay. Bivalve *Meretrix meretrix* populates both sides of the river and along the Costa do Sol, north of the city. Filter feeders like *Meretrix* accumulate heavy metal and are therefore good indicators of contamination. 60 clams were collected and analysed for heavy metals, giving a picture of today's levels of contamination. Inductively Coupled Plasma-Mass Spectrometry /Atomic Emission Spectrometry instruments were used for the chemical analysis and Principal Component statistical analysis was performed showing elevated levels of Pb and Cu in Matola and elevated levels of Cr and Fe at Villa do Pescadores, a fisherman's village, indicating anthropogenic influence.

***Contents***

# 1 Introduction

## *1.1 Mozambique: background*

The last couple of years there have been a rapid increase in population in the east African region. Marine environments are productive and therefore the place where the largest populations are found. Mozambique shows a rapid urbanisation and the industrial pressure on the coastal areas is continually increasing. Industries such as fisheries, maritime cultivation, transport and tourism together with deficient wastewater treatment are a threat to the marine life. Unsustainable land use resulting in erosion and contaminated runoff bring pollutants from inland. Populations along the coast depending on a healthy sea are likely to see their livelihood disappear if the situation doesn't change.

After many years of war Mozambique, is today at peace. Maputo, with a population of 1,3 million is likely to see a big increase in inhabitants. Very few scientific studies have been made until today and it is important that science becomes a factor in the country's development.

Mozambique is one of the poorest countries in the world and life expectancy is less than 40 years. Aids/HIV kills many people and drought and floods have taken turns devastating the country. Mozambique needs all possible aid to rebuild itself. To do this in an environmentally satisfactory way the need for studies of the present kind are essential.

## *1.2 Objective*

The heavy metal issue is believed to be a potential environmental disaster since the accumulation has been ongoing for decades and no one knows how long the pollutants will remain in the biosphere. Earlier studies of sediment composition and possible anthropogenic influence suggest that heavy metal levels are increasing in the Maputo River estuary area (Achimo, 2000, Basse et al, 2000). It is therefore interesting to investigate if organisms also show the same trends of increasing levels. The aims of this project are to observe and map the present levels of heavy metals outside Maputo using a mussel, *Meretrix meretrix* as an indicator. Initially the study object was *Padina boergeseeni*, a brown macro alga known to grow in the area. Earlier studies along the east African coast have been done on this species (Engdahl et al., 1998) however, after 2 weeks of searching for padinas along the cost not one single specimen had been found. Since heavy metal levels were expected to be very low (Mendez et al 2001, Wong et al., 1998) there was a need for advanced analytical instruments. Inductively Coupled Plasma-Mass Spectrometry/Atomic Emission Spectrometry allows several elements to be analysed and MS has a detection limit around 0.003 ppb for most elements. The instruments have earlier been proved sufficient for this kind of analysis (Bechman et al 2000, Sokolowski et al 2002). The amount of data produced demanded a powerful statistical method and therefore Principal Component Analysis was used.

## *1.3* Indicator-*Meretrix meretrix*

Fig 1 *Meretrix meretrix*

*Meretrix meretrix* was chosen as an indicator. This species, together with *Eumercia paupercula,* is found abundantly in the study area. Both species are filter feeders, hence accumulating sediment constituents.

*Meretrix meretrix* was chosen for four reasons. *Meretrix* is more accessible in the region and also bigger in size, facilitating collection and analysis ( minimum dry weight 0,2 g). Its size is up to 90 mm in shell length and it is easily distinguished from other species. There are earlier studies on this species and other *Meretrix* species (*M. lusoria*; Hung et al., 2000). *Meretrix* is collected for food and is therefore interesting also in a direct health perspective. *Eumercia paupercula w*as also collected at most stations but not analysed

# 2 Theory

## *2.1 ICP–MS/AES*

Inductively Coupled Plasma (ICP) is a step used in the two methods Mass Spectrometry (MS) and Atomic Emission Spectrometry (AES). Argon gas as a plasma reduces much of the interference encountered in ordinary combustion flames. Argon emitting few disturbing frequency signals makes it preferable before other gases. Ionised Argon gas releases electrons that are accelerated by radio-frequency load coils. Electrons colliding with the entire gas create a temperature up to 10 000 K.

In MS, the fact that ionised atoms with different weight travel in a vacuum tube at different speeds when accelerated by a certain energy level is used. It is both a qualitative and a quantitative method where the time of flight through the vacuum tube decides what elements are sampled and a detector measuring the current.

In AES, the unique wavelength each element emits is used. This radiation can either be ultraviolet or visible light. The intensity is proportional to the concentration of the analyte. Standard calibration curves are used to establish a relationship between the concentration and the intensity.

Both methods are sensitive to changes in physical conditions like temperature, sample introduction rate and calibrations need to be made frequently throughout the measurement.

### *2.2 Data analysis*

For the best overview of the relative concentration levels of metals and differences between samples a multivariate analysis named Principle Component Analysis (PCA) was performed on the data. This method allows different number of samples from different stations and detects outlying objects that poorly fit the model. It also visualises patterns between samples (Esbensen, 1997*)*. The Unscrambler software is used.

Geometrically described, the PCA method seeks out vectors in which the distance from the original values of the object to the vector-projected value is minimised through a least square fit method. These vectors are called principal components. The gain is that, since the data co-vary, the model can be described in fewer principal components than the original number of variables without loosing a considerable amount of information. The model of course improves the higher number of PCs included as long as the PC does not only describe noise. The first steps include centring and scaling the data. Centring, means, normalising each object, and scaling, means dividing each object by the standard deviation. The residual variance shows to what extent the PCs don't describe the model. The model is validated through full cross validation, meaning that a model is made using all objects but one, which is used for validation. This is done for all objects leaving one out at a time. Residual X variance is a measure on how well an object fits the model. A large Residual X

variance means a poor fit. Leverage value describes what impact an object has on the model. It ranges from 0-1, 1 meaning great impact on the model.

# 3 Materials and methods

## *3.1 Sample locations*

The object is to make a baseline study across the estuary of Matola River since this is likely to be the biggest source of anthropogenic contamination. The samples

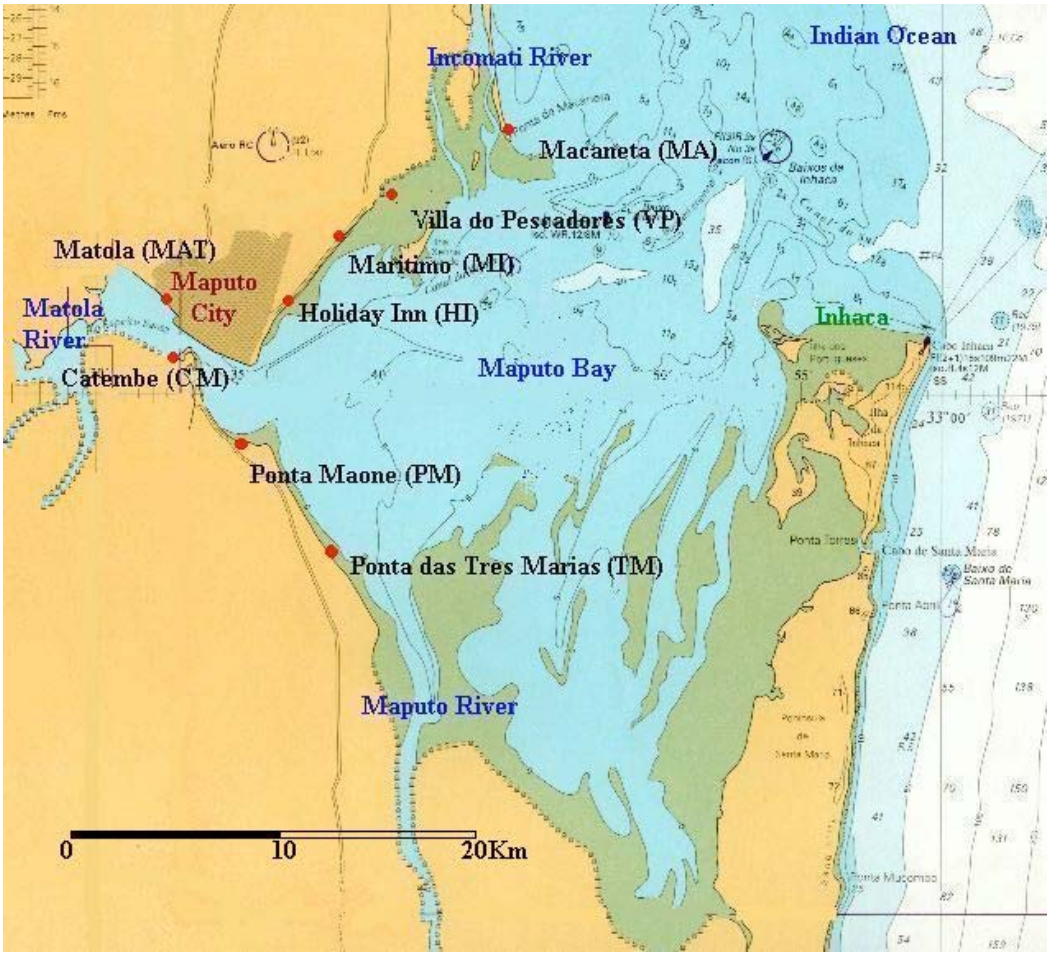


Fig. 2 Map over Maputo Bay showing location of collection stations.

were therefore collected at 10 stations, of which mussels from 8 stations were analysed (marked with red dot in fig 2), from Ponta de tres Marias 20 km SW from Maputo to Incomati River estuary 20 km north of Maputo. Three stations south of Matola River estuary and 6 stations northwards were chosen. The distance between the stations was on average three kilometres. Macaneta is situated by itself on the northern tip of Maputo Bay and will serve as a reference station since organisms here are probably not affected by Maputo River to a significant extent but only by Incomati River. Villa do Pescadores is located where the sandy beaches of Costa do Sol ends and the mangroves take over. People at this small village lives mainly from fishing. Maritimo station is in the middle of Costa do Sol with a sandy beach and intense collection of mussels. Holiday inn station is similar to Maritimo in terms of beach conditions but situated 1 km to the south. Matola station is located some 500 m from Matola river estuary mouth and close to the harbour. Sediments are mainly composed of mud. Catembe station is similar to Matola. Mussels were collected for food at all Stations.

### *3.2 Sample collection*

At each station 6-20 individuals of *Meretrix meretrix*, depending on availability, were collected either by hand or by locals. The mussel size varied between 40 and 60 mm. The distance from the shoreline was between 10 and 100 metres depending on tides, and the collection area was never more than 100 $m^2$. Tides limited the collection time to only 2-3 hours making it impossible to gather the needed amount of clams alone. Local help was therefore used. By scraping the top sediment layer with different metal or plastic devices the mussels were detected and collected. The sediment structure varied from muddy to sandy (Achimo 2000). The samples were transported without preparation to a laboratory in open plastic containers. Collection was carried out between November 1 and December 10 2001.

### *3.3 Sample preparation*

The samples were prepared at Eduardo Mondlane University. After freezing, the mussels were put in hot water, for a very short time, to open them and then rinsed thoroughly in distilled water. The entire soft tissue of the clam was separated from the shell. Shell length, soft tissue wet and dry weight of soft tissue was measured and the samples were dried in 70C for 24 h and frozen to –15 C awaiting transportation to Uppsala and chemical analyses.

### *3.4 Analysis preparation*

8 samples from each collection station were ground in a Teflon grinder together with two iron marbles and were intensively shaken for five minutes. This resulted in a fine powder and the sample was then considered homogenised. The samples were kept in a desicator to ensure constant humidity. In some cases the samples were not completely dry and could not be properly homogenised. Additional drying in 70° C for 24 h was then performed and the completely dry samples were then again ground. Four sets of cellulose material was also ground together with the iron marbles and later analysed the same way as the other material to detect any possible contamination from the marbles. Approximately 0.2-g of tissue material was weighed by an electronic balance and was wet digested in vessels in 4.0-ml concentrated pro analysis $HNO_3$, heated in two steps, first to 125° C for 45 minutes and then to 160° C for 2 h. Before opening, the vessels were cooled to room temperature. 12 samples were processed at a time. Each set of samples included samples from different stations and two blank samples (without any material, only acid matrix). The process

was conducted in thick glass tubes inside even thicker metal cylinders and tightly sealed to withstand the pressure from digestion gas products. The samples were finally diluted to 50.0 ml using MQ water and kept in closed plastic containers. The analysis of the blank samples did not show satisfactory results. Some background levels were high compared to MQ water and the acid matrix was changed to suprapure $HNO_3$ and used throughout the analysis. Some samples were destroyed during the process since they were not properly sealed during the heating process (Uhrberg, 1982).

### *3.5 Metal analysis*

An ICP-MS (Inductive Coupled Plasma-Mass Spectrometer) and an ICP-AES (Atomic Emission Spectrometer) were used to analyse the metal concentration of the samples. The AES was used for the majority of the elements. In the case of element signals under or close to the detection limit the MS was used. As reference material bovine liver undergoing the same wet digestion as the clams was used (National Bureau of Standards Certificate of Analysis. Standard Reference Material 1577a, Bovine liver). Calibration solutions containing known concentrations of each element were made and calibration curves (linear regression, least square method) were drawn for each element. Blank samples, and cellulose material ground with a Teflon grinder, were analysed for contamination.

## 4 Result and discussion

Earlier studies of surface sediments in the Maputo Bay area, using UCA ratios as a reference, suggest that levels of Cr, Fe, Co and Cu are elevated in the harbour area (Achimo, 2000, Basse et al., 2000). Two stations from this study, Matola and

Catembe, are situated within the sediment-studied area. Sediment composition affects the mussel soft tissue metal concentration (Inza et al., 1997) and it is reasonable to expect elevated levels also in the studied mussels.

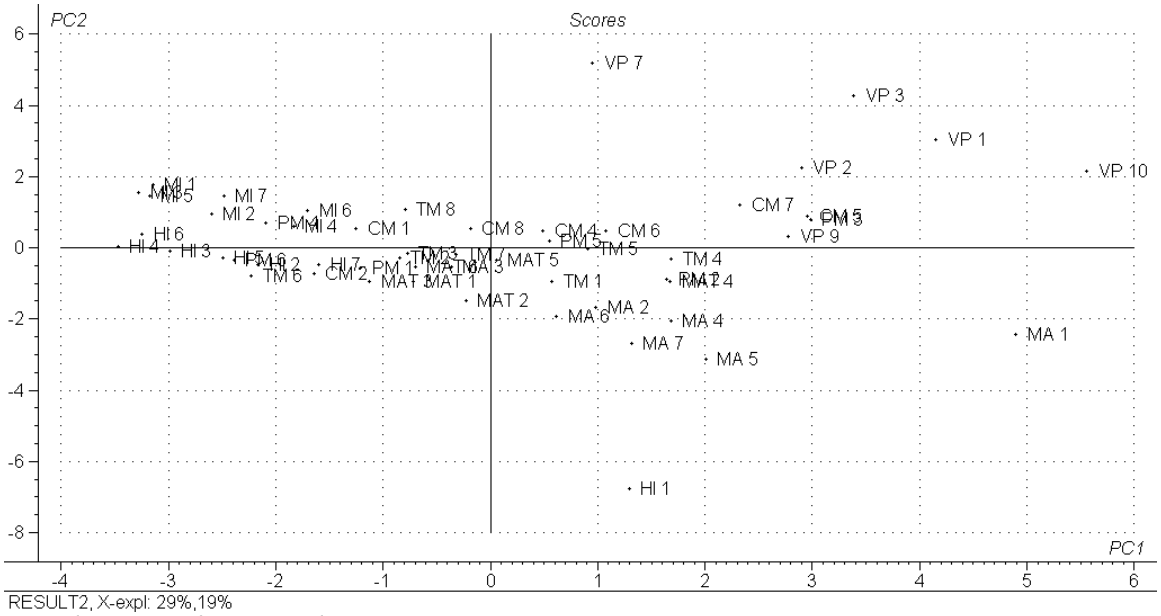

Fig 3. PC1 and PC2 scoreplot.

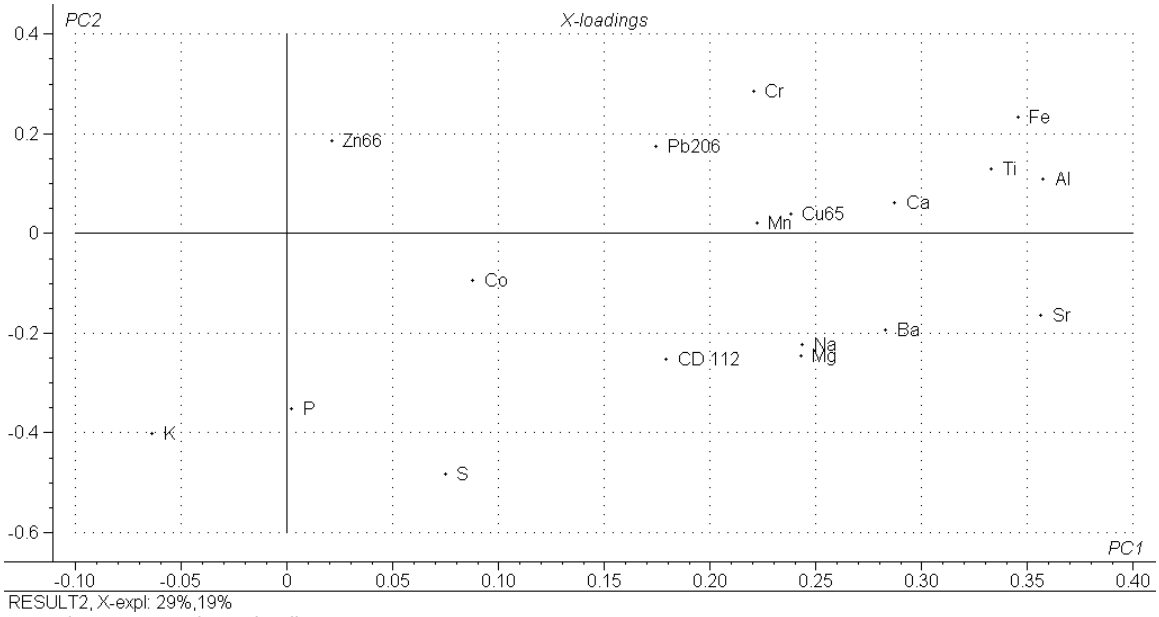

Fig 4. PC1 and PC2 loadings.

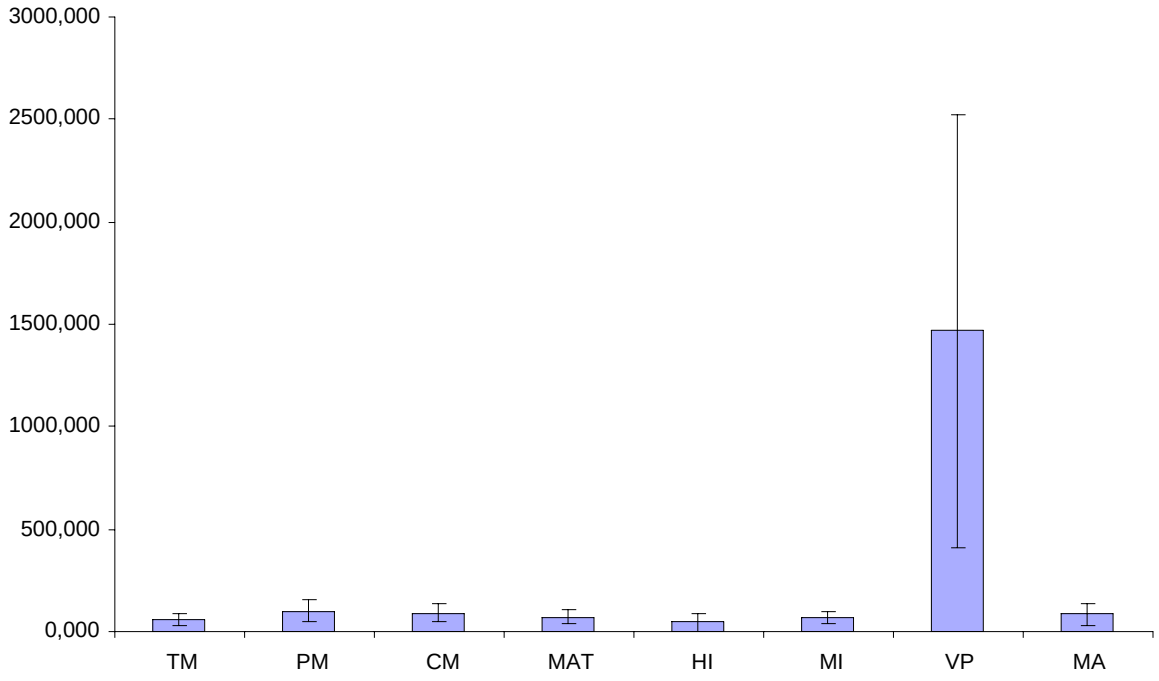


Fig 5. Cr concentration μg g-1

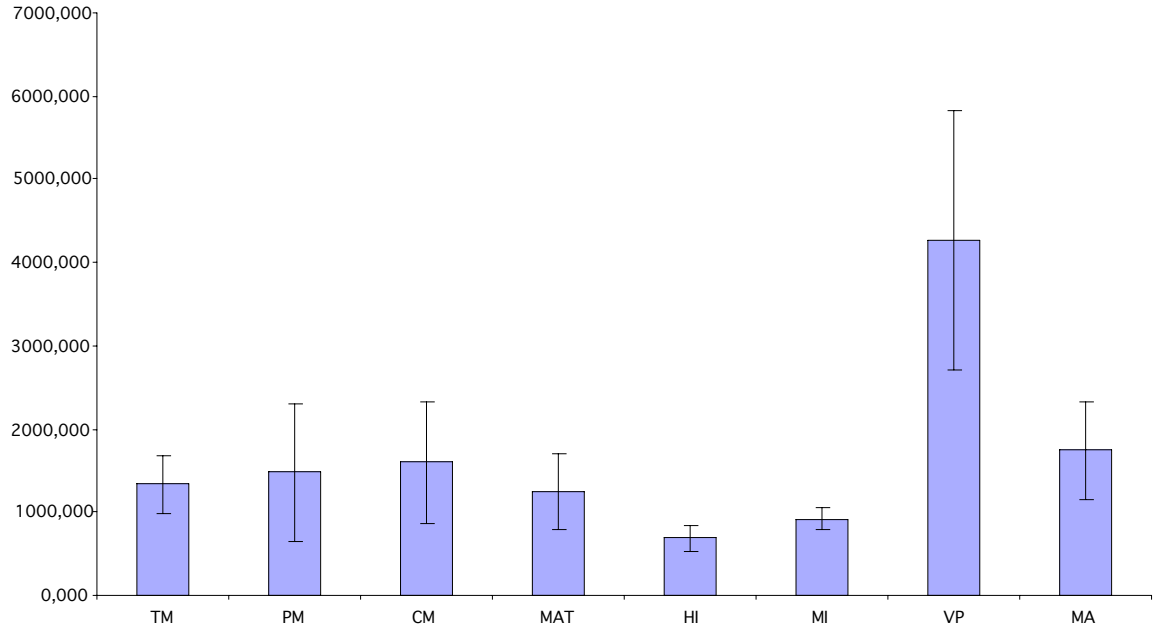


Fig 6. Fe concentration μg-1.

The biggest anomaly discovered in the statistical analysis is the Cr and Fe concentration at Villa Do Pescadores (VP). Fig 3 and Fig 4 show that VP samples correlate well with Cr and Fe along PC1. Cr and Fe are a factor 15 and 3, respectively, (Figs 5 and 6) higher than all other stations suggesting anthropogenic influence on a local scale. Fishermen at VP have a number boats parked in the area possibly emitting substances from paint and other mechanical use (Basse et al, 2000). A small creek, running through densely populated areas, also has its outlet close by and is another possible source of Fe and Cr. VP also shows relatively high concentrations of Pb, Al and Ca.

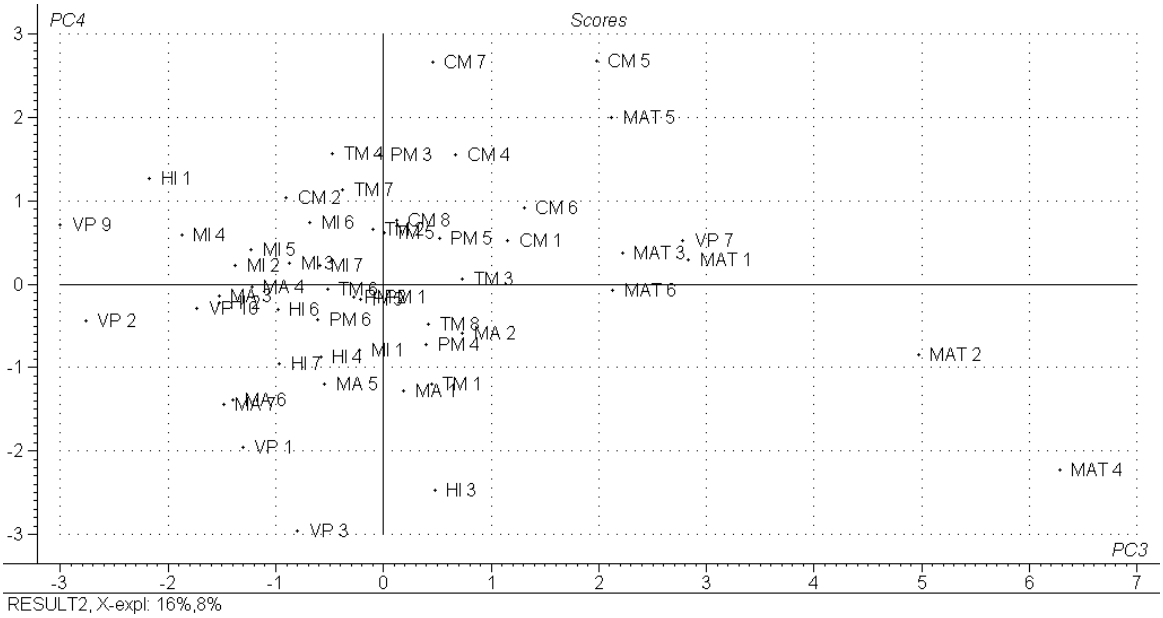


Fig 7. PC3 and PC4 scoreplot.

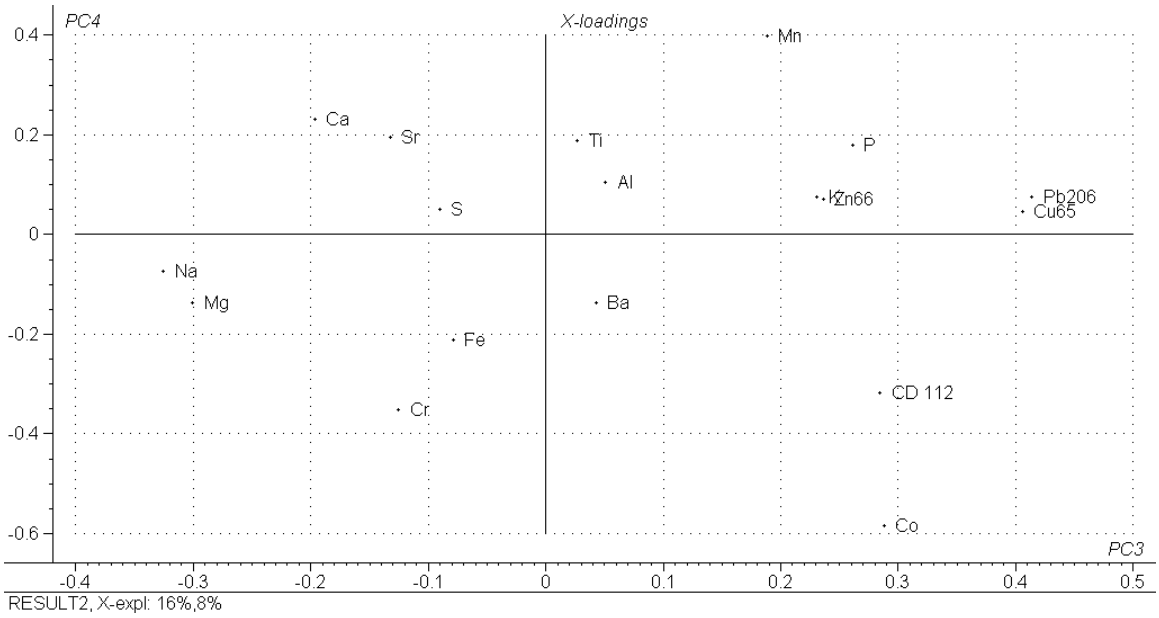


Fig 8 PC3 and PC4 loadings.

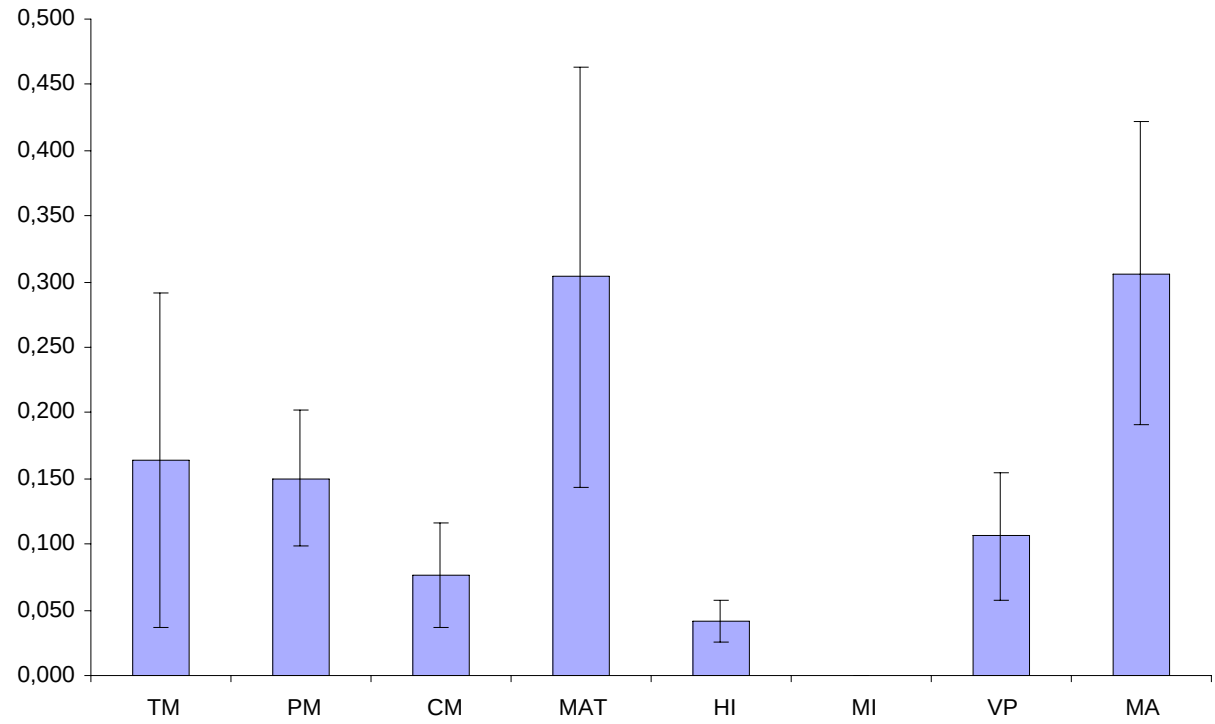


Fig 9 Pb concentration μg g-1.

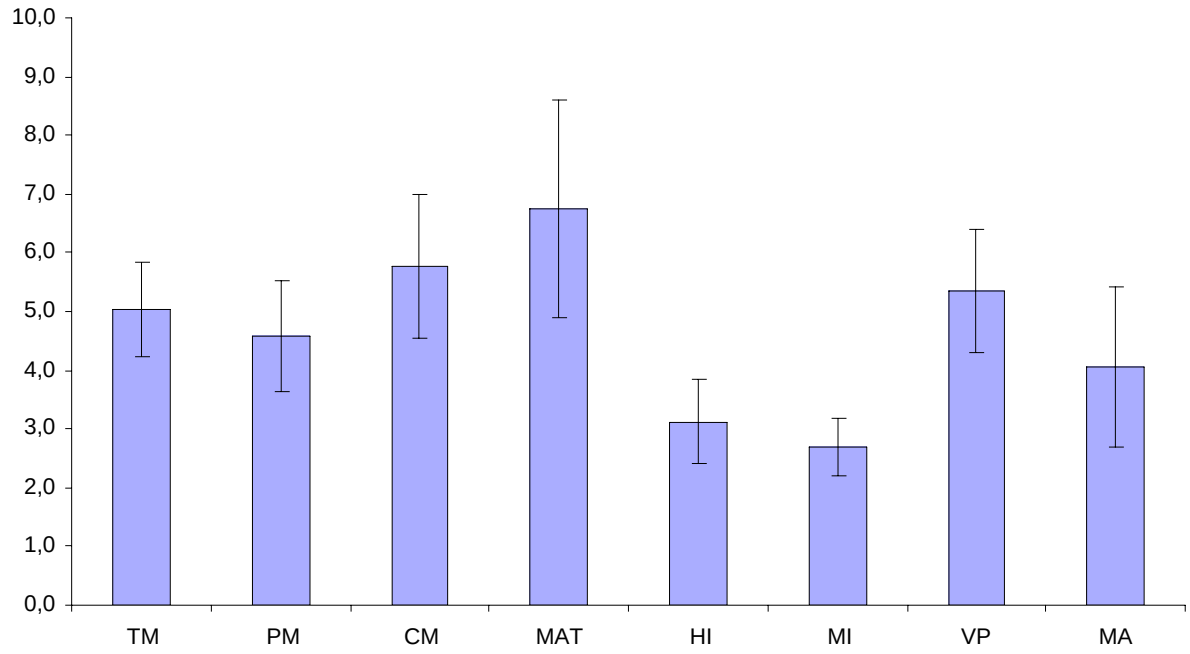


Fig 10. Cu concentration μg g-1.

PC3 in Fig 7 and Fig 8 suggests that Matola samples correlate with high concentrations of Pb and Cu, and low concentrations of Na and Mg. Fig 9 and Fig 10 showing the Pb and Cu concentrations at Matola support this. Like VP, Matola is situated in a region where boats are kept. Its vicinity to the harbour also makes it a target for anthropogenic waste. Sediment transport to this area is large. Influence of water from Matola River explains the low concentrations of Na and Mg.

Table 1. Mean concentration (m) and standard deviation (s) for every station (μg g-1).

| | Cd | Co | Cr | Cu | I | Li | Ni | Pb | Zn | Li | B |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TM m | 0,16 | 0,99 | 59,1 | 5,0 | 6,5 | 0,9 | 6,3 | 0,7 | 41,6 | 1,6 | 9,0 |
| s | 0,13 | 0,39 | 31,3 | 0,8 | 4,1 | 0,8 | 4,2 | 0,3 | 12,4 | 0,7 | 6,4 |
| PM m | 0,15 | 1,11 | 99,9 | 4,6 | 8,0 | 2,0 | 10,5 | 0,8 | 49,8 | 2,9 | 5,5 |
| s | 0,05 | 0,35 | 53,4 | 0,9 | 3,5 | 1,2 | 4,9 | 0,3 | 18,6 | 1,7 | 6,6 |
| CM m | 0,08 | 0,80 | 88,5 | 5,8 | 5,0 | 1,3 | 7,8 | 0,9 | 69,7 | 1,6 | 6,6 |
| s | 0,04 | 0,17 | 44,1 | 1,2 | 3,0 | 1,0 | 4,6 | 0,3 | 65,3 | 0,8 | 5,3 |
| MAT m | 0,30 | 1,71 | 71,2 | 6,8 | 2,1 | 0,6 | 8,0 | 1,3 | 67,5 | 1,3 | 4,3 |
| s | 0,16 | 0,92 | 33,9 | 1,9 | 1,6 | 0,7 | 4,3 | 0,4 | 17,8 | 0,6 | 4,2 |
| HI m | 0,04 | 1,29 | 47,4 | 3,1 | 2,8 | 0,7 | 5,9 | 0,5 | 49,8 | 0,8 | 9,1 |
| s | 0,02 | 0,76 | 44,7 | 0,7 | 1,8 | 0,8 | 3,9 | 0,1 | 14,0 | 0,5 | 8,3 |
| MI m | 0,01 | 0,60 | 66,5 | 2,7 | 1,8 | 0,4 | 3,8 | 0,5 | 41,0 | 0,5 | 6,2 |
| s | 0,03 | 0,24 | 28,4 | 0,5 | 0,7 | 0,2 | 1,1 | 0,1 | 8,6 | 0,4 | 2,7 |
| VP m | 0,11 | 1,11 | 1468,2 | 5,3 | 1,7 | 1,5 | 6,6 | 1,2 | 48,2 | 2,1 | 5,2 |
| s | 0,05 | 0,42 | 1054,4 | 1,1 | 0,6 | 0,4 | 1,4 | 0,8 | 7,7 | 1,1 | 3,7 |
| MA m | 0,31 | 1,24 | 84,7 | 4,0 | 1,2 | 1,3 | 5,7 | 0,6 | 36,5 | 1,6 | 4,8 |
| s | 0,12 | 0,40 | 51,8 | 1,4 | 0,2 | 0,4 | 2,1 | 0,2 | 9,4 | 0,8 | 2,2 |

| | Na | Mg | Al | P | S | K | Ca | Ti | Mn | Fe | Sr | Ba |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TM m | 20191 | 3173 | 1633 | 9243 | 12783 | 9187 | 3978 | 48,5 | 24,4 | 1332 | 33,4 | 4,9 |
| s | 1989 | 294 | 515 | 1936 | 1125 | 691 | 2461 | 11,9 | 9,6 | 350 | 8,6 | 1,3 |
| PM m | 20844 | 3155 | 2044 | 8564 | 12820 | 8398 | 3324 | 50,8 | 31,2 | 1477 | 39,7 | 6,1 |
| s | 5239 | 765 | 1272 | 471 | 1248 | 312 | 1077 | 24,9 | 21,2 | 824 | 10,7 | 2,3 |
| CM m | 20220 | 2723 | 2046 | 9511 | 11566 | 9507 | 3510 | 56,7 | 76,3 | 1597 | 39,5 | 5,8 |
| s | 4137 | 418 | 1001 | 683 | 770 | 748 | 1203 | 28,1 | 43,7 | 735 | 9,4 | 3,1 |
| MAT m | 12668 | 2675 | 1417 | 10789 | 12330 | 12779 | 2347 | 38,2 | 70,8 | 1249 | 31,4 | 7,0 |
| s | 3771 | 186 | 698 | 1606 | 544 | 1098 | 465 | 16,5 | 48,2 | 463 | 4,2 | 1,5 |
| HI m | 22159 | 3657 | 695 | 8340 | 13434 | 11158 | 2442 | 24,4 | 14,3 | 683 | 30,0 | 3,9 |
| s | 6685 | 1183 | 171 | 2099 | 3738 | 3214 | 976 | 5,1 | 8,0 | 168 | 14,6 | 1,4 |
| MI m | 14836 | 2749 | 1066 | 6611 | 10603 | 8791 | 2445 | 37,3 | 18,5 | 921 | 24,0 | 4,1 |
| s | 2709 | 378 | 204 | 533 | 1084 | 433 | 452 | 7,3 | 5,8 | 131 | 4,7 | 0,5 |
| VP m | 24695 | 4016 | 2449 | 5840 | 10147 | 6393 | 5649 | 61,7 | 46,0 | 4275 | 44,0 | 6,9 |
| s | 4971 | 816 | 821 | 1043 | 1161 | 739 | 1323 | 14,4 | 11,6 | 1554 | 9,6 | 1,2 |
| MA m | 28345 | 4296 | 1812 | 8483 | 14131 | 9811 | 3199 | 47,8 | 37,5 | 1739 | 42,0 | 14,4 |
| s | 3449 | 590 | 648 | 1561 | 1275 | 1003 | 569 | 9,5 | 8,4 | 578 | 8,4 | 3,9 |

The stations Maritimo and Holiday Inn showed overall lower metal levels than other stations (Table 1). Sediment transport is not as big here as to the other stations (Achimo, 2000) due to anti-clockwise littoral drift in the south and central Maputo Bay where Maputo River discharges, and a clockwise drift in the north at the Incomati River outlet. Not being a target for contaminated sediments possibly results in very low metal levels, and it is suggested that sediment composition plays a key roll in the heavy metal uptake by *Meretrix meretrix*.

Macaneta, located on the tip of Maputo Bay, mainly affected by seawater but to some extent also from Incomati River shows relatively large levels of Na, Ba and Mg, also indicating the salty environment. Salinity is around 0,4 % units higher than at the other stations (da Maia et al, 1997).

Table 2. Reference material comparison (μg g-1)

| Sample ID 1 | Cd | Co | Cu | Pb | Zn | Na | Al | Mg |
|---|---|---|---|---|---|---|---|---|
| lever 1 | 0,79 | 0,32 | 212 | 0,23 | 127 | 2276 | 14,47 | 644 |
| lever 2 | 0,64 | 0,37 | 211 | 0,10 | 125 | 2297 | 11,33 | 651 |
| Reference material certified value | 0,44 | 0,21 | 158 | 0,14 | 123 | 2430 | (2,00) | 600 |

| Sample ID 1 | P | S | K | Ca | Sr | Fe |
|---|---|---|---|---|---|---|
| lever 1 | 11814 | 7481 | 9301 | 134 | 0,04 | 194 |
| lever 2 | 11963 | 7568 | 9402 | 131 | 0,10 | 194 |
| Reference material certified value | 11100 | 7800 | 9960 | 120 | 0,14 | 194 |

Bovine liver was used as reference material. The differences between documented reference values and measured (Table 2) are likely to depend on matrix effects and

old, and possibly contaminated, reference material. Low signals and only two calibration points used for MS (AES calibration used 3) made the regression weak, which may also explain the differences. Levels are satisfactory for most elements with the exception of Cd, Co and Cu, which are around 50% higher for the analysed reference material. The concentration of Aluminium in the reference material was not certified.

Comparison between Cd, Cu, Pb, Zn and Cr levels of *meretrix* with other bivalves around the world (Hung et al., 2000, Mendez et al., 2001, Wong et al 1998, Saiz et al 1996,Locatelli et al 1999, Sokolowski et al 2002) indicate no high levels except for Cr levels at Villa de Pescadores. VP showing levels 15 times higher than normal suggests the need for additional studies from this station.

### *4.1 Conclusions*

Heavy metal levels are in general low at all stations except Villa do Pescadores where Fe and Cr concentrations are 3 and 15 times higher, respectively. In terms of health hazards to humans none of the elements Cd and Pb have dangerous levels at normal consumption of mussels (EU 466/2001 8 march 2001).

## 5 Acknowledgement

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Dr Adriano and Dr Aidate helped me changing the method and were of great help in practical issues. Louis drove me around Maputo Bay collecting clams. The Swedish school in Maputo helped me socially. Jens Dahlberg arranged excess to the DHI report.

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