A baseline study of metal contamination along the Namibian coastline for *Perna perna* and *Choromytilus meridionalis*.

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Abstract

The use of bivalves such as the brown mussel (*Perna perna*) and the black mussel (*Choromytilus meridionalis*) is common in the study of marine pollution and the effect of these pollutants on ecosystems and are important in both economic and ecological roles. Namibian marine ecosystems are threatened by pollution from mining, commercial fishing and population growth. The aims of this study were to determine baseline metal concentrations, spatial variation and variation between species. Metal levels in *C. meridionalis* from Guano Platform (GP) are the lowest of all the sites. The most polluted sites are Rocky Point (RP), Halifax Island (HIL) and between Walvis Bay and Swakopmund (WS). The bioaccumulation of metals between *P. perna* and *C. meridionalis* were not uniform for all metals. Overall the study indicates the condition of the coastline to be mostly normal, with Cd and Pb levels being of concern.

Keywords

Mussels, heavy metals, pollution, ICP-OES, bioaccumulation, bivalves.

Human development has brought about an assemblage of negative consequences for the natural environment, especially for the marine environment. Riverine systems continuously carry an array of pollutants from the continents to the oceans including raw sewage, domestic waste, agricultural waste and industrial waste to name a few (Meybeck, 2009). The contamination of marine ecosystems with these pollutants, both organic and metals, induce stress in marine life, especially in sedentary organisms such as bivalve molluscs (Sindermann, 2005).

The use of bivalves such as the brown mussel *Perna perna* (Linnaeus, 1758) and the black mussel *Choromytilus meridionalis* (Krauss, 1848) is common in the study of marine pollution and the effect of these pollutants on ecosystems (Vosloo et al. 2012; Angulo, 1996; Greenfield et al. 2011). Mussels are often used as indicators in trace metal analyses in marine habitats due to their filter-feeding abilities, the fact that they are bio-accumulators and ultimately because of their sedentary nature (NOAA, 1995).

Perna perna is a rock mussel species that is found not only in the tropical areas of southern Africa but also in the cooler waters along the Namibian coastline, caused by the cold Benguela current, whereas *C. meridionalis* is most common on the west coast of Africa (Van Erkom Schurink and Griffiths, 1990). According to Simon (1999) both *P. perna* and *C. meridionalis* are species that carry significant importance in both economic and ecological roles. *Perna perna* and *C. meridionalis* inhabit slightly different microhabitats: *P. perna* inhabits sand-free rocks whereas *C. meridionalis* inhabits areas where sand cover is often experienced (Marshall and McQuaid, 1993). It has been discovered that both species have diets that mainly consist of detritus (Griffiths, 1980).

Both *P. perna* and C. *meridionalis* not only serve as a high protein source for local Namibian people but also play an economic role in terms of the local markets (Bianchi et al. 1999). The excessive consumption of metal contaminated mussels can eventually lead to the poisoning of the consumer as it cannot be broken down by the human body into more harmless molecules (Kromhout et al. 1985). It is common for marine mussels to reside close to estuaries where they are more likely to be affected by the pollution carried into the ocean by rivers (Yap et al. 2004). Stress syndrome in bivalve molluscs can lead to shell defects, recession of the mantle and deterioration of the epithelium in the digestive tubule (Sindermann, 2005). Almeida et al. (2003) established that trace metal contamination also has effects on hormone levels in *P. perna* such as DOPA and 5HT, which in turn affects other physiological processes.

Unfortunately, as always, the human impact on pristine environments will lead to habitat destruction by means of pollution from mining, commercial fishing and population growth, and Namibia is no exception (Boyer et al. 2000). Constant exploration for oil has been going on for years and in 2013 the Brazilian company HRT finally found it, although volumes are not yet viable for commercial extraction (Immanuel, 2013). In case of oil spills, many fragile species can be affected and can ruin the pristine coastline with little human impact that the Namibians pride themselves in (Boyer et al. 2000).

In consideration of these factors, the importance of establishing baseline data for future reference is highlighted. No publications have been found to note the metal pollution levels along the coast of Namibia using bivalve molluscs. The aim of this study is to determine baseline trace metal concentrations by means of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Three objectives were identified in our study:

1) to provide a set of baseline data on metal concentrations along almost the entire length of the Namibian coastline.
2) to provide data on the possible impacts that Walvis Bay Harbour is having on the biota and
3) to compare the metal concentrations in the two sampled species namely *P. perna* and *C. meridionalis.*

Two working hypotheses were considered:

1) anthropogenic activities are having a negative effect on metal concentrations in mussels in Walvis Bay Harbour and
2) *P. perna* and *C. meridionalis* accumulate metals at similar concentrations.

Mussels of the species *P. perna* and *C. meridionalis* were collected from various sites along the Namibian coastline from 01/10/2012 to 12/10/2012. Figure 1 indicates the distribution of sampling sites along the Namibian coastline and which species were collected at each site. *Perna perna* were collected from Bosluis Bay (BB), Guano Platform (GP), Griffiths Bay, Lüderitz (GBL), Halifax Island, Luderitz (HIL), Mile 17 (M17), Rocky Point (RP), between Walvis Bay and Swakopmund (WS) and Walvis Bay Harbour (WBH). *Choromytilus meridionalis* samples were collected from GP, GBL and M17. *Choromytilus meridionalis* were collected from sites where they were present. Table 4 indicates the GPS coordinates of the sampling sites. Twenty samples from each site and of each species were collected where possible. Samples were air dried and sealed into air-proof containers and stored until use.

0.5g dry weight of each sample was digested using a Mars Microwave Digester. The samples were placed into individual digesting containers. To each container, 10ml of Suprapur 65% nitric acid as well as 1ml Suprapur hydrogen peroxide was added using a manual 1000µl pipette. Samples were left for 10 minutes at room temperature for the nitric acid and hydrogen peroxide to properly react before placing them into the digestion machine. Containers were placed into the Teflon holder and placed into the digester. The samples stayed in the high temperature and high pressure system for 70 minutes at a holding temperature of 200ºC including a 15 minute warm-up period followed by a 35 minute holding period and a 20 minute cool-down period. After digestion the bombs were emptied into their respective 50ml Falcon tubes. The samples were diluted gravimetrically to 50 grams with deionised Milli-Q water (Degger, 2010).

Samples were filtered using BOECO Germany 65g/m^2 Filter paper. From all sites, all 20 samples collected were read using the Spectro Arcos FSH 12 Inductively Coupled Plasma Optical Emission Spectrometer. The ICP-OES was calibrated using 10ppm and 50ppm standards. Certified Reference Material (CRM) (Dogfish Liver Tissue [DOLT]) was used for most of the metals tested, of which data were expressed in Table 3. The percentage recoveries of most of the metals tested were in the 80-110% range required. The recovery level of Pb was deemed unacceptable.

Data were statistically analysed using JMP and IBM SPSS 21. Raw data were normalised by using a log transformation after which descriptive analysis was conducted on the data. The log transformed data were then tested using the Tukey-Kramer Method (McDonald, 2009). The Tukey-Kramer method yielded Analysis of

Variance (ANOVA) for the determination of significant differences spatially and between the two species analysed. Pearson's correlation analysis was also performed to determine correlations between the two species from all of the different sites.

The mean metal concentrations of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn for all sites sampled are graphically represented in Figure 2. No concentrations were found to be below detection. All concentrations are presented in µg/g dry mass.

The highest concentration of Al (2.588µg/g) was found in *C. meridionalis* from GBL and the lowest in *P. perna* from WBH. Aluminium (Al) is not only found in marine sediments as aluminosilicates but also in other chemical junctions and complexes (Mao et al. 2011). Joyner (1964) reported that the concentrations of aluminium found in seawater can range between 2-150µg/L^{-1}. Studies have shown that aluminium can lead to neurotoxic symptoms in marine fauna (Mao et al. 2011). When compared to similar studies along the entire coastline of South Africa by Degger (2010), it is clear that conditions along the Namibian coastline are good. Degger (2010) shows that Al levels for Cape Town, Durban, East London, Mossel Bay, Port Elizabeth and Richards Bay harbours had mean concentrations that were 100-200 times the concentrations obtained in this study.

The highest Cd concentrations were discovered at HIL with *P. perna* having a mean of 1.29µg/g. The lowest Cd concentrations were found in *C. meridionalis* from GP (0.399µg/g). Griffiths Bay had the lowest concentrations of Co (0.469µg/g). *Perna perna* from RP had a higher Co concentration (0.907µg/g) than the rest of the sites. Cadmium (Cd) is known to have one of the highest toxicities of all metals and it is commonly found in polluted water (Choi et al. 2007). Bivalves that have lived in environments containing high levels of Cd for extensive periods of time have adapted to it by the development of Cd-binding metallothioneins, which lower the concentrations of Cd in the body (Choi et al. 2007). Degger (2010) found Cd concentrations that were profoundly higher than those in the Walvis Bay Harbour, such as Port Elizabeth Harbour with a mean Cd concentration almost 20 times of that found in Walvis Bay Harbour.

The concentrations of Cr were the highest at HIL with 0.971µg/g in *P. perna.* The lowest mean concentration was found in *C. meridionalis* from GP (0.527µg/g).

Chromium (Cr) is found in two oxidation states in the marine environment Cr (III) or Cr (VI) (Li et al. 2009). The reaction of Cr (III) is greatly enhanced in water of a neutral pH, whereas its counterpart can easily be accumulated by fauna (Li et al. 2009). The Cr concentrations obtained by Degger (2010) are relatively close to the concentrations obtained in this study, with the highest level being approximately 3μg/g dry mass. It has been found by many scientists that the concentrations of Cr can vary from 0.23μg/g dry mass in normal marine environments to 48.5μg/g dry mass in severely polluted environments (Mills, 2005). Results obtained from this study show all sites to be quite normal considering Cr concentrations. Chase et al. (2001) set out a guideline to Ni concentrations of concern which is only above 533μg/g dry mass, which is much higher than any of the results obtained in this study.

Species collected from WBH had the highest mean concentration of Cu (1.214μg/g), the lowest in *P. perna* from M17 (0.971μg/g). Copper (Cu) is a naturally occurring metal in seawater and according to Al-Subiai et al. (2011) a constant increase in the Cu contamination of seawater is imminent in the near future. Copper has negative effects on the DNA of marine life as it binds to DNA molecules and causes decreased resistance to disease and the development of morphological anomalies (Zorita et al. 2006; Lloyd and Phillips, 1999). High concentrations of Cu can become toxic and can cause an array of problems including negative changes in reproduction, growth and physiological processes (Vosloo et al. 2012).

Walvis Bay Harbour is the main port in Namibia and also the main fishing harbour (Ministry of Environment and Tourism, 2008). Walvis Bay Harbour showed a high Cu concentration which indicates the presence of antifouling paints. Antifouling paints are usually applied to ship hulls and would therefore be found in higher concentrations in the harbour as opposed to any of the other sites (Hall and Anderson, 1999). Cu is known to become toxic to invertebrates such as *P. perna* when found in concentrations 10 times higher than the amount necessary to sustain life, as copper is an element essential to life (Hall and Anderson, 1999).

The concentrations of Cu found by Degger (2010) were much higher than the results obtained from WBH, with concentrations ranging from negligible amounts to almost 115μg/g dry mass in Degger's study. Harbours along the South African coastline also had substantially higher concentrations of Fe than those found in the WBH, the highest being Mossel Bay harbour with approximately 700µg/g. Mn also showed dramatic differences between conditions in the South African harbours as opposed to WBH. Mn levels in the South African harbours were up to 120 times higher than in WBH. Nickel levels in South African harbours were similar to those in WBH.

The levels of Al were lower in the areas of WBH, which is not an anomaly mainly because Al concentrations in marine environments are naturally greatly variable (Joyner, 1964). A future problem identified is the development of aquaculture farms which will be in close proximity to the dredge dump which is allocated close to WBH (Ministry of Environment and Tourism, 2008).

Another similar study done by Yap et al. (2004) indicated similar levels of Cd from the Tolo Harbour in Hong Kong; however Cu levels in Tolo Harbour (6-24µg/g) were much higher than levels in the Walvis Bay Harbour. Levels of Pb in the Tolo harbour were approximately double the mean concentration in WBH. Zn concentrations in the Tolo harbour ranged from 90-135µg/g, which is more than 50 times the level from the WBH.

Fe concentrations were the highest in *P. perna* from M17 (2.857µg/g) and lowest in *C.meridionalis* from GP (2.275µg/g). The highest and lowest Mn concentrations were measured in *C. meridionalis* from M17 (1.302µg/g) and *P. perna* from GP (0.993µg/g) respectively. Iron (Fe) and Manganese (Mn) are naturally occurring elements in the marine environment due to the make-up of marine sediments (Shiller, 1997). Manganese, when consumed in excessive quantities by humans, can lead to neurological symptoms similar to Parkinson's disease (Levy and Nassetta, 2003). Manganese concentrations in seawater are known to range between 0.4-10µg/L (Zeri et al. 2000).

Ni concentrations were the highest in *P. perna* from RP (1.557µg/g). The lowest Ni concentrations were found in *C. meridionalis* from GP (0.71µg/g). Hédouin et al. (2007) reported the specific bioaccumulation of Nickel (Ni) in bivalves with the vast lack of knowledge in this area as a driving factor for the study. This study showed a directly proportional relationship between accumulated Ni and Ni available in the seawater. A study by Millward et al. (2012) concluded that the long term exposure of mussels to Ni can lead to cytotoxic and genotoxic effects.

Pb concentrations in *P. perna* from WS (2.084µg/g) and GP (2.072µg/g) were found to be the highest. The lowest Pb concentration was found in the *C. meridionalis* from M17 (1.86µg/g). Lead (Pb) has a tendency to influence naturally occurring metals and proteins in marine fauna such as calcium (Ca), zinc (Zn) and iron (Fe), causing these molecules to carry out alternative functions or fail to react to certain molecules (Company et al. 2011). The study by Company et al. (2011) revealed that elevated levels of Pb in marine ecosystems can lead to a reduction in the production of the δ-Aminolevulinate dehydratase enzyme in mussels, negatively influencing the heme biosynthetic pathway.

Zn concentrations were highest in *P. perna* from WS (2.422µg/g) and the lowest levels were found in *C. meridionalis* from GBL (1.976µg/g). Zinc is an essential metal to many organisms and a study done by Chan (1988) showed that Zn in *P. verdis* is accumulated up to a certain point, then it reaches a plateau and Zn concentrations can thus be partially regulated in the body of the mussel.

The Namibian government follows a set of standards laid out by the Food and Agricultural Organization (FAO) in conjunction with the World Health Organization (WHO) called the CODEX Alimentarius which determines the levels of heavy metals safe for human consumption, however not all heavy metals tested for in this study are found in this set of standards (CODEX, 2014). The Codex Alimentarius sets the limit of Pb in bivalve molluscs as 1µg/g which means that the mean concentration of Pb found in this study is almost double the maximum limit. The Codex Alimentarius places the maximum allowed limit of Cd in bivalve molluscs as 2µg/g which is more than double the mean concentration of Cd found in this study (CODEX, 2014). Therefore, even though most of the metal concentrations found in this study seem low compared to other studies, they could still render the mussels inedible.

The means, standard errors and ranges of all of the metals from all of the sites collectively is illustrated in Table 2. As can be seen in Table 5, the mean metal bioaccumulation of *P. perna* is spatially compared to determine statistically significant differences between sites. Sites that are connected by letters are therefore not significantly different from each other but they are significantly different from sites that they are not connected to.

The mean concentrations of Al show a strong correlation between all sites except GP and the WBH. These two sites have significantly lower concentrations than the rest of the sites. Al levels in *P. perna* from WBH are significantly lower than any of the other sites analysed.

Cd concentrations in *P. perna* revealed that species from HIL had considerably higher levels of Cd accumulated in their tissues than any of the other sites. BB, GP, WBH and WS were significantly lower than all the other sites considering Cd concentrations ($p>0.05$).

Cr concentrations reveal a division into two groups that are significantly different from each other. The first group includes HIL and RP which is significantly higher than group two which contains the rest of the sites.

Co concentrations revealed a clear division between the *P. perna* collected from RP and those collected from the six other sites. *Perna perna* from RP had Co levels that are significantly higher than any of the other sites.

Cu concentrations show WBH to have a significantly higher mean concentration than any of the other sites whereas BB, HIL and M17 are significantly lower than any of the other sites.

ANOVA analysis shows significantly lower levels of Fe from WBH while the rest of the sites are similar. Considering Mn concentrations, there were no significant differences between sites. Significantly lower concentrations of Ni were found in *P. perna* from M17, HIL and WBH. ANOVA analysis reveals little variation in mean concentration of Pb between sites. From Table 5 it is clear that GP, BB and M17 had significantly lower concentrations of Zn than the other sites.

Data concerning the metal bioaccumulation of *C. meridionalis* were only collected from three sites. Correlations between the samples collected from GBL, M17 and GP were analysed. Table 6 shows the significant differences of the different heavy metals between these three sites along the Namibian coastline.

The mean Al concentrations of samples collected from the three sites revealed that they were all significantly different from one another. GBL being significantly higher than the other two sites and GP being significantly lower than the other two sites.

M17 showed significantly higher concentrations of Cd, Co, Cr, Cu and Ni than GBL and GP. Cr concentration in *C. meridionalis* from GP was significantly lower than both GBL and M17.

M17 and GBL were not significantly different from each other considering Mn concentrations. M17 has a significantly lower concentration of Pb than that of GP and GBL, which do not differ significantly from each other. Concentrations of Zn did not show a substantial difference between sites.

Therefore GP is the least contaminated of the three sites having the lowest concentrations of 6 of the 10 metals tested in this study. GP is an ecologically important bird area just north of Walvis Bay. The richness of the area in invertebrates attracts a high number of shorebirds (NACOMA, 2013b). The Guano Platform mainly houses Cape cormorants, great white pelicans and the crowned cormorants (NACOMA, 2013b).

The presence of metals in marine environments can cause toxicity in marine animals when present in high concentrations (Kennish, 1997). Kremling et al. (1997) has highlighted the possibility of natural fluctuation of metals in the seawater due to changes in salinity, nutrient levels and oxygen availability. With the low rainfall experienced along the entire coast of Namibia, the possibility of significant pollution by means of storm water runoff is greatly reduced and therefore pollution sources can include rivers, mining activities and shipping activities around the harbours (Ministry of Environment and Tourism, 2008).

Halifax Island, WS and RP were found to be three of the most polluted sites in the study. The Lüderitz area is the site that experiences the most intense upwelling from all of the sites. It is also the only site in the study that is greatly affected by the southern Benguela upwelling system throughout the year. Lüderitz is undergoing the planning and implementation of mariculture of mussels, lobster and abalone and is also experiencing the increased utilization of offshore mining by Namdeb (NACOMA, 2013a).

Swakopmund is a town which is predominantly run by the mining sector because of the large amounts of Uranium found in the area (NACOMA, 2013b). Other mining activities in the area include marble, semi-precious stones, granite, sand and salt

(NACOMA, 2013b). Swakopmund is located where there is great seasonal variation in upwelling (Central to Northern Namibia) (Boyer et al. 2000). WS had the highest levels of both Zn and Pb. Future considerations should be made about the diamond mining activities that are continuing without consideration for sensitive areas such as the Cunene River Mouth (Ministry of Environment and Tourism, 2008). There is also concern about metal prospecting in the dune belt between Swakopmund and Walvis Bay as extraction licences have been awarded to a few companies already, and it has the possibility of becoming a significant source of marine pollution (Ministry of Environment and Tourism, 2008).

Perna perna populations from BB had the lowest levels of Cd and Co. Ni was the only metal tested for that was significantly higher than all sites except RP and GP. The low levels of pollution could in part be due to the fact that it is a marine protected area and that the area is closely regulated (Ministry of Environment and Tourism, 2008). The Cunene Region has no natural port and it is this fact that has slowed down the development of the area and therefore also explains the lowered levels of pollution found in this study (Ministry of Environment and Tourism, 2008).

Table 7 graphically presents the significant differences between *P. perna* and *C. meridionalis* from the same sites. For Al concentrations it was found that there were no significant differences between the two species for any of the sites. Cd concentrations differed significantly between the two species from GBL and HIL, as well as the two species from the GP. There was no significant difference between the two species from M17 in Cd concentration.

Cr concentrations also showed significant differences between species for the sites inspected. Cu concentrations showed no significant difference between the two species from GP or GBL and HIL. The study did however show *C. meridionalis* from M17 to have significantly higher levels of Cu than *P. perna* from the same site.

The mean concentrations of Co show significant ($p<0.05$) differences between the two species for all sites. From GBL, HIL and GP, *P. perna* had significantly higher levels of Co than *C. meridionalis* and M17 showed the opposite. This could possibly be due to high natural variation of the metal in seawater.

ANOVA analysis of Fe concentrations shows no difference between species from GBL and HIL, or GP. On the other hand, M17 shows a significantly higher concentration in *P. perna* than in *C. meridionalis*. Results showed that Mn concentrations were higher in *C. meridionalis* from each site and that there were no significant differences between different species from each site. Concerning Ni concentrations, there were significant differences between all species from all sites.

P. perna from GP had significantly higher levels of Pb than *C. meridionalis* from that site. It is interesting that the adjacent GP and M17 *P. perna* populations had significant differences in Pb levels whereas the *C. meridionalis* populations did not.

Zn concentrations show a significant difference between *P. perna* and *C. meridionalis* from GBL and HIL; however the other two sites show no significant differences between species.

Cd, Cr and Zn concentrations in both sampled species show *P. perna* to have significantly higher concentrations than *C. meridionalis* in two of the three sites, with only Mile 17 showing different results. Ray (1984) highlights the fact that concentrations of Cd can be higher in coastal areas due to pollution and weathering. Studies by Ray (1984) also showed levels of Cd much higher than the concentrations we obtained along the Namibian coastline. There are several factors that have an effect on the bioaccumulation of Cd which include biotic (body size, sex, age) and abiotic (salinity, pH, temperature) factors (Ray, 1984).

A greater variation in metals can be seen in the samples collected from M17 than the other two sites. This could be because of natural variation or different bioaccumulation rates of the two species. The concentrations of Mn showed *C. meridionalis* to have higher mean concentrations than *P. perna* from the same sites; however these differences were not statistically significant and therefore it is not of major concern. Mean concentrations of Ni showed that *P. perna* of all sites had higher concentrations than their counterparts; however they were only significantly higher in *P. perna* from GP and M17, but not from GBL or HIL. It is interesting to note that the *P. perna* from adjacent sampling sites GP and M17 have significant different levels of Pb whereas *C. meridionalis* from those sites did not.

This study has shown that levels of pollution in GP and BB remain the lowest of all the sites because of restricted access and protected areas. The most polluted sites are HIL, RP and WS. The reason for this could include anthropogenic substances carried into the ocean by the Swakop River or the high nutrient load created by the intense upwelling along the southern coastline. Conditions in WBH were found to be better than anticipated with only Cu levels being significantly higher than the rest of the sites.

When comparing the bioaccumulation of metals between *P. perna* and *C. meridionalis,* it was discovered that results are not uniform for all metals. *Perna perna* had significantly higher bioaccumulation in most sites for Cd, Cr and Zn. However, the bioaccumulation of Mn shows *C. meridionalis* to have higher concentrations than *P. perna,* although not statistically significant. Ni concentrations showed that *P. perna* had higher concentrations than *C. meridionalis* for all sites, but these differences were also statistically insignificant. It is important to also note that the results of the other metals did not show any significant differences between species, and for those metals the two species may be of equal use as sentinel organisms. Therefore Hypothesis 1 is accepted as human impacts are having negative effects on the metal concentrations in the WBH. Hypothesis 2 is rejected as the levels of bioaccumulation for some metals are significantly different between the two species. Pearson's correlation analysis showed only one significant correlation between the two species for Fe from one site.

Overall, the study indicates the marine environment in relation to heavy metal contamination as normal, with only the Cd and Pb levels being of concern as they are above CODEX levels and can be dangerous when ingested. Special attention must be given in the future to mining activities, the conditions in the harbour and the effect of these activities in the pollution of the rich and diverse marine ecosystem to ensure that the conditions do not deteriorate any further in the current age of development.

Table 1: Showing the mean size and standard deviation of mussels (n=20) from all sites.

Species	Collection Site	Mean Size (cm)	Standard Deviation
P. perna	WBH	55.4	5.580323
	GP	62.6	11.08783
	M17	88.65	16.26738
	RP	69.65	6.373971
	BB	62.75	8.251515
	WS	56.05	6.726626
	HIL	50.55	5.545043
C. meridionalis	GP	73.8	8.749857
	M17	64.55	8.440823
	GBL	59.05	5.563048

Heavy Metals	Range	Mean	Standard error
Aluminium	1.02-3.21	2.30	0.0283
Cadmium	0.12-1.55	0.88	0.0219
Cobalt	0.36-1.21	0.68	0.0115
Chromium	0.36-1.20	0.75	0.0116
Copper	0.73-1.40	1.07	0.0075
Iron	0.89-3.32	2.60	0.0216
Manganese	0.64-1.98	1.10	0.0122
Nickel	0.35-1.95	1.09	0.0243
Lead	1.55-2.38	1.95	0.0092
Zinc	1.37-2.92	2.20	0.0167

Table 2: The range, mean and standard error of the metals collected from seven sites along the Namibian coastline

Table 3: Metal recovery percentages from the Certified Reference Material (CRM)

	Cd	Cu	Fe	Ni	Pb	Zn
CRT Value	24.3 ± 0.8	31.2 ± 1.1	1833 ± 75	0.97 ± 0.11	0.16 ± 0.04	116 ± 6
ICP-OES Reading	29.0	22.3	1546.4	14.9	40.4	2.3
ICP-MS Reading	23.3	29.5	0.0	0.9	0.2	91.6
OES % Recovery			84.4			
MS % Recovery	98.1	94.6		88.3	127.3	78.9

Table 4: GPS Coordinates of Sampling Sites.

Location	Abbreviation	Latitude (S)	Longitude (E)
Walvis Bay Harbour	WBH	22.92487	14.51802
Mile 17	M17	22.53721	14.50174
Halifax Island	HIL	26.65077	15.08011
Rocky Point	RP	18.83375	12.38696
Bosluis Bay	BB	17.37603	11.75860
Between Walvis Bay and Swakopmund	WS	22.67578	14.52393
Guano Platform	GP	22.88186	14.54006
Griffiths Bay	GBL	26.65528	15.12824

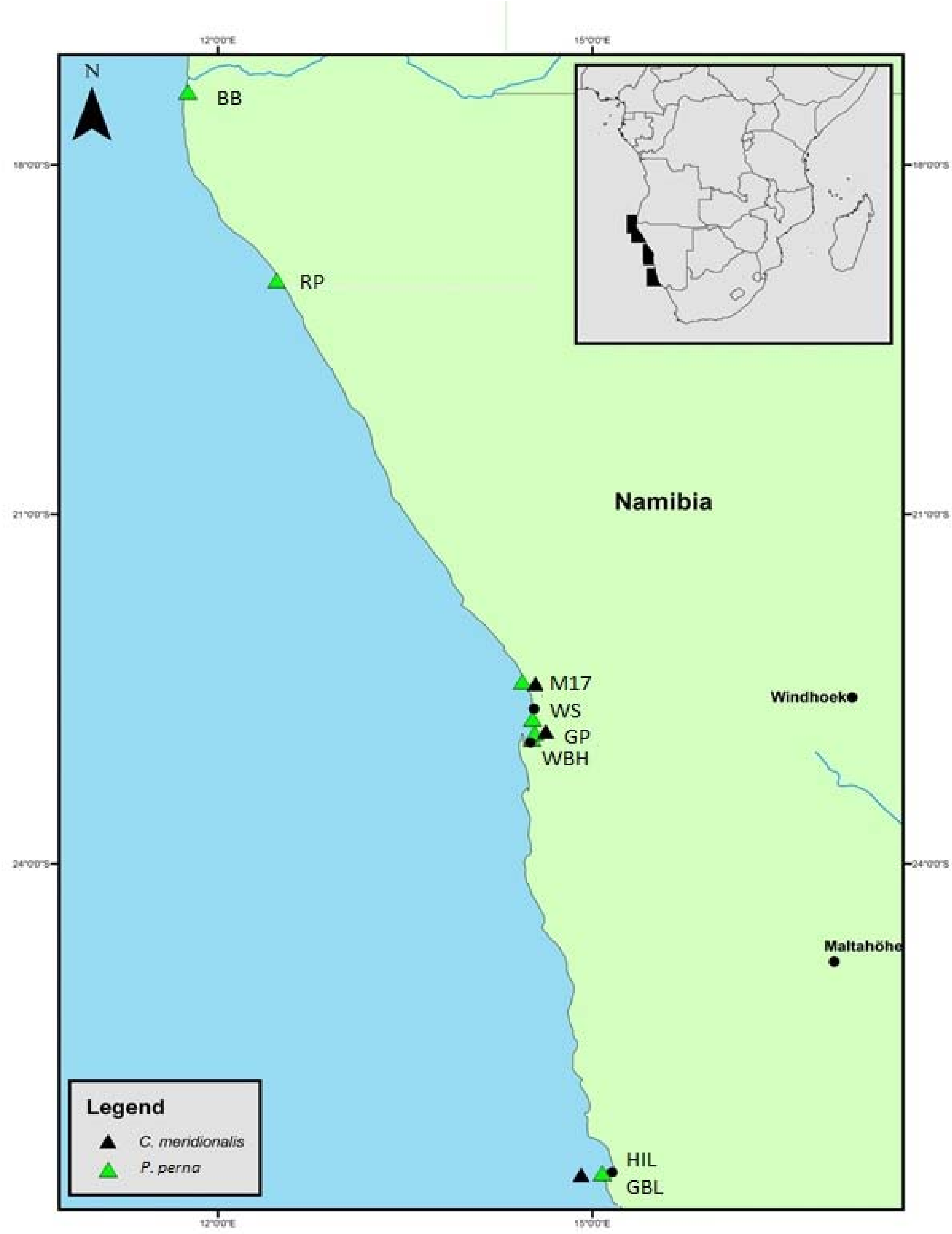

Figure 1: A map of the Namibian coastline showing collection sites of *Perna perna* and *Choromytilus meridionalis*.

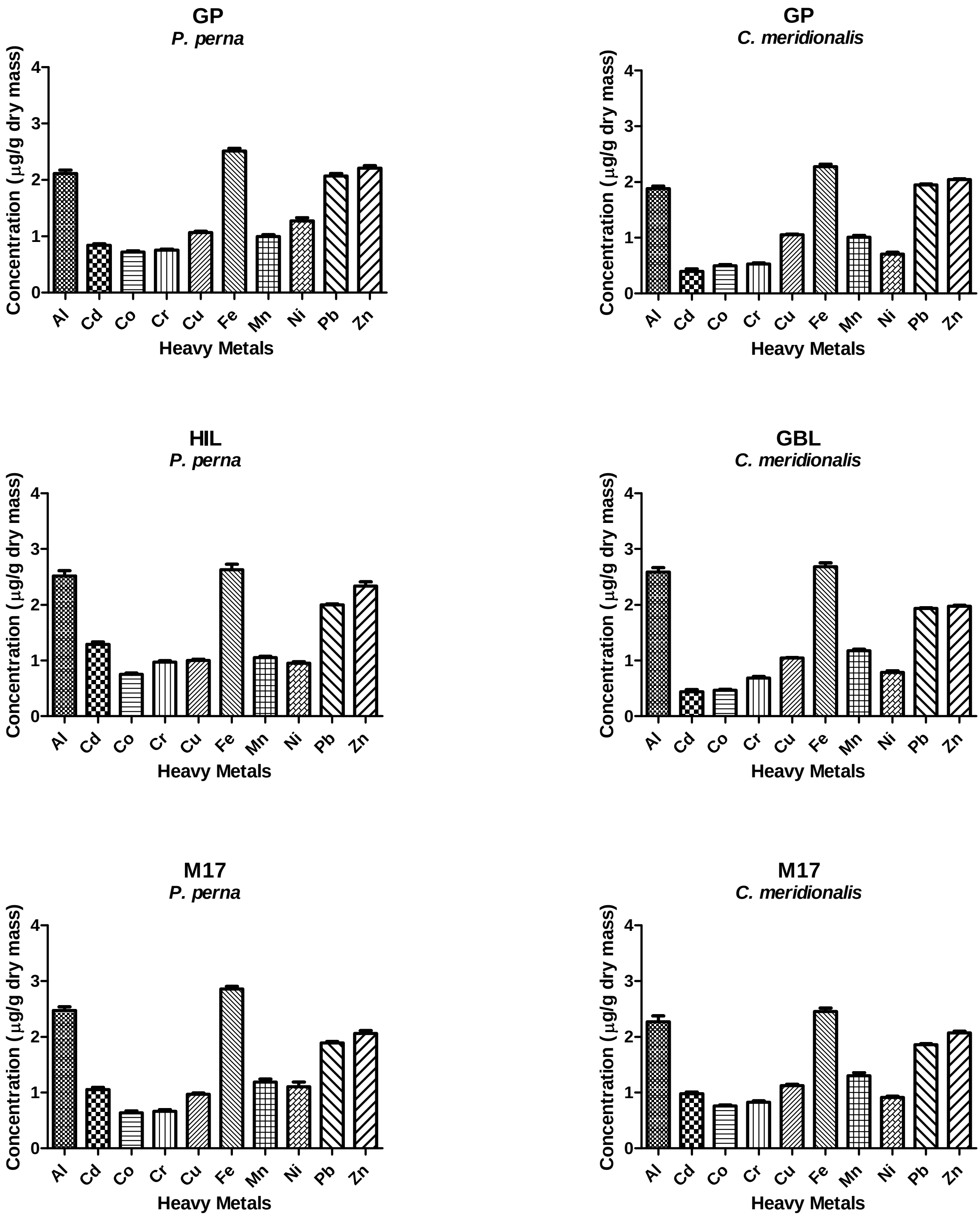

Figure 2: Graphical representation of mean heavy metal concentrations and standard error accumulated in brown mussels (*P. perna*) and black mussels (*C. meridionalis*) collected from seven sites along the Namibian coastline.

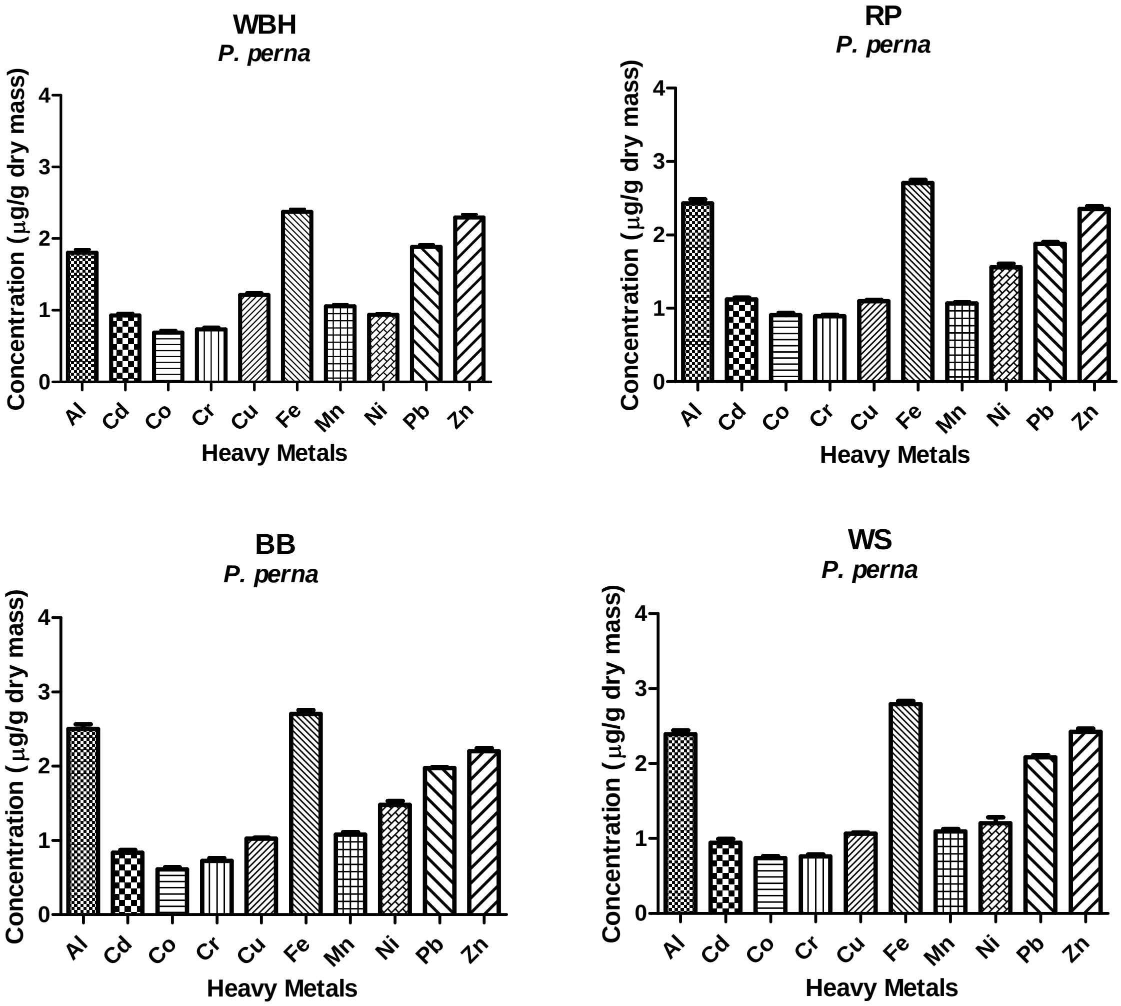

Figure 2 Continued: Graphical representation of mean heavy metal concentrations and standard error accumulated in brown mussels (*P. perna*) and black mussels (*C. meridionalis*) collected from seven sites along the Namibian coastline

Table 5: Spatial patterns of heavy metal bioaccumulation in brown mussels (*P. perna*) collected from seven sites along the Namibian coastline. Common superscripts denounce no significant differences (p<0.05).

	HIL	RP	M17	BB	WS	WBH	GP
Al	a	a	a	a	a		
							b
						c	
Mean	2,51789	2,42958	4,475	2,50046	2,39079	1,80408	2,11324
SD	0,424415	0,246798	0,8891	0,287209	0,226487	0,18684	0,272519
Cd	a						
		b	b				
			c		c	c	
				d	d	d	d
Mean	1,29056	1,12187	1,05015	0,83605	0,94614	0,92771	0,84056
SD	0,196536	0,098092	0,428	0,141578	0,215424	0,097999	0,110724
Cr	a	a					
			b	b	b	b	b
Mean	0,97106	0,89061	0,66277	0,72483	0,76142	0,73393	0,75458
SD	0,099452	0,085743	0,3393	0,157691	0,114699	0,112868	0,080215
Co		a					
	b				b	b	b
			c		c	c	c
			d	d		d	d
Mean	0,75152	0,90731	0,419	0,61099	0,73988	0,68975	0,71975
SD	0,099662	0,11758	0,3052	0,112839	0,111499	0,109103	0,074734
Cu						a	
		b		b	b		b
	c			c	c		c
	d		d	d			
Mean	1,00092	1,09696	0,97096	1,02226	1,06465	1,21402	1,06519
SD	0,085783	0,074842	0,452	0,05443	0,073404	0,109483	0,096323
Fe	a	a	a	a	a		
	b	b		b			b
						c	c
Mean	2,62804	2,70857	2,85765	2,70606	2,79156	2,37411	2,51066
SD	0,440405	0,151585	1,0424	0,212612	0,182766	0,17145	0,214863
Mn	a	a	a	a	a	a	
	b	b		b	b	b	b
Mean	1,05321	1,06386	1,1887	1,07678	1,09341	1,05582	0,99374
SD	0,092412	0,078185	0,4188	0,140791	0,136506	0,130841	0,138811
Ni		a		a			
				b			b
			c		c		c
	d		d			d	
Mean	0,95036	1,55692	1,10927	1,47875	1,20588	0,9338	1,27168
SD	0,119729	0,204743	0,2276	0,227572	0,328344	0,099391	0,242759
Pb	a			a	a		a
	b		b	b		b	
		c	c	c		c	
Mean	1,99674	1,87953	1,88775	1,97495	2,0844	1,88506	2,07208
SD	0,089857	0,091129	0,6685	0,049667	0,118682	0,144655	0,178826
Zn	a	a			a	a	
	b	b		b		b	b
			c	c			c
Mean	2,33903	2,35341	2,06316	2,20259	2,42158	2,29343	2,20259
SD	0,322272	0,158261	0,9341	0,166257	0,186129	0,200241	0,195392

	HIL	RP	M17	BB	WS	WBH
Al	a	a				a
		b			b	b
				c	c	
			d	d		
Mean	1,2557	2,51789	1,2341	2,11324	1,2198	4,475
SD	0,8708	0,42441	0,7927	0,27252	0,9506	0,8891
Cd		a				
					b	b
				c	c	
	d		d			
Mean	0,5565	1,29056	0,5555	0,84056	0,5773	1,05015
SD	0,3823	0,19654	0,3541	0,11072	0,4643	0,428
Cr		a				
				b	b	
	c			c		c
			d			
Mean	0,4489	0,97106	0,4423	0,75458	0,4587	0,66277
SD	0,3069	0,09945	0,277	0,08022	0,3692	0,3393
Co		a		a	a	
				b		b
	c		c			
Mean	0,4033	0,75152	0,3936	0,71975	0,4074	0,419
SD	0,2742	0,09966	0,2433	0,07473	0,3136	0,3052
Cu			a	a	a	
	b	b	b	b		
	c	c				c
Mean	0,5525	1,00092	0,5349	1,06519	0,5953	0,97096
SD	0,3978	0,08578	0,3474	0,09632	0,5021	0,452
Fe	a	a				a
	b	b		b	b	
			c	c	c	
Mean	1,3747	2,62804	1,3442	2,51066	1,3899	2,85765
SD	0,9854	0,4404	0,8736	0,21486	1,1199	1,0424
Mn	a				a	a
	b	b				b
		c	c	c		
Mean	0,5546	1,05321	0,5386	0,99374	0,5775	1,1887
SD	0,3859	0,09241	0,3472	0,13881	0,4575	0,4188
Ni				a		a
		b				b
	c	c			c	
	d		d			
Mean	0,6876	0,95036	0,6593	1,27168	0,603	1,10927
SD	0,4879	0,11973	0,4423	0,24276	0,4644	0,2276
Pb		a		a		
	b	b	b			
	c		c		c	c
Mean	1,0145	1,99674	0,9757	2,07208	1,0612	1,88775
SD	0,7713	0,08986	0,6685	0,17883	0,9254	0,6685
Zn		a		a		
			b	b	b	b
	c		c		c	c
Mean	1,1997	2,33903	1,1703	2,20259	1,2597	2,06316
SD	0,8509	0,32227	0,7514	0,19539	1,0219	0,9341

Table 6: Spatial variation in heavy metal bioaccumulation in the black mussel *(C. meridionalis)* as sampled from three sites along the Namibian coastline. Common superscripts denounce no significant differences ($p<0.05$).

	GBL	GP	M17		GBL	GP	M17
Al	a			**Fe**	a		
			b			b	b
		c					
Mean	1,2557	1,2341	1,2198	**Mean**	1,3747	1,3442	1,3899
SD	0,8708	0,7927	0,9506	**SD**	0,9854	0,8736	1,1199
Cd			a	**Mn**	a		a
	b	b				b	
Mean	0,5565	0,5555	0,5773	**Mean**	0,5546	0,5386	0,5775
SD	0,3823	0,3541	0,4643	**SD**	0,3859	0,3472	0,4575
Cr			a	**Ni**			a
	b				b	b	
		c					
Mean	0,4489	0,4423	0,4587	**Mean**	0,6876	0,6593	0,603
SD	0,3069	0,277	0,3692	**SD**	0,4879	0,4423	0,4644
Co			a	**Pb**	a	a	
	b	b					b
Mean	0,4033	0,3936	0,4074	**Mean**	1,0145	0,9757	1,0612
SD	0,2742	0,2433	0,3136	**SD**	0,7713	0,6685	0,9254
Cu			a	**Zn**	a	a	
	b	b				b	b
Mean	0,5525	0,5349	0,5953	**Mean**	1,1997	1,1703	1,2597
SD	0,3978	0,3474	0,5021	**SD**	0,8509	0,7514	1,0219

Table 7: A comparison of heavy metal bioaccumulation between brown mussels (*P. perna*) and black mussels (*C. meridionalis*) collected from four sites along the Namibian coastline. Common superscripts denounce no significant differences ($p<0.05$).

Acknowledgments

We would like to thank the University of Johannesburg and Spectrum for the use of Facilities. We would like to thank the Namibian MFMR for assistance in field collections. We would also like to thank Mrs Eve Kroukamp from Spectrau for analytical assistance and the National Research Foundation of South Africa for funding assistance.

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