The application of active and passive bioaccumulation methods to measure metallic and organic pollutants along the Namibian coastline

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ABSTRACT

Human activities and natural weathering processes are responsible for the input of elements and organic pollutants into marine environments. There is currently no ongoing marine pollution monitoring program in Namibia and there are limited studies on marine pollution along the Namibian coastline. Since the 1970s, mussels have been used as bioindicators because they are widely distributed, plentiful, and stationary. The main aim of this study was to use active and passive biomonitoring tools to determine the element and organic contaminant concentrations present in the coastal areas of Swakopmund and Walvis Bay Harbour in Namibia. Artificial mussels (AMs), semi-permeable membrane devices (SPMDs), resident- and transplanted mussel bioindicators (*Choromytilus meriolionalis*) were deployed at two exposure sites (Walvis Bay Harbour & Swakopmund) for six weeks. Element concentrations (Al, As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Se, U, and Zn) were determined using inductively coupled plasma mass spectrometry (ICP-MS) techniques. Chromium concentration was determined using an atomic absorption spectrometer (AAS) and mercury (Hg) concentrations with a flow injection mercury system (FIMS). The concentrations of organochlorine pesticides (OCPs) and polychlorinated biphenyl (PCB) were analysed in both SPMD and mussels following QuECHERS extraction methods and using a GC-20230 Plus coupled with a GCMS-TQ8050 NX triple quadruple mass spectrometer. The element levels in all AMs were significantly lower ($p<0.05$) when compared to mussels and did not show a significant increase in uptake during the deployment period. Transplantation studies are very effective as a biomonitoring tool, but for more comprehensive data interpretation, resident mussels should be assessed in conjunction with transplanted mussels. The transplanted and resident mussels from Walvis Bay Harbour had significantly ($p<0.05$) higher Cu concentrations than the transplanted and resident mussels collected at Swakopmund. Walvis Bay Harbour's resident mussels had significantly lower element concentrations for the most part when compared to Swakopmund's resident mussels. This was attributed to the adaptation of resident mussels to their environment through the regulation of elements. The differences are also attributed to constant exposure since resident mussels remain submerged and therefore constantly exposed to metals compared to the transplanted mussels that are subjected to tidal influences and exposed to the air during low tide. There were notable fluctuations in the element levels in both the AMs and the transplanted mussels during the deployment period for both study sites. The results indicated that there are clear trends in the spatial and bioaccumulation indicators for elements in mussels at the two study sites. There were also clear differences between the uptake patterns of OCPs in the mussels and the SPMDs. Although analyses for PCBs were done, they were not detected in the SPMDs or the mussels. When comparing the results of this study in Namibia to element concentrations in mussels

from South African harbours, the levels for most of the elements were in the same range. However, passive biomonitoring devices (AMs and SPMD) displayed much lower levels than those found in other studies. For monitoring purposes, passive (AM and SPMD) should be used in combination with the active biomonitoring organisms (transplanted and resident mussels) to acquire an accurate representation of contaminant exposure in the marine environment. This study could potentially contribute to the development of a Namibian coastal biomonitoring plan.

Keywords: *Choromytilus meridionalis*, bioaccumulation, artificial mussels, semi-permeable membrane device, organochlorine pesticides, Namibia

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Chapter 1: General Introduction

1.1 Background information

As the world's human population grows there is an increase in anthropogenic activities. Nekhoroshkov *et al*. (2021) stated that the input of elements into the marine environment is not only due to natural environmental processes but also increased anthropogenic activities along coastlines such as those of Namibia, South Africa and Mozambique. Due to the expansion of anthropogenic actions (industrial, exploration and misuse of natural resources), an increase of metals occurs and has become problematic (Wang, 2002; Vellemu and Omoregie, 2014). This is especially true for Namibia where in 2019 the Fishrot scandal came to light, where the Minster of Fisheries and various other parties were bribed to increase fishing quotas (Coetzee, 2021). This is a clear indication of misuse of natural resources that occurred in Namibia.

The main contributing factors to Namibia's economy are fisheries, mining, and exports (salt, petroleum, ore) (Namport, 2023), therefore, it is important that these natural reserves are protected (Sahlén and Stage, 2012). Approximately 3000 vessels enter the commercial port of Walvis Bay every year, averaging around 5 million tonnes of cargo (Namport, 2023). The Namibian government is aware of the importance of the ocean and the role that fisheries play in their economy; thus, they have formed a part of various conventions focused on conserving and sustainably managing the ocean and its inhabitants (Finke *et al*., 2020). Namibia is a signatory of both the MARPOL Convention (Ministry of Works and Transport, 2019), as well as the Abidjan Convention (Royeppen and Kornegay, 2015). The MARPOL Convention is a global initiative for the prevention of pollution from ships and aims to reduce the greenhouse gas produced by these vessels by 50% by 2050 (Ministry of Works and Transport, 2019). The Abidjan Convention is administered by the United Nations Environment Program (UNEP), in which they aim to liaise in the safeguarding, management and development of the Atlantic coast in Western, Central and Southern Africa (Royeppen and Kornegay, 2015). In 2018, Namibia was one of three African countries to join the international Ocean Panel initiative. The first goal implemented is for each of the 14 countries to sustainably manage their national marine areas successfully by the year 2025 (Lubchenco *et al*., 2020). However, despite being part of these various conventions, there is still limited information on the presence of contaminants within the marine environment along the Namibian coastline and there is no biomonitoring plan in place.

1.2 Environmental contaminants along the Namibian coastline

The earliest reported studies focussing on environmental contaminants along the Namibian coastline reported on the bioaccumulation of metals and metalloids (henceforth referred to as elements) in Namibian seabirds (Burger and Gochfeld, 2001) and organochlorine pesticides (OCPs) in seal blubber (Vetter *et al*., 1999). Orani *et al*. (2018) analysed metals in marine sediments, while the only other studies on metals in marine organisms were reported for metals in mussels (Dahms *et al*., 2014; Vellemu and Omeregie, 2014) and mercury (Hg) in demersal fish off Namibia (Erasmus *et al*., 2018). Recently, Klopper *et al*. (2020) stated that elements are deposited in the ocean via wind-derived dust from the mining regions in central and northern Namibia.

1.2.1 Element contaminants

Element contaminants can enter a system naturally or through anthropogenic activities. Elements that are pliable, conductive, and ductile with a shiny appearance are commonly known as metals, e.g. aluminium (Al), copper (Cu), iron (Fe), zinc (Zn), etc. Metalloids, such as selenium (Se) and arsenic (As), have metallic and non-metallic element characteristics such as being semiconductors and less shiny when compared to metals (Newton, 2015; Chapman *et al*., 1996).

Elements are naturally introduced by natural processes, such as weather-related conditions (strong winds from the East carry sand from the dunes to the ocean) (Soboil, 1996), organic break-down or even natural disasters via the atmosphere and rivers (Valavanidis and Vlachogianni, 2014; Nekhoroshkov *et al*., 2021). Elements that naturally occur in marine environments are Al via wind-blown dust and Hg from volcanic eruptions (Valavanidis and Vlachogianni, 2014). The main anthropogenic contributors, which introduce element contaminants into marine environments, are urban and agricultural runoff, dredging of shipping canals, antifouling coatings, human waste discharge through sewage treatment plants, industrial discharges, power stations, dockyards, and recreational activities (Valavanidis and Vlachogianni, 2014; Firth *et al*., 2019b; Nekhoroshkov *et al*., 2021). Areas, such as harbours, contribute to an increase in contaminants in the marine environment, as they concentrate pollutants from surrounding urban and industrial areas (Wepener and Degger, 2020).

The increase in these contaminants ultimately affects marine organisms that inhabit those environments. Marine organisms such as fish and mussels can accumulate these elements through bioaccumulation directly from the surrounding environment or through their diet.

Bioconcentration occurs due to the uptake of substances by an organism from the water column, while the absorbing of substances through food and the environment is known as bioaccumulation (Chapman *et al*., 1996). The concentrations of the measured substances from the diet are presumed to be higher or equal when compared to the concentrations from the water (Chapman *et al*., 1996). This is specifically true for methylmercury (MeHg) and various other elements in their alkylated forms (Chapman *et al*., 1996). The degree of bioaccumulation is dependent on the bioavailability and total concentration of every element present within the environment. Element bioaccumulation can vary between individuals, as well as between species, due to differences in biological processes and specific uptake strategies, as trace metals cannot be metabolised (Luoma and Rainbow, 2005; Krishnakumar *et al*., 2018; Capelle *et al*., 2021).

Although some elements such as cobalt (Co), Cu, Fe, manganese (Mn), molybdenum (Mo) and Zn are important for biological processes, including metabolism and growth (Reinecke *et al*., 2014; Chapman *et al*., 1996). The increased accumulation of these above-mentioned elements can ultimately negatively affect marine organisms, since all elements can be toxic when accumulated beyond a certain threshold, (Chapman *et al*., 1996; Reinecke *et al*., 2014). Moreover, some elements e.g. cadmium (Cd), lead (Pb) and Hg do not even have any benefit to marine organisms (Ivanina and Sokolova, 2015).

1.2.2 Persistent organic pollutants (POPs)

Research conducted on the exposure of marine organisms to organochlorine pesticides (OCPs) in Namibia is very limited as the only study to date has focused on seal blubber (Vetter *et al*., 1999). Other studies conducted in southern Africa are by Degger *et al*. (2011b) on OCP levels in mussels from selected sites along the west and east coasts of South Africa and by Firth *et al*. (2019a) on POP levels in framed mussels (*Choromytilus meridionalis* and *Mytilus galloprovincialis*) from aquaculture facilities in Saldanha Bay, South Africa.

Persistent organic pollutants are released into the environment through anthropogenic activities such as incinerating organic matter in the presence of chlorine, discharge gasses from both vehicles and ships, chemical industries, etc. (Tesar, 2000; Degger *et al*., 2011b; Firth *et al*., 2019a). These POPs are represented by a variety of contaminants that include: OCPs such as 1,1,1-trichloro-2,2-bi(p-chlorophenyl) ethane (DDT) and Hexachlorocyclohexane (HCH); polycyclic aromatic hydrocarbons (PAHs) that originate from petroleum compounds; polyhalogenated biphenyls that include Polybrominated biphenyl (PBBs) and Polychlorinated biphenyl (PCBs); and polyfluorinated compounds. Organochlorine pesticides are artificial chemicals used to produce insecticides and are known to be lipophilic

and hydrophobic (Richardson *et al*., 2001; Degger *et al*., 2011b). These OCPs are comprised of various compounds such as HCH or benzene hexachloride (BHC), and DDT. Technical-grade DDT is comprised of three isomers (o' p-DDT; p' p-DDT; o' o-DDT) as well as the by-products 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDD) and 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (ATSDR, 2019). Hexachlorocyclohexane used in agricultural pesticides consists of 4 isomers (alpha, beta, delta and gamma) (ATSDR, 2005). The compound DDT is still used to combat and control malaria outbreaks in developing countries to this day, while developed countries have banned these OCPs for the last three decades (Sibali, 2008; Stockholm Convention, 2014; Firth *et al*., 2019a). Countries such as Namibia, Botswana, Zimbabwe and Mozambique are all members of the southern African coalition to control malaria with the use of DDT (Sadasivaiah *et al*., 2007). Since these compounds are lipophilic and bind to lipids, it is possible for these compounds to bioaccumulate (Ahmed, 2015). These OCPs do not degrade easily, thus categorising them as POPs as they are extremely toxic (El-Shahawi *et al*., 2010; Tang, 2013).

1.3 Bioaccumulation monitoring

Recognising the scarcity of elemental and pesticide studies along the Namibian coast, the development and introduction of various bioaccumulation monitoring strategies will benefit the country. Furthermore, the study of various monitoring methods will aid researchers to provide a new baseline for marine pollution monitoring, providing vital information to determine and achieve the relative goals set out by the global conventions to which Namibia is partied to.

1.3.1 Prospective organisms for transplantation studies

Ideal bioindicator organisms should concentrate elements and provide a time-integrated estimate of the bioavailable fraction of elements in water (Wu *et al*., 2007). When selecting a bioindicator species, it is important to consider their local and international distribution and that every species has different pollutant accumulation approaches (Leung *et al*., 2008; Wepener, 2008, 2013; Degger *et al*., 2011a). It is also important to ensure that there are enough individuals of the chosen species within its population and that removing a large quantity will not negatively impact the ecosystem. If the organism is known to be stationary and resilient by adapting or evolving to tolerate its environmental conditions and staying within the study site boundaries, it will be beneficial (Catharino *et al*., 2011).

When considering the Namibian coastline, the Benguela Current is a cold nutrient-rich environment, due to wind-driven upwelling there is a frequent source of dissolved nutrients from deeper waters (Javis *et al*., 2022). Many different invertebrate species, specifically

bivalves, benefit from occasional hydrogen sulphide outbreaks as these feed the large layers of sulphide-oxidising bacteria (*Thiomargarita namibiensis*) on the seabed. The bacteria not only detoxify the environment but also provide sustenance to these invertebrate species. Physiological and behavioural adaptation have equipped these invertebrates to cope with stressors that accompany a hydrogen sulphide outbreak (Javis *et al*., 2022). Therefore, it essential that indicator species for transplantations studies are selected from the same coastline.

1.3.2 Passive bioaccumulation monitoring using resident mussels

As mussels are filter feeders, they filter large amounts of seawater, thus absorbing any contaminants present in both the water and particles. Mussels are impervious to various contaminants; therefore, they can survive in polluted environments (Krishnakumar *et al*., 2018). Mussels bioaccumulate pollutants within their tissues, thus they can be used as indicators of exposure to pollutants (Wepener and Degger, 2020). Mussels are therefore commonly used as bioindicators of chemical pollutants and are part of large international pollutant monitoring programs such as the "Mussel Watch" (O'Connor, 2002).

Historically, pollution monitoring studies in South Africa have been conducted near key urban settlements along the Western Cape, Eastern Cape and KwaZulu-Natal using mostly bivalves as bioaccumulation indicator species, e.g. *Perna perna* (Brown mussel) and *M. galloprovincialis* (Mediterranean mussel) and these studies mainly focused on the accumulation of elements (Wepener and Degger, 2019). Vellemu and Omoregie (2014) conducted a study using *C. meridionalis* as a bioindicator species to monitor Pb concentrations within the Namibian marine environment. Dahms *et al*. (2014) also conducted a study along the Namibian coastline focussing on element concentrations, such as: Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn in *P. perna* and *C. meridionalis*.

1.3.3 Active bioaccumulation monitoring using transplanted organisms

There are many benefits in using active biomonitoring methods rather than traditional passive monitoring methods. Active biomonitoring methods reduce the number of different variables (water and sediment characteristics) that need to be considered when conducting a monitoring study such as pH, salinity, and clay content as well as total organic carbon, respectively (Teunen *et al*., 2021). Active biomonitoring uses transplanted organisms rather than resident organisms from study sites, as resident organisms adapt to pollutants in their ecosystem, thus building a tolerance for them (Greenfield *et al*., 2014). The indicator organisms are collected

from an unpolluted area and deployed (transplanted) in cages at the predetermined sites for a set time period. As transplanted organisms are kept in cages that are attached to a floatation device such as a navigation buoy it drifts in the water column with water freely moving through the cage, thus ensuring continuous exposure to contaminants at the site. The time frame for the exposure differs for species and can range between four to eight weeks (Wepener, 2008, 2013). Different study sites can be compared due to the certainty that the same species of organism is deployed at all of the study sites (Giarratano *et al*., 2010).

1.3.4 Passive monitoring using artificial mussels

Artificial mussels (AMs) are passive sampling devices that were developed to monitor water quality and exclude the need to kill bioindicators, as well as make it possible to observe time-integrated results (Wu *et al*., 2007). The benefits of these devices are that they are cost-effective, do not require power while in use, can be deployed anywhere in the world since it has no geographical limits, and physiological processes do not impact them like they do the bioindicator organisms (Kibria, 2012; Kibria *et al*., 2023). These devices are suitable for both marine and freshwater environments as they accumulate bioavailable elements from the water column that they are exposed to (Wu *et al*., 2007). This sampling device is constructed with Perspex® tubing fitted with an internal spacer containing Chelex®-100 beads and deionised water (freshwater) or artificial seawater (marine environment) enclosed by a polyacrylamide gel on each side (Wu *et al*., 2007). The polyacrylamide gels work the same as a membrane, thus diffusion takes place and water containing bioavailable elements will move from a high concentration to a low concentration and these elements will bind to the Chelex®-100 beads. The polymer ligand reacts to changes in the element concentrations in the ecosystem. Factors such as temperature and pH do not influence the uptake patterns of artificial mussels (Wu and Lau, 1996; Claassens *et al*., 2016). The bio-indicators biological processes are more complex due to mechanisms such as element regulation, while the Chelex®-100 beads processes are more straightforward and present the expected outcomes (Wu and Lau, 1996). The AM devices were originally developed for marine environments (Wu *et al*., 2007; Leung *et al*., 2008; Degger *et al*., 2011a; Gonzalez-Rey *et al*., 2011) but have also been applied in freshwater studies (Claassens *et al*., 2016, Kibria *et al*., 2016). A summary of the application of AMs to monitor elements in the marine environment is presented in Table 1.1.

Table 1.1: Published literature pertaining to artificial mussels and where it has been implemented successfully in marine environments**.**

Study area	Metals studied	Reference
Marine environments (development)	Cd, Cr, Cu, Pb and Zn	Wu *et al*., 2007
Scotland and Iceland	Cd, Cr, Cu, Pb and Zn	Leung *et al*., 2008
South Africa	Cd, Cr, Cu, Pb and Zn	Degger *et al*., 2011a
Portuguese coast	Cd, Cr, Cu, Pb and Zn	Gonzalez-Rey *et al*., 2011
China coast	Cd, Cr, Cu, Hg, Pb and Zn	Degger *et al*., 2016
Bay of Bengal, Bangladesh	Cd, Cu, Fe, Mn, Ni, Pb, U and Zn	Kibria *et al*., 2016
Estero de Urias lagoon, Gulf of California	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn	Ruiz-Fernández *et al*., 2018
Sarıçay Stream, Turkey	Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U and Zn	Genç *et al*., 2018
Australian coast	As, Cd, Cu, Cr, Hg, Pb, Se, Zn	Shen *et al*., 2020
Artificial seawater (Laboratory environment)	Cs, Sr and U	Yang *et al*., 2023

1.3.5 Passive monitoring using semi-permeable membrane device

Semi-permeable membrane devices (SPMD) are similar to AMs, as they are also passive devices designed specifically for lipophilic organic pollutants such as OCPs in marine environments (Richardson *et al*., 2001; Esteve-Turrillas *et al*., 2007). The SPMD can be used to determine the level of pollutants in both water and sediment (Vrana *et al*., 2001). Huckins *et al*. (1990) developed the SPMD by inserting a lipid-rich compound, triolein (triglyceride) into a polyethene tube. The mechanism of this passive device is based on the assumption that when the device is exposed to the water column if there are any contaminants that are lipophilic present it will diffuse through the polyethylene tubing and the compounds are caught within the lipid (Richardson *et al*. 2003; Degger *et al*., 2011b).

Using SPMDs instead of biological samples can be beneficial as they negate biogenic hydrocarbons and lipids that interfere with the analysis, as well as physical factors (temperature, climate, physiological processes) that can alter the outcome of the results (Richardson *et al*., 2001). As SPMDs accumulate contaminants without the interference of the factors mentioned above, they are able to take up contaminants that may not be present in the bioindicator tissue. The potential of using passive monitoring tools, such as AMs and SPMDs to monitor elements and OCPs along the South African coastline has been demonstrated by Degger *et al*. (2011a, b).

1.4 Study rationale

Based on the above information the present study was conducted to gain more knowledge on the use of different bioaccumulation monitoring tools (transplanted, resident, and artificial devices), to determine the element and OCP concentrations along selected sites of the Namibian coastline. The data will provide an assessment of current contaminant exposure, which could provide valuable information towards the development of an integrated marine monitoring program.

Namibia has only one deep water harbour, located in Walvis Bay, that is of high economic significance for the country (Figure 1.1) (Hughes *et al*., 1992). The Walvis Bay Harbour handles 1500 to 2500 vessels annually (Namport, 2023). In 1892 Swakopmund was the main harbour of Namibia, the site now reflects historical harbour activity (Simon and Ekobo, 2008).

Figure 1.1: Images of Walvis Bay Harbour. A - shows the deployment area circled in red. B - is a close up of the deployment site.

The Swakop River is situated to the west of the watershed, flowing westwards till it reaches the Atlantic Ocean. At Swakopmund, it is easy to identify the Swakop River, as it clearly mouths into the ocean, while the Kuiseb River seldomly flows into the lagoon at Walvis Bay or vanishes into the sandy delta (Javis *et al*., 2022). Figure 1.2 A shows the Swakopmund Jetty that was used as a deployment site for this study. It is located approximately 1 km from the Swakop River.

Figure 1.2: Images of the Jetty restaurant in Swakopmund. A - shows the entire Jetty and the red circle indicates the deployment basket location. B -a close up of the deployment site.

Mussels for the transplantation study were collected from the reference site at Mile 17 (Figure 1.3).

Figure 1.3: Image of Mile 17 where the mussels were collected for transplantation purposes.

1.4.1 Hypotheses

1. The accumulation of elements by AMs and transplanted mussels will show similar uptake patterns during the 6-week exposure period (Chapter 2).
2. The accumulation of elements by resident mussels will be lower than the concentrations of elements by transplanted mussels (Chapter 2).
3. The accumulation of OCPs in transplanted and resident mussels will differ from OCPs in SPMDs due to biological factors such as biotransformation processes (Chapter 3).

1.4.2 Aim of the study

The main aim of this study was to assess the element and OCP levels at Walvis Bay Harbour and Swakopmund coastal areas in Namibia using a combination of artificial (AMs and SMPDs) biomonitoring tools and an indicator mussel species (*C. meriolionalis*) as indicators of exposure.

1.4.3 Objectives of the study

1. Compare element levels in resident and transplanted mussels at two study sites; i.e. Walvis bay Harbour and Swakopmund. Conduct field studies comparing elements in AMs and transplanted mussels from the two selected sites over a 6-week exposure period.
2. Conduct field studies deploying SPMDs and transplanted mussels in preselected study sites from the Namibian coast after a 6-week period to compare their accumulated OCP concentrations as well as compare the OCP levels in resident and transplanted mussels.
3. Compare the element and OCP concentrations of this study to the concentrations of previous studies conducted in Namibia and internationally.
4. Provide recommendations on the use of AMs and SPMDs together with mussels in an integrated marine monitoring program for Namibia.

1.5 Dissertation outline

The current chapter (Chapter 1) is a general introduction to this study, thus providing the background and overview of the study, as well as the research hypotheses, aim and objectives. Chapter 2 presents the results of element accumulation in resident, transplanted and AMs from Walvis Bay and Swakopmund. In Chapter 3 the OCP levels in SPMDs are compared to levels measured in resident and transplanted mussels from Walvis Bay and

Swakopmund. Chapter 4 concludes the study and provides recommendations for future studies. Lastly, Chapter 5 presents the references that were used in this study.

Chapter 2: Application of artificial mussels as an element biomonitoring tool under Namibian conditions

2.1 Introduction into the use of AM's to monitor elements and comparing it with resident and transplanted mussels

Element inputs to the marine environment originate from natural and anthropogenic sources. Natural inputs consist of discharge from rivers due to geogenic weathering in the catchment, atmospheric-derived dust deposition, from arid and semi-arid environments, and hydrothermal circulation through mist-ocean ridges (Krishnakumar *et al*., 2018). Elements that naturally enter marine environments are e.g. aluminium (Al) via dust being blown and mercury (Hg) from volcanic eruptions (Valavanidis and Vlachogianni, 2014). Element contaminants such as cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn) in the marine environment are derived from urban and agricultural runoff, and a large range of other coastal anthropogenic activities such as dredging of shipping canals, antifouling coatings, chemical manufacturing, discharge of human waste via sewage treatment plants, industrial discharges either intentionally or accidentally, power stations, dockyards, and recreational activities (Valavanidis and Vlachogianni, 2014; Ivanina and Sokolova, 2015; Krishnakumar *et al*., 2018; Nekhoroshkov *et al*., 2021).

Elements can be divided into essential and non-essential elements (Newman, 2015). Certain elements, such as cobalt (Co), Cu, chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), Ni, selenium (Se), and Zn occur naturally within marine environments, although these element concentrations are low, they are essential for physiological processes within organisms (Newman, 2015; Krishnakumar *et al*., 2018). Other elements such as Al, arsenic (As), Cd, Pb, Hg, Ni and tin (Sn) are non-essential elements with no known biological function (Krishnakumar *et al*., 2018). However, essential elements can become harmful to organisms if the concentrations exceed natural tolerance limits (Newman, 2015). Copper for example, is ubiquitous in nature and is used as a micronutrient by many organisms but can reach detrimental levels in the marine environment as it is used in antifouling paints for ships (Omoregie *et al*., 2019). Copper can be taken up from the water column through filter-feeding marine organisms such as mussels or plankton. Sedimentation may cause Cu to accumulate in sediments, exposing sediment-dwelling organisms to the element (Omoregie *et al*., 2019; Sánchez-Marín, 2020). The uptake of elements from the marine environment is influenced by physico-chemical conditions, such as salinity, as it influences the speciation of elements (Namieśnik and Rabajczyk, 2015). Salinity and the presence of organic ligands in the form of dissolved organic matter are the main contributing

factors to the presence of free Cu ions in the seawater, which determines bioaccumulation and ultimately the toxicity (Sánchez-Marín, 2020).

Bivalves, such as mussels, have been used as indicator organisms since the 1970s in biomonitoring studies to determine the quality of marine environments (Catharino *et al*., 2012). Mussels are regarded as effective element bioaccumulation indicator organisms since they reflect both exposure to free element ions found in the water column, as well as particle bound elements due to their filter mode of feeding (Krishnakumar *et al*., 2018). Mussels therefore accumulate elements very effectively and provide a time-integrated estimate of the bioavailable fraction of elements within the water (Wu *et al*., 2007). When selecting an indicator species, it is important to consider their local and international distribution and the fact that there may be species specific pollutant accumulation strategies (Leung *et al*., 2008; Degger *et al*., 2011a). Marine monitoring studies along the South African coastline have mainly focussed on key urban settlements and associated harbours of the Western Cape, Eastern Cape and KwaZulu-Natal provinces (Wepener and Degger, 2019, 2020). These studies focussed on two bivalve species, i.e. *Perna perna* (brown mussel) and *Mytilus galloprovincialis* (Mediterranean mussel) as bioindicators of element exposure in the marine environment. *Choromytilus meridionalis* (black mussel) has been used as a bioindicator species for element monitoring within the Namibian marine environment (Dahms *et al*., 2014; Vellemu and Omoregie, 2014).

Many studies have shown that there are disadvantages to solely using resident mussel populations as indicators of marine pollution. Long term exposure may result in resident mussels adapting to their environments (Greenfield *et al*., 2014; Capelle *et al*., 2021). In addition, the accumulation of elements by live organisms is also influenced by numerous factors such as physical (temperature and salinity) and biological (size, reproductive stage, age, and gender) factors (Wu and Lau, 1996; Mubiana *et al*., 2006; Degger *et al*., 2011a). To overcome these limitations Degger *et al*. (2011a) and Greenfield *et al*. (2014) recommended that complementary monitoring should be undertaken using artificial (passive) sampling devices and transplanted mussels. Transplantation studies involve the relocation of mussels from a relatively unpolluted site to the polluted monitoring sites, thereby negating the influence of long-term exposure of resident mussels to the ambient conditions (Greenfield *et al*., 2014). The advantages of using passive monitoring devices as an alternative to the use of live individuals in biomonitoring programmes are well known. Artificial mussels (AMs) are passive sampling devices that have successfully been deployed to determine the element concentrations in coastal and freshwater environments (Wu *et al*., 2007; Degger *et al*., 2011a; Labuschagne *et al*. 2021). The AMs have semi-permeable gel layers that make it possible for

element ions to diffuse from the surrounding environment and to be adsorbed onto the Chelex-100 beads (Labuschagne *et al*. 2021) thereby reflecting the bioavailable element fraction exposure at a particular site.

A large portion of the Namibian coastline is situated within the Skeleton Coast Park and Naukluft Park where freshwater flows into the marine environment from non-perennial rivers such as the Swakop River (Vellemu and Omoregie, 2014). Since there are various mining activities that take place inland from this coastal region, there is a threat of element contamination of the marine environment (Coakley, 2003). Coastal regions that receive runoff from the catchments and where there are increased human activities of e.g. harbours are particularly under threat of elements due to being in the proximity of the pollutant sources (Degger *et al*., 2011a, 2016; Omoregie *et al*., 2019; Wepener and Degger, 2020). There has been a Namibian national marine pollution contingency plan in existence since April 2017, but this plan mostly focuses on what to do in the event of an oil spill. It therefore does not include the monitoring of other contaminants that may originate from the increase in urban and industrial development along the Namibian coastline.

The aims this chapter were therefore to:

i) Conduct field studies comparing element concentrations in AMs and transplanted black mussels (*C. meridionalis*) from two research sites along the Namibian coast over a 6-week time period.

ii) Compare element concentrations in resident black mussels to the concentrations in the transplanted and artificial mussels at the two study sites.

2.2 Materials and methods

2.2.1 Site selection

The only formal marine protected area along the Namibian coastline is known as the Namibian Islands. In 2014 the Convention of Biological Biodiversity deemed six different areas of ecological and biological significance that are protected by various conservation and management methods. Three of these areas (Cape Fria, Namib Flyway and Walvis Ridge) are only within Namibian borders while the other three are transboundary (Namibe, Orange Cone and the Orange Seamount and Canyon Complex) (Javis *et al*., 2022). All the study areas within this study fell inside the Namib Flyway area (see Figure 2.1).

Relevant sampling permits to collect the indicator species (*C. meridionalis*) from the reference and two research sites (permit number: RPIV0101252022-1) were obtained. This study received ethical approval from the North-West University's Faculty of Natural and Agricultural Sciences research ethics committee (ethics number: NWU-01309-22-A9).

In December 2022, black mussels were collected for the transplantation study from the reference site (Figure 2.1) at Mile 17, also known as Rock Bay (22˚27'13''S 14˚27'29''E). The black mussels (between 4 – 6 cm) were removed from the rocky tidal pools during low tide. Care was taken to ensure that byssal threads were not damaged and mussels were then kept alive till they were deployed at the pre-determined research sites. The two research sites were Walvis Bay Harbour (22˚55'47''S 14˚30'58''E) and the Jetty Restaurant (22˚40'50''S 14˚31'09''E) in Swakopmund. Both owners of these locations gave their consent for research to be conducted on their premises.

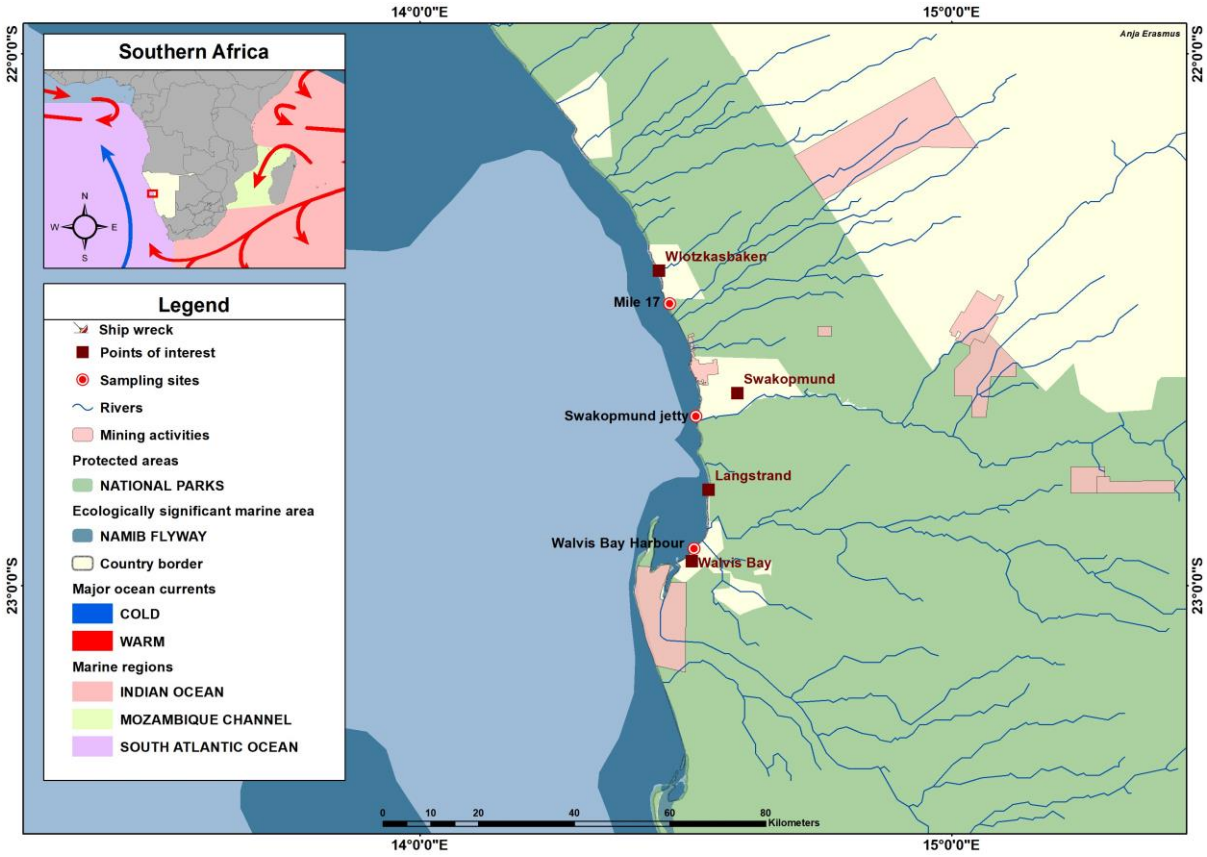

Figure 2.1: Map of the Namibian coastline indicating the two research areas and the location where sampling was done.

At the harbour site (Figure 2.1), the baskets containing AMs and the transplanted mussels were hung next to the dock, ensuring they were deep enough not to be exposed during low tide (i.e. 2 – 3 m), while not touching the bottom. This specific area of the harbour is approximately 5 to 6 m deep. At the second location, the Jetty restaurant (Figure 2.1), the basket was secured to the jetty at a depth of 2 to 3 m. The resident mussels from this site were collected near the pillars of the jetty.

2.2.2 Deployment and retrieval protocol

At each site, 60 AMs were placed in plastic cages (35 x 26 x 20 cm, mesh size of 1.5 cm^2) and secured to structures at a depth of 2 m. A second cage containing 60 transplanted mussels (mussels from a reference site - Mile 17) of similar shell length (4 – 5 cm) were deployed simultaneously at each site. Ten AMs together with 10 transplanted mussels were retrieved from the baskets from each site every week for six weeks for element analysis. At the end of the 6-week exposure period, 10 resident mussels were collected for comparison with the transplanted mussels. The mussels' (transplanted and resident) shell lengths were measured in the field after they were retrieved from the deployment site. The tissue from the mussel (approximately 3 g wet weight) was dissected out from the shell with a pre-cleaned

scalpel and put in a falcon tube. Samples were then stored at -20°C for further analyses (Degger *et al*., 2016). The 10 AMs that were retrieved every week were stored with dampened cotton wool in each opening, to keep the gels moist and placed in a marked plastic bag which was kept in the fridge at 4°C until it could be analysed in the laboratory.

2.2.3 Preparation of artificial mussels

The AM (Figure 2.2) was developed by Wu *et al*. (2007) and consists of a Perspex tube (length of 5 cm and diameter of 3 cm) that is enclosed on one side with parafilm to ensure no solution spills out while the gel polymerizes. The gel layers are made from the combination of three solutions: Firstly, 15 g acrylamide (Acrylamide for electrophoresis, ≥99% (HPLC) powder, Sigma) and 0.5 g N, N-methylene bis-acrylamide (BioReagent, suitable for electrophoresis, 99%, Sigma) were added to 100 ml MillQ ultrapure water and mixed thoroughly, and 4 ml of this solution was pipetted into the Perspex tubes. The second solution consisted of 10 g ammonium peroxydisulfate (Reagent grade, 98%, Sigma) that was added to 100 ml ultrapure water. A volume of 160 µl of the second solution was pipetted into the Perspex tube before adding lastly 40 µl of N, N, N', N'-Tetramethylenediamine (BioReagent, for molecular biology, ≥99% (GC), Sigma) (Labuschagne *et al*., 2021). Once the gel is polymerized, the tubes were then submerged in ultrapure water for an hour. This allowed the gels to swell until they were firm. The perspex spacer was inserted into the tube and 200 mg of Chelex-100 beads were added. for the purpose of this dissertation, 5 ml artificial seawater was added to the internal spacer (length of 1 cm and diameter of 2.6 cm). The second gel was transferred onto the internal spacer, ensuring no air was trapped between the gel layers.

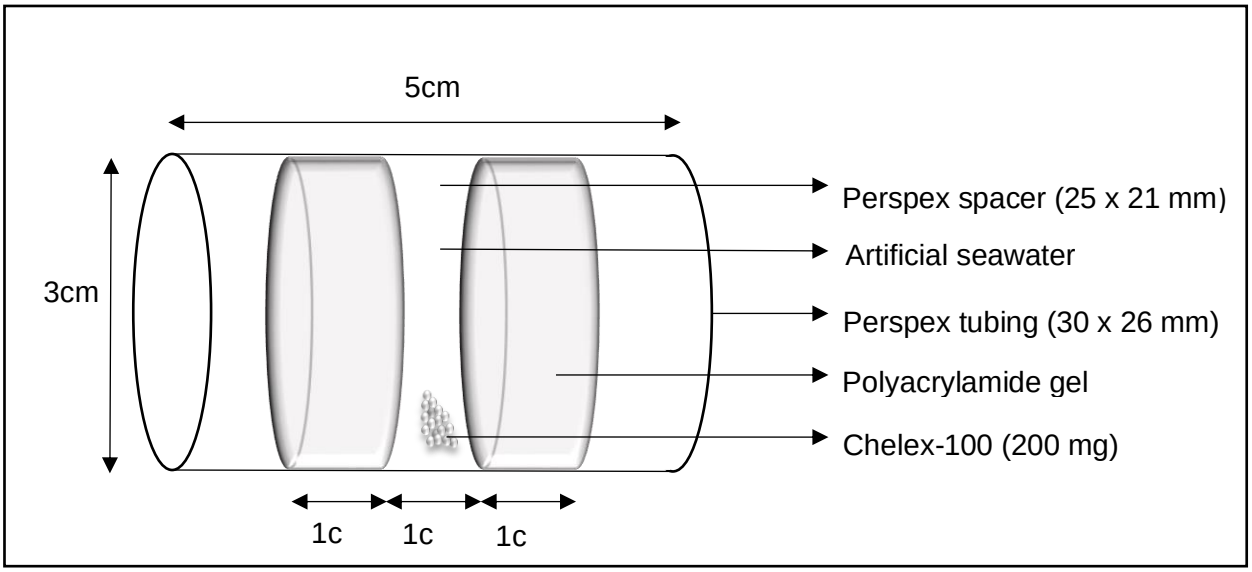

Figure 2.2: The artificial mussels design was adopted and adapted from Wu *et al*., (2007).

2.2.4 Element recovery from Chelex-100

The Chelex-100 beads were retrieved from the AM, by carefully removing one of the polyacrylamide gel plugs. The Chelex-100 beads were rinsed with MilliQ ultrapure water and pipetted into an acid-pre-washed 15 mL polypropylene falcon tube. The samples were centrifuged for 2 min at 1000 g and the supernatant was removed with a pipette. The beads were rinsed twice with 5 mL of ultrapure water. After the supernatant was removed again, 4.5 mL 6 M HNO_3 (sub-boiled from 65%; p.a. quality) and 0.5 mL HCl (Merck, 37%) were added to the polypropylene falcon tube containing the beads. The Chelex-100 beads were left in the acid mixture for approximately two hours. After two hours, the supernatant was removed and added to new (15 mL) polypropylene falcon tubes. The samples were diluted to a ratio of 1:10 volume with 1% nitric acid and MilliQ water solution, before being analysed with Agilent 7900 inductively coupled plasma mass spectroscopy (ICP-MS) (Labuschange *et al*., 2021).

2.2.5 Biological tissue sample preparation for element analyses

At the laboratory, samples were placed in a -80°C freezer for 24 hr, then transferred to a freeze drier (FreeZone 6, Labconco) for ± 72 hrs. Then 0.2 g (dry weight) of the samples was digested in a mixture of 7.5 mL nitric acid (sub-boiled 65%, Merck), and 2.5 mL hydrogen peroxide solution (30%, Sigma-Aldrich). These samples were digested with an Ethos Easy MAXII-44 Microwave Digester system, at standard digestion power of 1000 W at 200°C for 45 min. Following digestion, samples were transferred to a volumetric flask and brought to a volume of 50 mL with 1% nitric acid in MilliQ water. The solution was decanted into Falcon tubes and covered with aluminium foil, as the samples are light-sensitive, and was kept in the dark until they were analysed (Degger *et al*., 2016; Labuschagne *et al*., 2021).

Prior to element analysis, all the digested solutions were diluted 1:5 with 1% HNO_3. The concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Uranium (U) and Zn were determined using ICP-MS (Agilent 7900) equipped with an autosampler system. Concentrations of Chromium (Cr) were analysed using graphite furnace atomic absorption spectrometry (GF-AAS) with a PerkinElmer Analyst 600 equipped with Zeeman-effect background correction (Erasmus *et al*., 2020). A volume of 600 µL of the sample solution was pipetted into a AAS vial, where an autosampler was used to inject 20 µL into the graphite furnace. The calibration of the AAS resulted in a regression line with the correlation factor being (r^2 = 0.9995).

Concentrations of total mercury were analysed with a Flow Injection Mercury System (FIMS 400, PerkinElmer). The FIMS operated with two carriers, a gas carrier (argon) at 80 mL/min and a liquid carrier (3% HCl), and a reducing agent [52 mM sodium borohydride ($NaBH_4$)].

Seven dilutions of the Hg standard (1000 µg/mL Hg standard in 10 % HNO_3, PerkinElmer) were used to adjust the FIMS to obtain a regression line with a correlation factor of ($r^2 = 0.9989$) before analysing the samples (Erasmus *et al*., 2022). The concentrations in tissues were expressed as µg/g dry weight while concentrations in the AM samples concentrations were expressed as µg element/g Chelex-100.

2.2.6 Quality assurance and control

The quality assurance and control of the element analysis was performed using applicable certified reference material (CRM) for all the elements (Table 2.1). Reference material ERM-CE278k (mussel tissue certified reference material, European Commission, Belgium) was used to authenticate the methodical procedure of biota samples. All the recovery rates recorded for the CRM were within the acceptable ranges, with the exception of Cr (129%) and Fe (74%) (Table 2.1). For the purposes of this dissertation a correction factor was not applied to account for the over- and under recoveries of these two elements. Therefore it should be borne in mind when interpreting the data. The limit of detection (LOD) for each specific element in mussel tissue samples was calculated as three times the standard deviation of the blank measurements. The calculation of the limit of quantification (LOQ) was performed by multiplying the standard deviation of the average by nine (Erasmus *et al*., 2022).

Table 2.1: Recovery rates (%), limit of detection (LOD) and limit of quantification (LOQ) of the elements of interest obtained for certified reference material used in the analysis.

Elements	Recovery rates (%)	CRM (ERM-CE278k) mg/kg	LOD	LOQ
As	90.23	6.7	0.001899	0.006331
Cd	93.04	0.336	0.154405	0.463215
Cr	129.36	0.73	2.343386	7.030158
Cu	91.17	5.98	0.509303	1.52791
Fe	74.36	161	4.745174	14.23552
Hg	112.44	0.071	0.000327	0.000981
Mn	88.52	4.88	0.131323	0.393968
Ni	88.60	0.69	0.274954	0.824863
Pb	97.88	2.18	8.684111	26.05233
Se	103.04	1.62	0.034115	0.113716
Zn	91.63	71.4	4.077985	12.23396

2.2.7 Statistical analyses

A range of uni- and multivariate statistical methods were used to compare the concentrations of elements from the different biomonitoring tools (AM and mussels). The data were tested for normality and homogeneity of variance using Shapiro-Wilk and Kolmogorov-Smirnoff normality test with Dallal-Wilkinson-Lillie for p-value (Zar, 1996), respectively, prior to applying *post-hoc* comparisons. Regression analysis along with Pearson's and Spearman's (where appropriate) correlation analysis were used to determine the relationships between the temporal element uptake profiles and spatial element uptake profiles across sites of the AMs and *C. meriodionalis*. If the data were normally distributed, Pearson's correlation analysis was applied and if the data were not normally distributed, Spearman's correlation analysis was applied. The data were log-transformed [y=log (x+1)] before undertaking a two-way analysis of variance (ANOVA). Tukey's post-hoc and Šídák's multiple comparisons tests were applied to test for significant differences in element concentrations between the AMs, transplanted mussels and resident mussels over the 6-week deployment period and the two exposure sites, where the alpha was set at ($p < 0.05$). A multivariate discriminant function analysis (DFA) was performed to evaluate spatial and temporal element uptake patterns in the AMs, transplanted and resident mussels. The Eigen-structure of the multivariate matrix was used to determine the canonical variates that were analysed for every sample; the first two variates determine patterns in the data as they are plotted on the DFA.

2.3 Results

2.3.1 Element bioaccumulation

All the recovery rates recorded for the CRM were within the acceptable ranges, thus indicating the analytical techniques were valid. All the concentrations are presented in µg/g dry mass in whole *C. meriodionalis* tissue. The bar graphs show the mean ± standard error of element concentrations (Al, As, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Se, U and Zn) in dried whole mussel tissue at both Walvis Bay and Swakopmund during the 6-week deployment period (Figures 2.3 and 2.4).

2.3.2 Differences between sites

Concentrations of As (Figure 2.3 B), Cd (Figure 2.3 C), Co (Figure 2.3 D), Cr (Figure 2.3 E), Fe (Figure 2.3 G), Mo (Figure 2.4 B), Ni (Figure 2.4 C), Se (Figure 2.4 E) and U (Figure 2.4 G) in Walvis Bay's resident mussels were all significantly lower ($p<0.0001$) than the concentrations in Swakopmund's resident mussels. Walvis Bay's resident mussel's concentrations of Cu (Figure 2.3 F) were significantly higher ($p<0.0001$) than the concentrations of Swakopmund's resident mussels. Walvis Bay's transplanted mussel's concentrations of Cu (Figure 2.3 F, $p=0.0015$) and Mo (Figure 2.4 B, $p=0.0365$) were significantly higher than the concentrations of Swakopmund's transplanted mussels. Only Se in transplanted mussels from Swakopmund were significantly higher ($p=0.0013$) than in transplanted mussels at Walvis Bay (Figure 2.4 E).

When compared to the element concentrations in mussels from the reference site (Mile 17) the resident mussel levels of all the elements from Walvis Bay were significantly higher ($p<0.05$), except for Cu (Figure 2.3 F) which was significantly lower ($p<0.0001$) and Al (Figure 2.3 A), Mn (Figure 2.4 A), Mo (Figure 2.4 B), Pb (Figure 2.4 D) and Zn (Figure 2.4 G) that had similar levels as the resident mussels. The levels of Cr (Figure 2.3 E, $p=0.0403$) and Hg (Figure 2.3 H, $p=0.0057$) in resident mussels from Swakopmund were significantly lower than the reference site's mussel's concentrations, while Fe (Figure 2.3 G, $p<0.0001$), Mo (Figure 2.4 B, $p=0.0002$) and U (Figure 2.4 F, $p<0.0001$) concentrations were significantly higher than the reference site's mussels. The As (Figure 2.3 B), Cr (Figure 2.3 E), Hg (Figure 2.3 G) and Ni (Figure 2.4 C) concentrations in mussels from the reference site were the only significantly higher ($p<0.05$) elements when compared to the transplanted mussel levels from both study sites, as well as Se (Figure 2.4 E, $p=0.0017$) at Walvis Bay and U (Figure 2.4 F, $p=0.0260$) at Swakopmund. However, Cu (Figure 2.3 F, $p=0.0066$) and Pb (Figure 2.4 D, $p<0.0001$) levels of the reference site mussels were significantly lower than Walvis Bay's transplanted mussel levels. When comparing the reference site's mussel element concentrations to Swakopmund's

transplanted mussels it is evident that Pb (Figure 2.4 D) levels were significantly lower ($p=0.0010$) in the reference sites mussels.

2.3.3 Differences between element indicators

Swakopmund

Except for Cu (Figure 2.3 F), U (Figure 2.4 F) and Zn (Figure 2.4 G) element concentrations in AMs were significantly lower ($p<0.05$) than in the resident and transplanted mussels. All element concentrations except for Cd (Figure 2.3 C), Cu (Figure 2.3 F) and Pb (Figure 2.4 D) in resident mussels were significantly higher ($p<0.05$) than in transplanted mussels (Figures 2.3 and 2.4).

Walvis Bay

All element concentrations, with the exception of Al (Figure 2.3 A) and U (Figure 2.4 F) in the AMs were significantly lower ($p<0.05$) than in the resident and transplanted mussels. The transplanted mussels Cd (Figure 2.3 C), Cr (Figure 2.3 E), Fe (Figure 2.3 G), Ni (Figure 2.4 C) and Pb (Figure 2.4 D) concentrations were significantly ($p<0.05$) higher than the residents, while the resident mussels concentration of Cu (Figure 2.3 F), Hg (Figure 2.3 H) and Mn (Figure 2.4 A) were significantly ($p<0.05$) higher than the transplanted mussels (Figure 2.3 and 2.4).

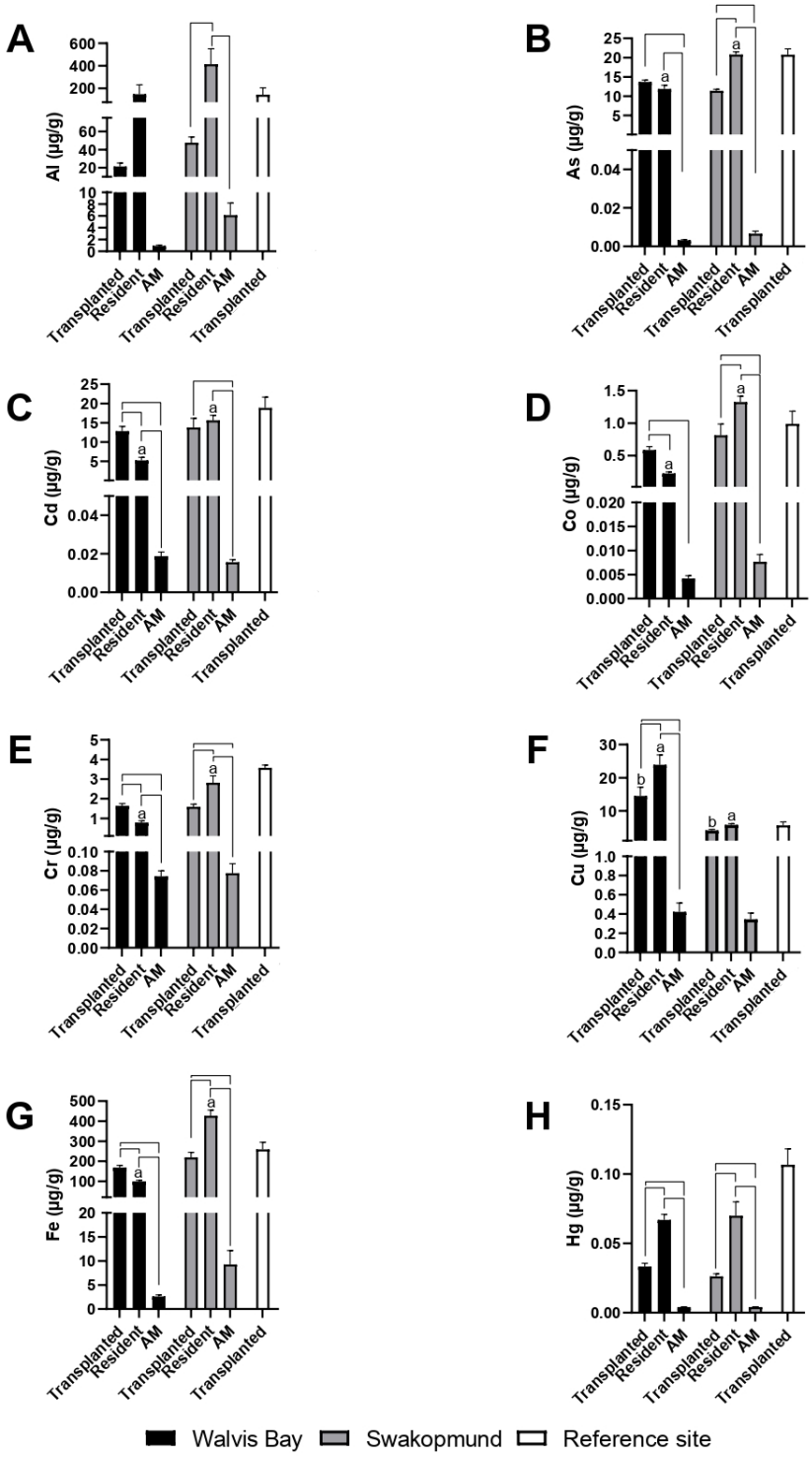

Figure 2.3: Spatial patterns of element accumulation (mean ± standard error) in *Choromytilus meriodionalis* of transplanted, resident and artificial mussels (AM) at Walvis Bay (black bars) and Swakopmund (grey bars) after 6 weeks deployment. The initial element concentrations (mean ± standard error) in the *Choromytilus meriodionalis* collected at the reference site (white bar). Element concentrations are presented as µg/g dry weight for transplanted, resident mussels, and µg/g Chelex for the artificial mussels. Significant differences ($p<0.05$) between indicators within a site are indicated with joining lines, while differences for the same indicator between sites are indicated with common alphabetical superscript above the bar.

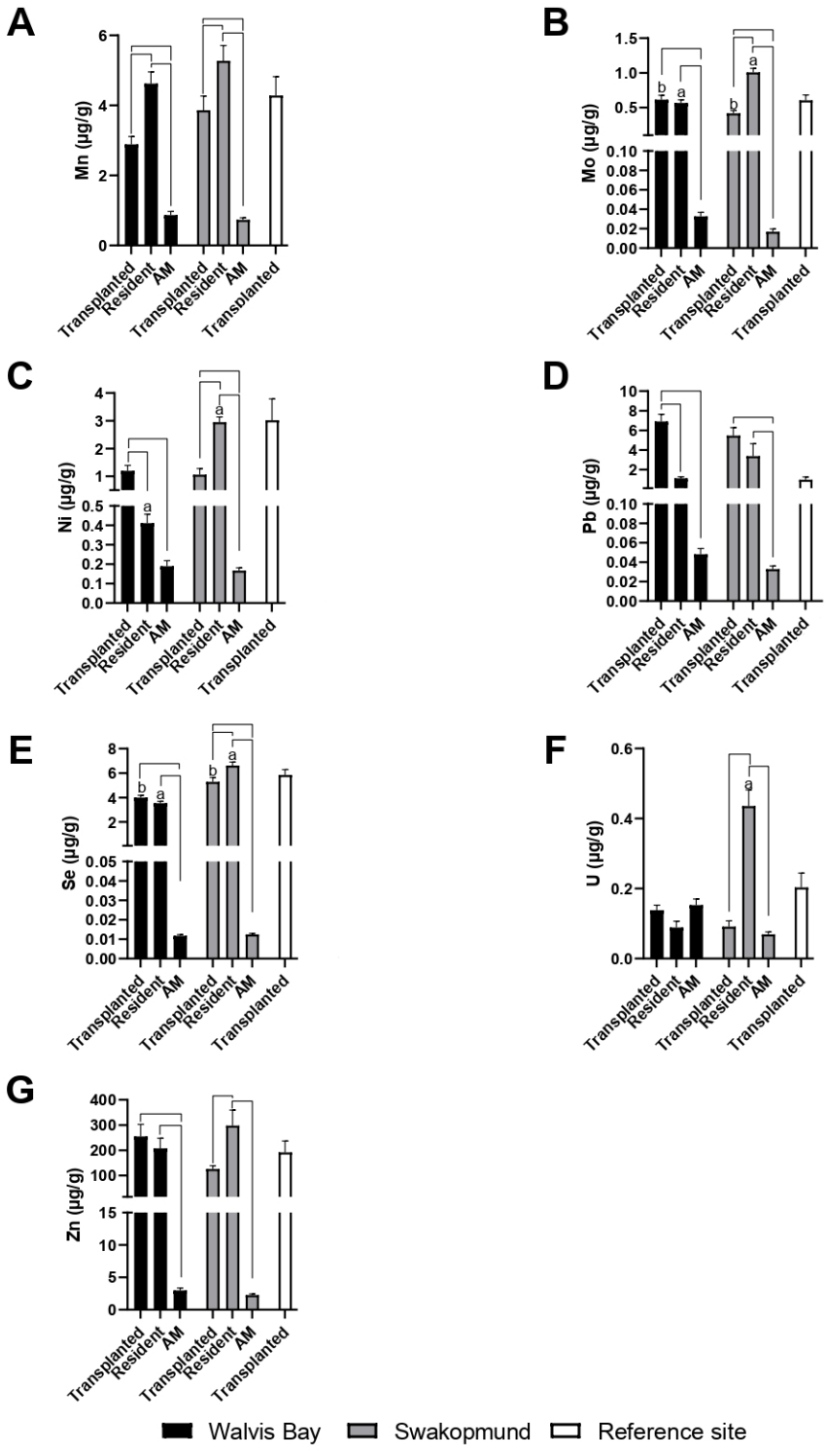

Figure 2.4: Spatial patterns of element accumulation (mean ± standard error) in *Choromytilus meriodionalis* of transplanted, resident and artificial mussels (AM) at Walvis Bay (black bars) and Swakopmund (grey bars) after 6 weeks deployment. The initial element concentrations (mean ± standard error) in the *Choromytilus meriodionalis* collected at the reference site (white bar). Element concentrations are presented as µg/g dry weight for transplanted, resident mussels, and µg/g Chelex for the artificial mussels. Significant differences ($p<0.05$) between indicators within a site are indicated with joining lines, while differences for the same indicator between sites are indicated with common alphabetical superscript above the bar.

Table 2.2: Two-way Analysis of Variance table comparing element concentrations of three different bioaccumulation indicators between two sites at week 6. Asterisks (*) indicate interactions that are not significant.

Element	Bioaccumulation indicators (df=2)	Site (df=1)	Bioaccumulation indicators and site interaction (df=2)
Al	F= 9.693; p= 0.0003	F= 3.005; p=0.0891*	F= 2.224; p= 0.1186*
As	F= 448.6; p< 0.0001	F= 21.46; p< 0.0001	F= 51.54; p< 0.0001
Cd	F= 76.18.; p< 0.0001	F= 16.54; p= 0.0002	F= 13.25; p< 0.0001
Co	F= 67.98; p< 0.0001	F= 53.49; p< 0.0001	F= 31.79; p< 0.0001
Cr	F= 65.32; p< 0.0001	F= 22.78; p< 0.0001	F= 25.44; p< 0.0001
Cu	F= 40.74; p< 0.0001	F= 49.37; p< 0.0001	F= 15.77; p< 0.0001
Fe	F= 151.0; p< 0.0001	F= 100.2; p< 0.0001	F= 64.08; p< 0.0001
Hg	F= 99.17; p< 0.0001	F= 0.1183; p= 0.7323*	F= 0.5727; p= 0.5676*
Mn	F= 107.6; p< 0.0001	F= 4.286; p= 0.0435	F= 1.868; p= 0.1648*
Mo	F= 174.5; p< 0.0001	F= 4.875; p= 0.0318	F= 29.91; p< 0.0001
Ni	F= 67.96; p< 0.0001	F= 52.44; p< 0.0001	F= 65.51; p< 0.0001
Pb	F= 40.17; p< 0.0001	F= 0.2316; p= 0.6324*	F= 3.718; p= 0.0311
Se	F= 392.0; p< 0.0001	F= 76.14; p< 0.0001	F= 30.29; p< 0.0001
U	F= 26.38; p< 0.0001	F= 13.44; p= 0.0006	F= 50.89; p< 0.0001
Zn	F= 25.81; p< 0.0001	F= 0.1864; p= 0.6678*	F= 4.225; p= 0.0201

2.3.4 Interactions between sampling sites and bioaccumulation indicators

The interaction between the sampling sites and the bioaccumulation indicators (Table 2.2) showed that for all elements, except Al, Hg and Mn, there were significant interactions in element concentrations between the different bioaccumulation indicators (transplanted, resident, and artificial mussels) and sampling sites. In Table 2.2 it shows that there were significant interactions ($p < 0.05$) for all the elements except for Al, Hg, Pb and Zn when comparing the different sites.

2.3.5 Element concentrations in transplanted mussels and AMs over the 6-week deployment period

Swakopmund

Based on the graphs (Figure 2.5 and 2.6) and then also the correlations for Swakopmund there were notable fluctuations in the element levels in both the AMs and the transplanted mussels over the deployment period (week 0 to 6). While Al, Cd, Ni, Pb, Se, U and Zn levels increased over time, only Pb (Figure 2.6 D) was significantly ($p<0.05$) higher after 6-weeks (Table 2.3). The levels of As Co, Cr, Hg and Mn decreased with only As (Figure 2.5 B), Cr (Figure 2.3 E) and Hg (Figure 2.5 H) decreasing significantly ($p<0.05$) after 6-weeks deployment (Table 2.4). For the AMs only Pb (Figure 2.6 D) decreased significantly ($p<0.05$) over the 6-weeks deployment period. Other elements such as As, Co, Fe, Mo, Se and U reached the highest levels after two weeks of deployment, whereafter they decreased (Table 2.3). Whilst there was a positive relationship between As, Cr, Mo and Ni concentrations in the AMs and transplanted mussels; these were not significant (Annexure Figures A.1 and A.2).

Walvis Bay

The correlations for Walvis Bay (see Annexure Figures A.3 and A.4) indicated that there were notable fluctuations in the element levels in both the AMs and the transplanted mussels over the deployment period (Figure 2.7 and 2.8). Cadmium, Co, Cr, Fe, Mo, Ni, Se and U levels in the transplanted mussels increased and reached their highest levels after the first few weeks of deployment, whereafter they decreased. The levels of Cu, Pb and Zn increased over the 6-week deployment with only Pb (Figure 2.8 D) increasing significantly ($p<0.05$) after the six weeks (Table 2.4). The levels of Al, As, Hg and Mn decreased over the deployment period with only As (Figure 2.7 B), Cr (Figure 2.7 E) and Hg (Figure 2.7 H) decreasing significantly ($p<0.05$) over the six weeks. For the AMs only Al (Figure 2.7 A) and Hg (Figure 2.7 H) increased significantly ($p<0.05$) over the 6-weeks deployment period. Other elements such as As, Cd, Co, Cu, Fe, Mo, Ni Se and Zn reached the highest levels after two to four weeks of deployment, whereafter they decreased (Table 2.4). Whilst there was a positive relationship between Cd, Co, Cu, Cr, Fe, Mn, Mo, Ni and Zn concentrations in the AMs and transplanted mussels, only Co and Fe were significant (Appendix A).

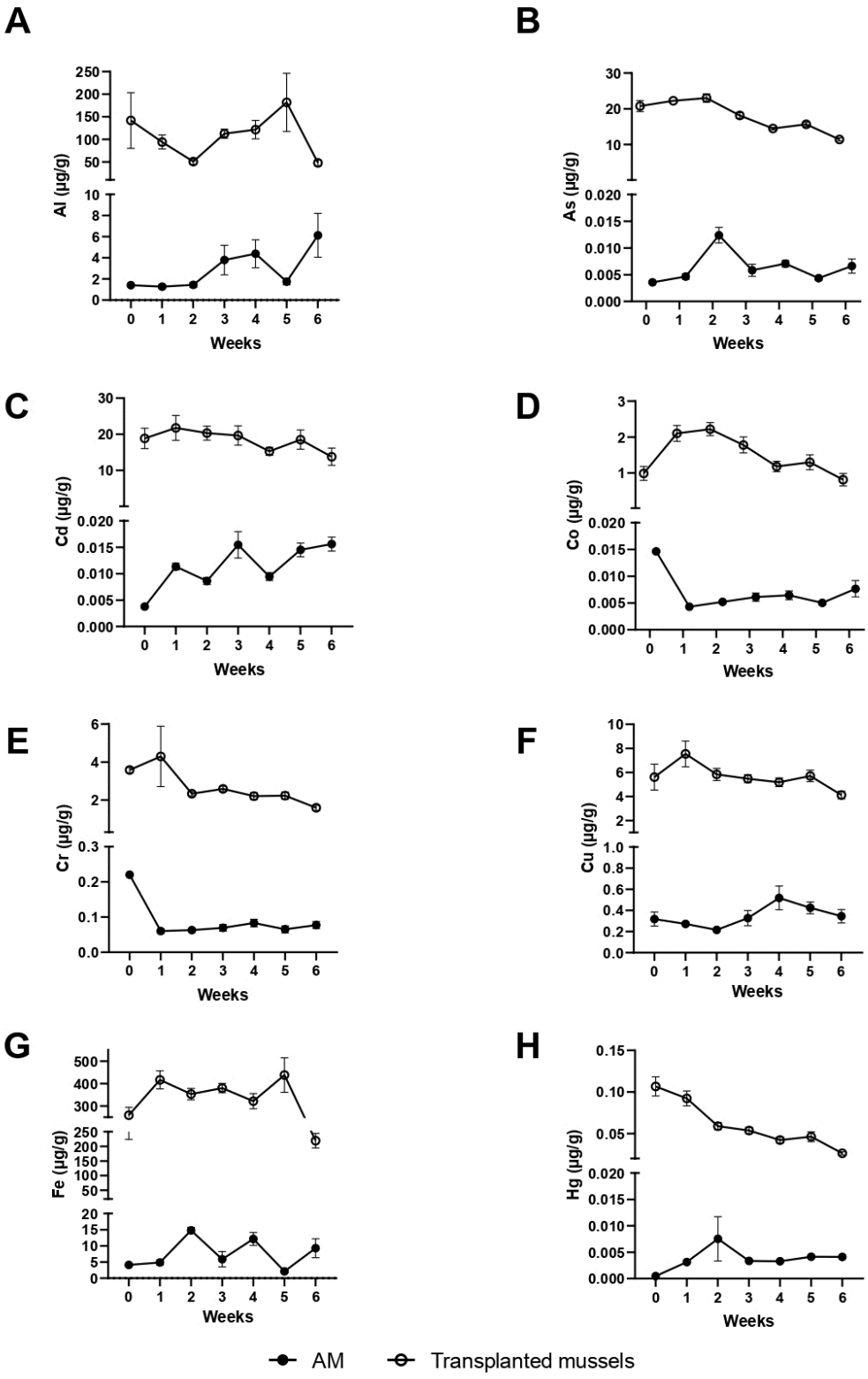

Figure 2.5: Element uptake (mean ± standard error) in *Choromytilus meriodionalis* (open circles) and artificial mussels (closed circles) deployed in Swakopmund over a 6-week period. All concentrations are presented as µg/g dry mass for transplanted mussels and µg/g Chelex for the artificial mussels (AM). Refer to Table 2.4 for significant differences between transplanted and artificial mussels over the 6-week deployment period.

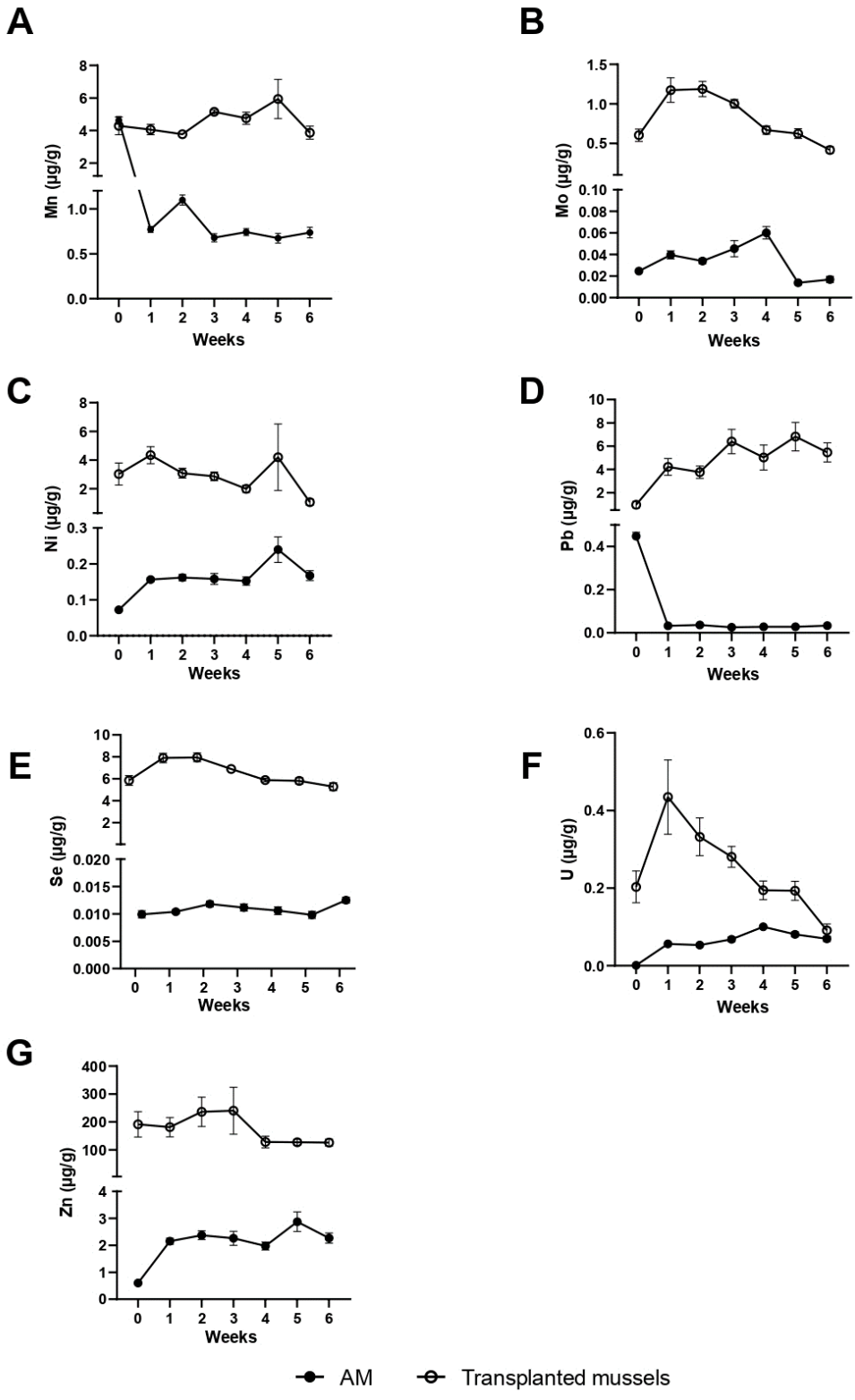

Figure 2.6: Element uptake (mean ± standard error) in *Choromytilus meriodionalis* (open circles) and artificial mussels (closed circles) deployed in Swakopmund over a 6-week period. All concentrations are presented as µg/g dry mass for transplanted mussels and µg/g Chelex for the artificial mussels (AM). Refer to Table 2.4 for significant differences between transplanted and artificial mussels over the 6 week deployment period.

Table 2.3: Matrix indicating elements in transplanted black mussels (TM) and artificial mussels (AM) from Swakopmund which displayed significant temporal differences (ANOVA; $p < 0.05$).

	Week 0		Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
	AM	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**
Week 0			Cd, Co, Cr, Hg, Mn, Ni, Pb, U, Zn	Pb	As, Cd, Co, Cr, Fe, Mn, Ni, Pb, U, Zn	Co, Cr, Mo, Pb, Se	Cd, Co, Cr, Hg, Mn, Ni, Pb, U, Zn	Cr, Hg, Mo, Pb	As, Cd, Co, Cr, Hg, Fe, Mn, Mo, Ni, Pb, U, Zn	As, Cr, Hg	Cd, Co, Cr, Hg, Fe, Mn, Mo, Ni, Pb, U, Zn	Cr, Hg, Pb	Cd, Co, Cr, Hg, Mn, Ni, Pb, Zn	As, Cr, Hg, Pb
Week 1					As, Fe, Mn		Hg		U	As, Hg	Fe, Hg, Mo, U	As, Se	Hg, Mo	As, Co, Cd, Fe, Hg, Mo, Ni, Se
Week 2							Mn	Al, Mn	Mn, Mo, U	Al, As, Co, Mo, Se	As, Cd, Cu, Fe, Mn, Mo	As, Mo	Cd, Mn, Mo	As, Co, Hg, Mo, Se
Week 3									U	As, Mo	Hg, Mo	Mo	Hg	As, Cr, Fe, Hg, Mo, U
Week 4											Fe, Hg, Mo		Cd, Hg, Mo, U	As
Week 5														As
Week 6														

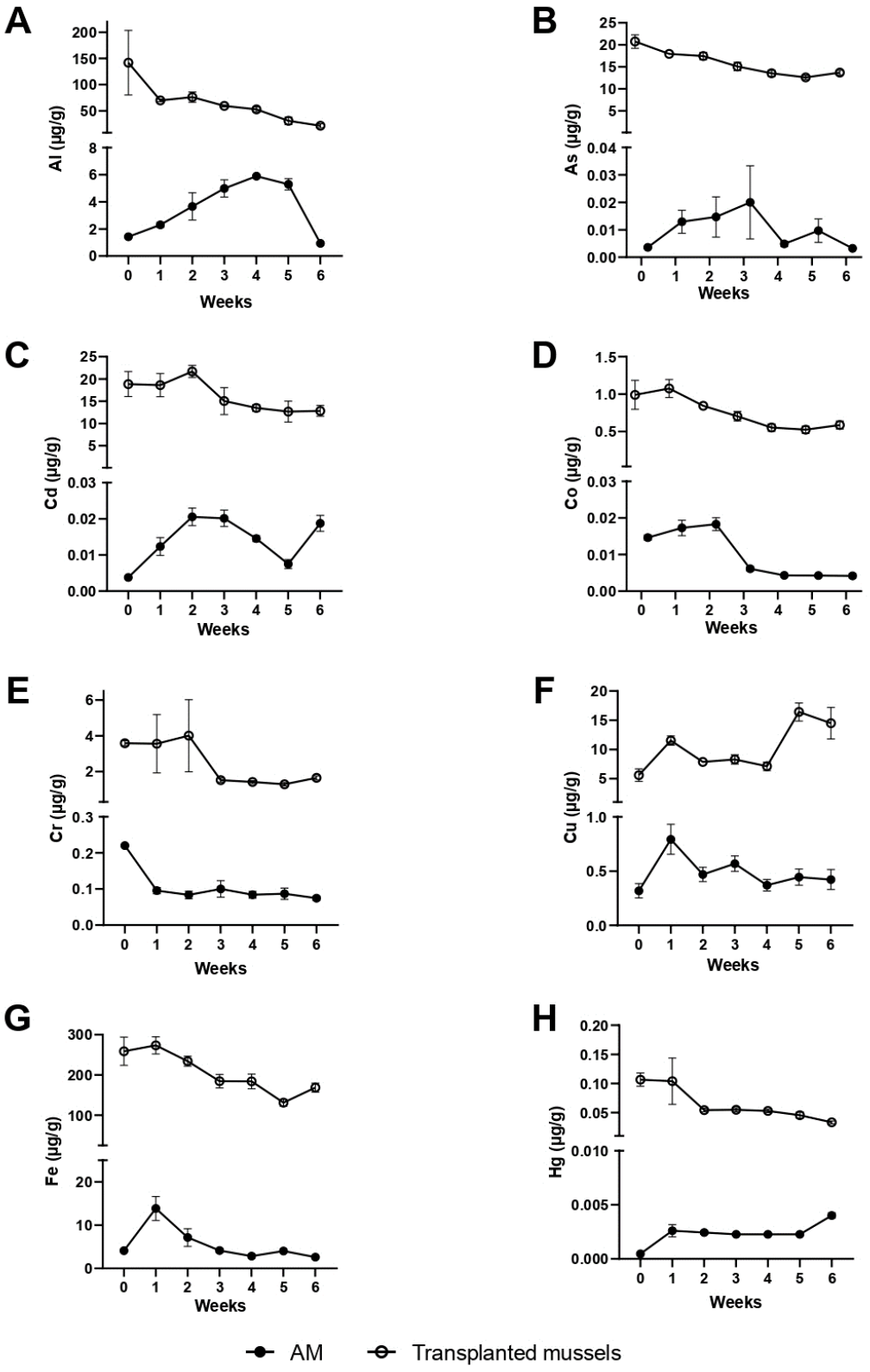

Figure 2.7: Element uptake (mean ± standard error) in *Choromytilus meriodionalis* (open circles) and artificial mussels (closed circles) deployed in Walvis Bay Harbour over a 6-week period. All concentrations are presented as µg/g dry mass for transplanted mussels and µg/g Chelex for the artificial mussels (AM). Refer to Table 2.5 for significant differences between transplanted and artificial mussels over the 6-week deployment period.

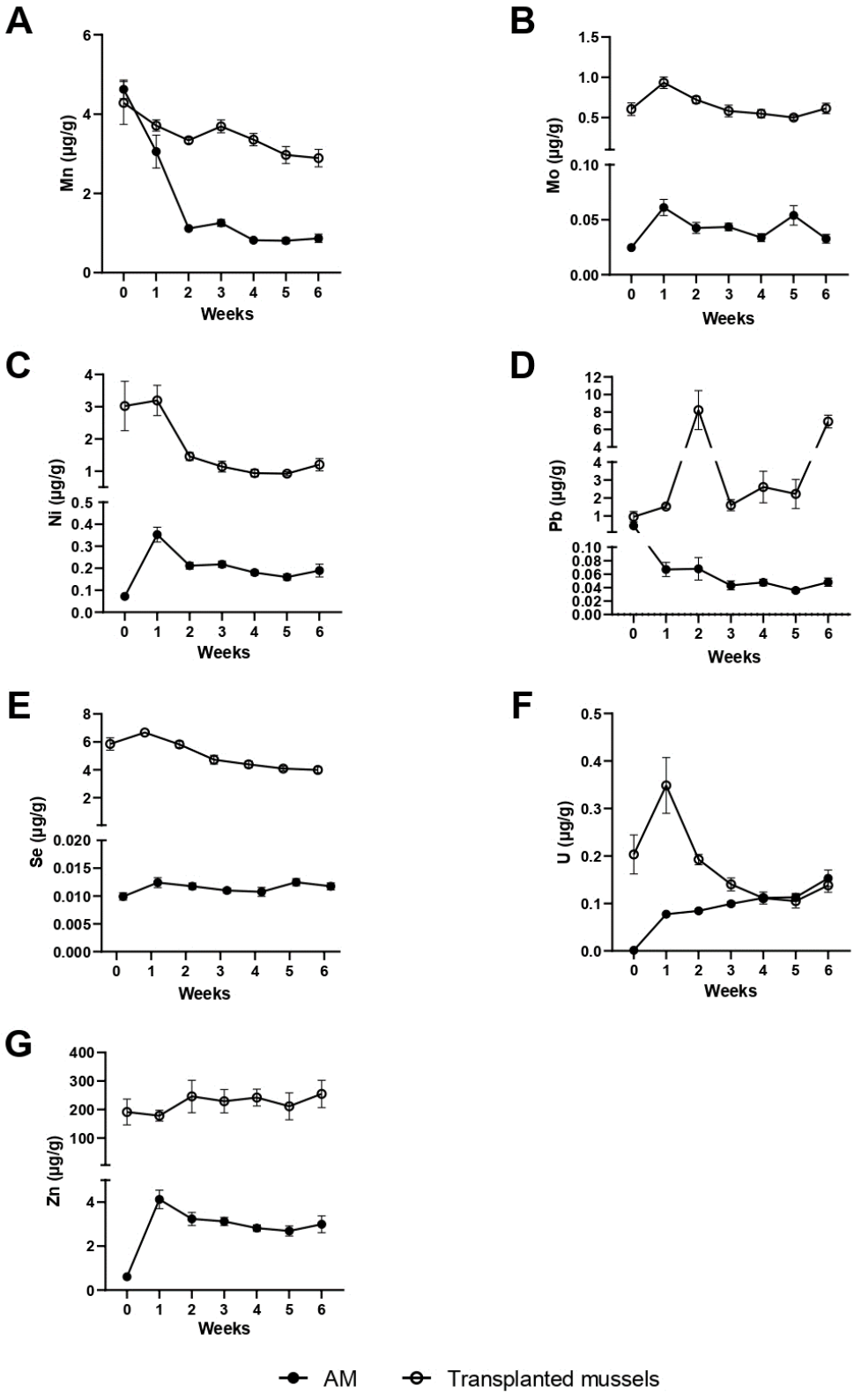

Figure 2.8: Element uptake (mean ± standard error) in *Choromytilus meriodionalis* (open circles) and artificial mussels (closed circles) deployed in Walvis Bay Harbour over a 6-week period. All concentrations are presented as µg/g dry mass for transplanted mussels and µg/g Chelex for the artificial mussels (AM). Refer to table 2.5 for significant differences between transplanted and artificial mussels over the 6-week deployment period..

Table 2.4: Matrix indicating elements in transplanted black mussels (TM) and artificial mussels (AM) from Walvis Bay Harbour which displayed significant temporal differences (ANOVA; $p < 0.05$).

	Week 0		Week 1		Week 2		Week 3		Week 4		Week 5		Week 6	
	AM	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**	**AM**	**TM**
Week 0			Al, Cr, Mo, Ni, Pb, U, Zn	Cu	Cd, Cr, Hg, Ni, Pb, U, Zn	Hg	Al, Cd, Cr, Hg, Mn, Mo, Ni, Pb, U, Zn	Cr	Al, Cd, Cr, Hg, Mn, Ni, Pb, U, Zn	Cr	Al, Cr, Hg, Mn, Ni, Pb, U, Zn	Cu, Cr	Al, Cd, Cr, Hg, Fe, Mn, Ni, Pb, U, Zn	As, Cr, Hg, Pb
Week 1					Mn, Ni	Cu	Al, Fe, Mn	Cu, Se	Al, Fe, Mn, Ni, U	As, Se	Al, Mn, Ni, U, Zn	Al, As, Fe, Mo, Se	Al, Fe, Mn, Mo, Ni, U	Al, As, Fe, Pb, Se
Week 2									Mn, U	Cd, U	Mn	Al, As, Cu, Fe, Mo, Ni, Se, U	Hg, U	Al, As, Cd, Hg, Se
Week 3									Mn		Cd, Mn	Cu	Al, Hg	Al, Hg, Pb
Week 4											Cd	Cu	Al, Hg	Al, Hg, Pb
Week 5													Al, Cd, Hg	Fe, Pb
Week 6														

2.3.6 Spatial patterns in element concentrations

The results of the DFA and structure matrix indicated a clear differentiation between the elements and concentrations in AMs and the resident and transplanted mussels at the two research sites (Figure 2.9). This reflects the significantly lower element concentrations in the AMs when compared to the mussels at the two sites. Following the removal of the AM data from the analysis, clear spatial and indicator trends for the mussels at the two sites were observed (Figure 2.10). Function 1 axis (55.7% of the variation explained) differentiated between the Walvis Bay resident and transplanted mussels; and the Swakopmund resident and transplanted mussels, as well as mussels from the reference site. The elements accounting for the separation were Cu and Ni, followed by Ni and Mn (Table 2.5). The differences in elements from the resident, as well as reference mussels and the transplanted mussels, account for 29.4% of the variation in the data along Function axis 2. Aluminium, As and Hg in the Swakopmund resident and reference mussels are responsible for the differentiation from the transplanted mussels (Table 2.5).

Table 2.5: The Standardized Canonical Discriminant Function Coefficients of the first two functions of a Discriminant Functions analysis (DFA), based on different elements measured from three different biomonitoring devices used along the Namibian coastline after 6 weeks.

	All three indicators		**AMs removed**	
Elements	**Function 1**	**Function 2**	**Function 1**	**Function 2**
Al	0.352	-0.149	0.407	**0.824***
As	**1.097***	**-.618***	0.272	**0.817***
Cd	0.683	0.209	0.074	0.225
Co	-0.291	0.295	0.195	-0.602
Cr	-0.259	0.31	0.241	-0.535
Cu	-0.084	**-.853***	**-0.886***	-0.015
Fe	**-1.113***	0.573	0.139	-0.637
Hg	0.255	-0.188	-0.064	**0.780***
Mn	-0.508	-0.263	-0.572	-0.329
Mo	0.193	-0.34	-0.494	0.318
Ni	0.152	0.502	**0.761***	0.39
Pb	0.04	-0.007	-0.301	-0.785
Se	0.843	0.292	0.596	-0.176
U	-0.131	0.174	0.157	0.089
Zn	0.113	0.197	0.044	0.493

*. Largest absolute correlation between each variable and any discriminant function

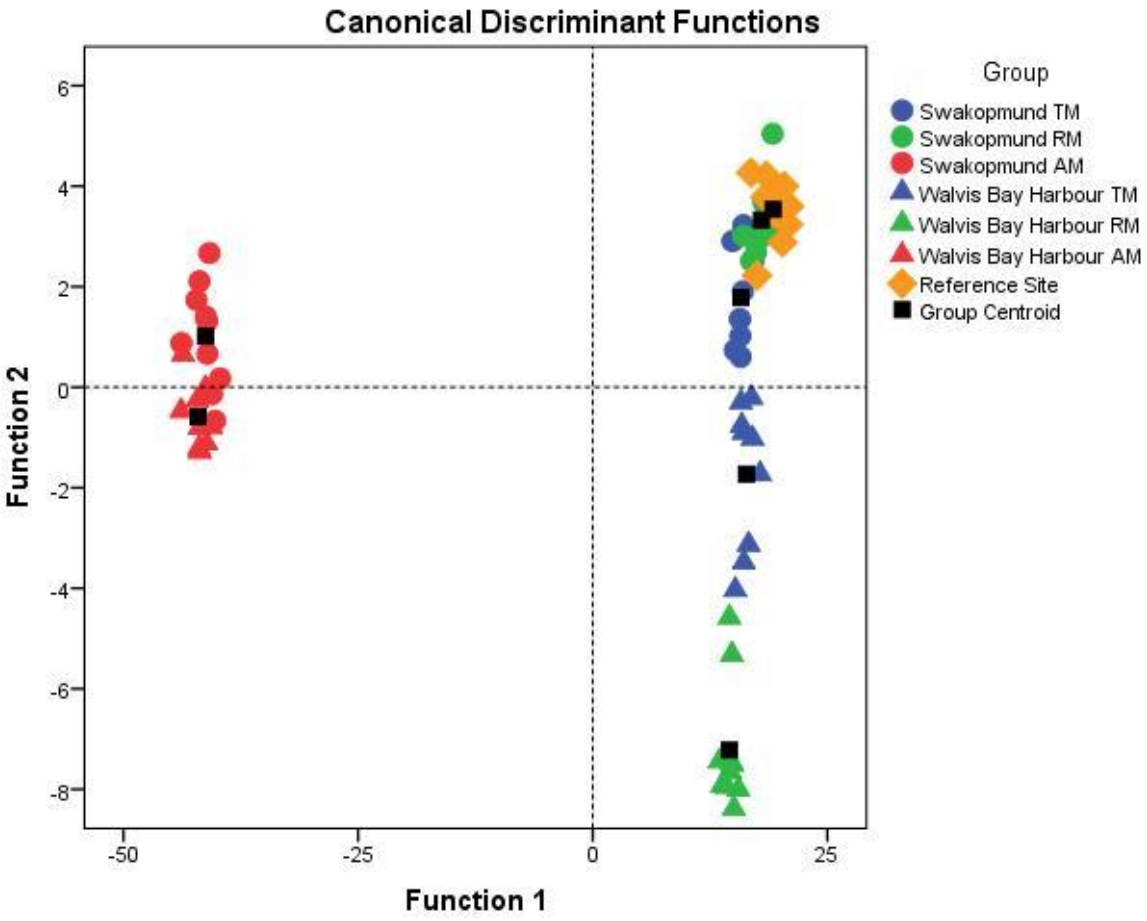

Figure 2.9: Canonical variates were derived from a discriminant function analysis (DFA), using elements measured from transplanted, resident and artificial mussels along the Namibian coastline. Function 1 (97.3%) and 2 (1.2%) refer to the first two canonical functions of a multivariate data set, explaining a total of 98.5% of the variation in the data. The function 1 driver was Cd its concentration increased from left to right and the function 2 drivers were Cu and Ni concentrations increased from top to bottom.

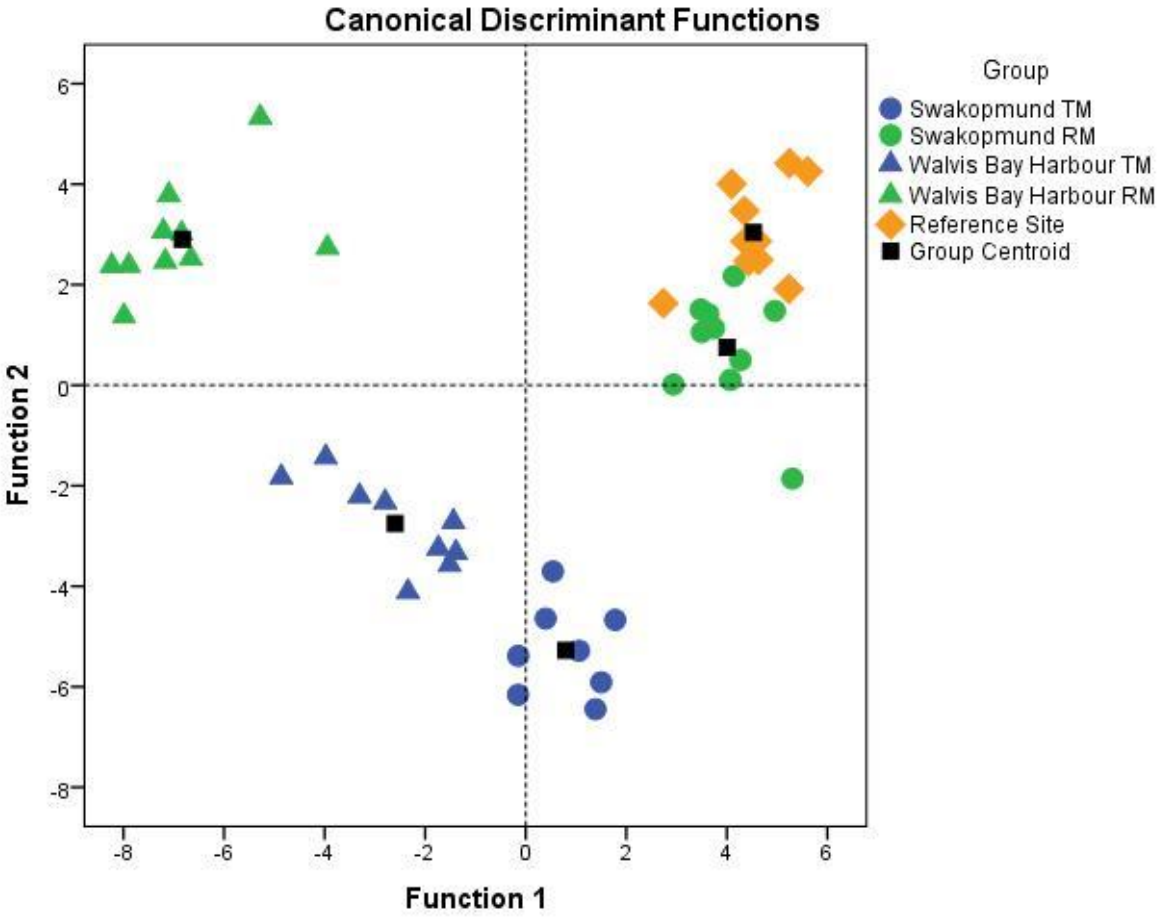

Figure 2.10: Canonical variates were derived from a discriminant function analysis (DFA), using elements that were measured from transplanted and resident mussels along the Namibian coastline. Function 1 (55.7%) and 2 (29.4%) refer to the first two canonical functions of a multivariate data set, explaining a total of 85.1% of the variation in the data. The function 1 drivers were Cu and Ni and function 2 drivers were Al and As concentrations increased from left to right and from top to bottom.

2.4 Discussion

There were distinct differences in element concentrations in resident and transplanted mussels within and between the sampling sites. The two study sites' uptake patterns differed from each other, while Swakopmund's resident mussels' concentrations were similar to the mussels from the reference site.

2.4.1 Site specific element concentrations

Walvis Bay Harbour is the main port of Namibia and all types of cargo are transported from this port. Some of the cargo is ore from mines situated inland (Nekhoroshkov *et al*., 2021). This study showed that Walvis Bay had high concentrations of Cu for both the transplanted and resident mussels, as well as high concentrations of Pb in the transplanted mussels. The concentrations of Cu in the Walvis Bay harbour samples for both the transplanted and resident mussels increased over the 6-week period. The high concentrations of Cu and Pb at the harbour site could be attributed to fish processing factories, shipping activities such as repairs and antifouling painting, and lastly recreational activities conducted in and around the harbour area (Omoregie *et al*., 2019).

The Pb concentrations of the transplanted mussels that were deployed at Walvis Bay Harbour were the highest when compared to both its resident mussels and the transplanted and resident mussels concentrations from Swakopmund. This could be due to ships being cleaned at the harbour site, thus exposing the transplanted mussels (with lower Pb) to higher levels of Pb in the surrounding environment. The resident mussels from this site have been continuously exposed to higher levels of Pb and probably adapted to the increased exposure, hence the lower concentrations. Lead is a non-essential element and is accumulated by mussels in their tissues, and although they can regulate other elements, Pb is not one of those elements. Mussels that are situated close to areas and influenced by industrial or urban activities have higher Pb levels (Yan *et al*., 1997; Sparks *et al*., 2014; Mejdoub *et al*., 2018) than non-industrialized areas. The marine environment is exposed to Pb due to paint being scrapped from ships (Lloyd, 1992; Vellemu and Omoregie, 2014). In marine embayments, such as Walvis Bay, where there is restricted water circulation and therefore an increase in elements as they are not regularly flushed by the currents (Sulochanan *et al*., 2007; Vellemu and Omoregie, 2014).

Swakopmund resident mussels had higher element concentrations than their transplanted mussels, specifically elements such as Al, Fe, U and Zn. The Swakopmund site is situated northwards up the coast from the Walvis Bay study site. The current (Benguela) flows from south to north, possibly transporting element concentrations as observed in the mussels that

were exposed at those sites (Vetter *et al*., 1999; Vellemu and Omoregie, 2014). The main source of Fe in marine environments is rock erosion from rainfall, while other sources are due to anthropogenic activities (industrial waste, run-off from urban areas and atmospheric deposition) (Giarratano *et al*., 2010; Sparks *et al*., 2014). The high levels of Fe in the marine environment may possibly be due to its natural occurrence (Fatoki *et al*., 2012) as the dune sand from the Namib desert has high levels of iron-oxide that is transferred into the ocean by wind (White *et al*., 2007). The Fe and Zn concentrations of the transplanted and resident mussels are the highest of all the essential elements recorded in this study. Mussels mainly bioaccumulate Zn through their diet of phytoplankton, macroalgea and detritus, rather than from the water column (Sparks *et al*., 2014; Nekhoroshkov *et al*., 2021). Even though the highest Zn levels in transplanted and resident mussels were recorded at this site they were still below the maximum allowable safe level of 300 µg/g for consumption (Sparks *et al*., 2014). Many studies have shown that mussel species such as *Perna viridis*, *Mytilus edulis* and *Mytilus galloprovincialis* are able to regulate their Zn levels (Chan, 1988; Riget *et al*., 1997; Ross *et al*., 2003; Garland *et al*., 2011). It can be assumed that *C. meridionalis* sampled in this study can also regulate Zn levels as the results showed a decrease in the transplanted mussels from Swakopmund over the 6-weeks. Whereas the AMs Zn levels increased over the same time frame.

The element concentrations in mussels from the reference site were similar to levels recorded in Swakopmund resident mussels. Concentrations of As and Hg were higher in the mussels from the reference site. Both As and Hg are two non-essential elements that biomagnify between organisms. Arsenic is present in marine environments, and concentrations increase as the trophic levels increase (Shilla *et al*., 2019). Arsenic has a wide variety of isotope forms and the level of toxicity for each one is different. Arsenite is known as an inorganic form of arsenic that can be absorbed through an organism's muscles, digestive tract and abdominal cavity. While inorganic arsenic is absorbed, the organic form of arsenic does not accumulate and is regulated and excreted frequently (Bosch *et al.,* 2016). The most toxic form of arsenic is its organic form which can be bioaccumulated by organisms (Goyer and Clarkson, 2001).

2.4.2 Comparisons with other studies

When comparing the element concentrations of Walvis Bay Harbour from this study to the element concentrations measured by Dahms *et al*. (2014) in the same area (see Annexure Table B), it is evident that Al, Cd, Cu, Fe, Mn and Zn has increased over the decade. In contrast there has been a decrease in Co, Ni and Pb over the ten years. An increase in elements such as Cu is due to inputs from anthropogenic activities in proximity of the study site (Bezuidenhout *et al*., 2020). As this study's site was within a harbour it can be assumed

that the anthropogenic activities in the surrounding areas could be responsible for increase for the element concentrations.

The reference site (Mile 17) concentrations for Al, Cd, Cr, Cu, Fe, Mn, Ni and Zn have increased since 2012 when the *C. meridionalis* was sampled, while Co and Pb has decreased (Dahms *et al*., 2014). Notable was the 100 fold increase in Zn when compared to the Dahms *et al*. (2014) study. Compared to the Vellemu *et al*. (2014) study, *C. meridionalis* from Walvis Bay Harbour and Swakopmund accumulated higher concentrations of Pb over the years, as well as the Cu levels (Omoregie et al., 2014) have increased at both sites during the current study. Mussels sampled during a more recent study by Nekhoroshkov *et al*. (2020) showed that Al, Co, Cr, Fe, Mn and Ni had decreased, while As has increased during the current study. Higher concentrations of As could be due to an increase in the salinity of the environment (Nekhoroshkov *et al*., 2021). The concentrations of Se, U and Zn for Walvis Bay were lower in the current study, while Se and Zn levels for Swakopmund from the current study were similar to the concentrations measured by Nekhoroshkov *et al*. (2021). Nekhoroshkov *et al*. (2021) reasoned that the high levels of Zn could be attributed to the mussel's dietary intake, as well as increased anthropogenic inputs.

However, the element levels in mussels from the current study were higher to those recorded in mussels from Saldanha Bay (Firth *et al*., 2019b). In 2020, another study was conducted in Saldanha Bay, South Africa where the element concentrations were similar to those measured in mussels from Walvis Bay Harbour (Bezuidenhout *et al*., 2020). Certain elements (Cd, Cu, Pb and Zn) occur in food particulates which can contribute to the levels found in the mussels as they filter feed on these particulates (Szefer *et al*., 2004). As it has been documented that the Benguela current flows past Saldanha Bay and this study's research sites. The current is nutrient rich, thus elements accumulate in the suspended matter and could explain an increase in elements (Bezuidenhout *et al*., 2020).

When compared to mussels from larger international harbours (Annexure Table B) the Walvis Bay Harbour resident mussel's element concentrations were much lower. A study conducted on *Perna viridis* in Victoria Harbour, Hong Kong, had higher concentrations for Al, Cr, Cu, Fe, Hg, Mn, Ni and Pb than Walvis Bay Harbour, whereas Walvis Bay Harbour had higher concentrations of Cd and Zn (Liu and Kueh, 2005). The element concentrations in mussels from Namibia's main harbour were higher overall or similar to the element concentrations found in mussels from Cape Town and Durban harbours during 2009 (Wepener and Degger, 2020). This is in contrast to the mussels from Cape Town and Durban harbours having much higher element concentrations than in Walvis Bay Harbour during 2008 (Wepener and Degger, 2020). As the results for the same sites for two different years varied so much it can be

assumed the organism regulate their concentrations (Wepener and Degger, 2020). This could be the reason for this studies results showing that certain element concentrations decreased over the exposure period. The Walvis Bay Harbour resident mussel element concentrations for As, Cr, Hg, Pb and Zn were lower than the concentrations found in *M. galloprovincialis* from Port Melbourne in Australia (Shen *et al*., 2020), except for Cd which was higher in Walvis Bay Harbour. The concentrations for Cu and Se were similar for both Walvis Bay Harbour and Port Melbourne (Shen *et al*., 2020). They explained that the reason for the element concentrations being high in Port Melbourne is due to industrialization and urbanization located in proximity of the Yarra River estuary (Shen *et al*., 2020), and this could be the reason as to why this study's concentrations were also high as the Walvis Bay site was in the harbour which is surrounded by urban settlements.

2.4.3 Transplantation and bioaccumulation

The results of this study showed that Swakopmund's resident mussel element concentrations were higher than the transplanted mussel's from the same site, while for Walvis Bay, the resident mussels element concentrations are mostly lower than the transplanted mussels. According to Greenfield *et al*. (2014), transplanted organisms react more efficiently to being exposed to conditions at the study sites, as the resident organisms may have adapted to the local conditions. Due to the resident organisms' adaptations, they develop a tolerance to contaminants in their environment, thus using the resident mussels as indicator organisms will most likely not provide accurate monitoring results (Greenfield *et al*., 2014). Transplantation studies also make it possible to compare the uptake patterns of the same species at different study areas as there is certainty that the same organisms can be found at multiple sites (Giarratano *et al*., 2010).

It is important to note that tidal influences can impact the element concentrations of the mussels, as timely intervals of submergence can impact the bioavailability of elements present in the water column (Mubiana *et al*., 2006). The results showed that Swakopmund's resident mussels were more similar to the mussels from the reference site. This can be due to the similarities of the tidal influences that both these sites experienced. The Swakopmund resident mussels were located on the rocks that were not consistently submerged, whereas the Walvis Bay resident mussels were continuously submerged. There is quite contradictory information regarding the impact of tidal influences on mussels' element accumulation tendencies or potential. According to Mubiana *et al*. (2006), shore height may possibly be a main contributing factor to high levels of elements in mussels, although Lin *et al*. (2020) stated that organism size, seasonal variations and inter-species differences play a larger role in determining element accumulation (Lobel *et al*., 1990; Mubiana *et al*., 2006; Weng and Wang, 2014; Tang

et al., 2017). It has been reported that mussels collected from the upper-shore had higher levels of Cd, Pb and Zn (Phillips, 1976; Lobel and Wright 1982; Lin *et al*., 2020). To date, tidal height is not considered when conducting intertidal sampling as it is not presumed to influence or change the bioaccumulation of the organisms (Lin *et al*., 2020). Although not grasping the effects tides possibly have on the intertidal ecosystem could lead to the misinterpretation of biomonitoring data (Lin *et al*., 2020).

Notable was that the Hg levels in mussels from the reference site were higher than the resident mussel levels at the two exposure sites. However, following transplantation the Hg levels decreased to below the resident mussel and the original source site. The source of the Hg is from the upwelling along the nearshore that leads the production of methyl mercury (MeHg) which is released into the water column. This phenomenon is a common cause of increased metal levels in the nearshore marine environment (Erasmus *et al*., 2018; Wepener and Degger, 2020). The high levels of Hg from the reference site may be attributed to methylated Hg being adsorbed to phytoplankton (Lindqvist *et al*., 1991; Watras and Bloom, 1992; Mason *et al*., 1996; Chen *et al*., 2008) and then being biomagnified by the mussels that are higher in the foodweb (Boening, 2000; Erasmus *et al*., 2018; Al-Sulaiti *et al*., 2022). An explanation for the decrease in Hg of the transplanted mussels in Walvis Bay Harbour and Swakopmund could be due to the decreased Hg exposure at these sites during the transplantation exposure period and therefore the depuration of Hg over time (Eugene *et al*., 2013).

The transplanted mussels element concentrations decreased at the study sites. The reason for this may be due to a combination of the mussels regulating their element levels, as well as not being exposed to the same level of elements as at the reference site. The high concentration of U in the resident mussels at Swakopmund could be attributed to the large-scale uranium mining that takes place along the banks of the Swakop River. It is noteworthy that the Swakop River flowed into the ocean for the first time in 10 years just prior to the sampling event. The boating activity of large shipping vessels may contribute to the elevated levels of Cd, Cu and Zn present in the environment (Fatoki *et al*., 2012) and this could possibly be the reason for elevated Zn and Cu levels in the mussels for this study.

2.4.4 Artificial mussels and bioaccumulation monitoring

The AMs element concentrations were consistently lower than the transplanted and resident mussels' concentrations from both sites. These results are supported by the findings of many other studies (Degger *et al*., 2011a; Gonzalez-Rey *et al*., 2011; Leung *et al*., 2008; Wu *et al*., 2007). According to Shen *et al*. (2020) AM concentrations for Hg and Cr were higher than what was detected in the *M. galloprovincialis* in both winter and summer at from Port Melbourne in

Australia. The element concentrations (As, Cr, Cu, Hg, Pb, Se and Zn) for the AMs from Port Melbourne are much higher than the AM concentrations for this study (Shen *et al*., 2020).

The reason for the lower element concentrations in the AMs could be due to the AMs only accumulating bioavailable free element ions in the water column. This contrasts with mussels taking up both dissolved element ions and particle bound elements from filter feeding (Krishnakumar *et al*., 2018). The results of this study therefore indicate that dissolved metals are relatively low at the two Namibian sites when compared to other more element polluted sites.

2.5 Conclusions

This study showed that element concentrations in *C. meridionalis* have increased over the last decade. This was expected as the anthropogenic activities have increased, specifically the shipping activities in the harbour. The resident mussels' element concentrations were lower when compared to other larger harbours around the world. Some of the high elements concentrations could possibly be attributed to natural phenomena such as upwelling while others are possibly due to anthropogenic activities such as shipping. It is evident that the mussels regulate their element levels as many of the transplanted mussel's element levels decreased over the fixed deployment period. The AMs cannot replace bioindicators as bioaccumulation monitors completely as they do not consider the uptake of particle bound elements or the biological regulation processes that the mussels have, and as a result accumulate much lower concentrations of elements. Thus, AMs must be used in conjunction with transplanted and resident mussels to be able to provide an accurate depiction of the marine environment's condition.

Chapter 3: Application of various biomonitoring tools to monitor organochlorine pesticides and polychlorinate biphenyls concentrations from research sites along the Namibian coast.

3.1 Introduction into the use of SPMD's to monitor organic compound concentrations and comparing it with resident and transplanted mussels

South Africa is the largest importer of pesticides in Africa, with an estimated value of $3.4 million between 2008 and 2017 (Tolera, 2021). This results in the continuous introduction of pesticides into rivers and ultimately the marine environment through diffusion and point-source pollution (Vogt *et al*., 2019; Wepener and Degger, 2019; Erasmus *et al*., 2020). The Orange River drains vast areas of South Africa and also forms a 400 km natural border with Namibia. As the Orange River drains into the Atlantic Ocean, contaminants enter the Atlantic Ocean at Oranjemund and are then transported by the Benguela current up along the Namibia coastline to Cape Cross (Vetter *et al*., 1999).

Furthermore, anthropogenic activities on the coast can introduce contaminants, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) into the marine environment, which can negatively affect the aquatic biota that inhabit these systems (Fey *et al*., 2019). The marine environment is also exposed to pesticide residues from various other sources such as atmospheric deposition, agricultural runoff and sewage waste discharge (Ojemaye *et al*., 2020). Secondary sources of contaminants include weathering of soils that have been exposed to OCPs and reach the ocean due to stormwater runoff. These contaminants that enter the marine ecosystem settle in the sediments and accumulate in both the sediments and marine organisms (Girones *et al*., 2020; Ojemaye *et al*., 2020).

Many OCPs that are released into the marine environment are classified as persistent organic pollutants (POPs) (Jayaraj *et al*., 2016). These contaminants do not degrade easily and are extremely volatile and quite persistent (Jayaraj *et al*., 2016; Olisah *et al*., 2020). They are characterised by their intended use as biocides, fungicides, bactericides, insecticides, and herbicides. Herbicides are the most commonly used of all different types of pesticides (Ojemaye *et al*., 2020; Olisah *et al*., 2020). Examples of insecticides are aldrin, endrin, chlordane, DDT, heptachlor, etc. (Olisah *et al*., 2020). Many of these POPs have been banned globally under the Stockholm Convention (Bodin *et al*., 2011). Notwithstanding the ban, there is still wide-scale use of many of these compounds in Africa (Sadasivaiah *et al*., 2007) and

many are still detected in marine environments (Følsvik *et al*., 2002; Karacik *et al*., 2013; Marrucci *et al*., 2013).

Polychlorinated biphenyls are also characterised as POPs and can be grouped into three classes of toxic polyhalogenated aromatic hydrocarbons (Barone *et al*., 2021). These compounds are known for their high chemical and metabolic persistence (Barone *et al*., 2021). Polychlorinated biphenyls enter the environment through vehicle emissions, incineration, coal energy plants, large forest fires and oil or petroleum spills (Cloutier *et al*., 2017). They also form part of dielectric fluid that functions as a fire resistant liquid found in transformers as well as capacitors (Cloutier *et al*., 2017).

According to Phillips (1980; 1995) and Phillips and Rainbow (1990), organochlorines tend to have a highly hydrophobic nature and prefer to attach to suspended particulates in aqueous solutions/systems, or with the lipids within the biota, and herbicides are lipophilic (Ying and Williams, 2000; Wyss *et al.,* 2006; Jurado *et al.,* 2011; Marin-Morales *et al.,* 2013; Yu *et al.* 2016). Therefore, mussels are often used as indicator of contamination in marine environments as they filter feed large volumes of water and particulate matter, absorbing the dissolved contaminants in their tissues. Mussels are capable of metabolising OCPs as they have low enzyme activity (León *et al*., 2013). These compound concentrations are dependent on the quantity of dissolved fractions in the water column, food particulates and the organisms excretion and regulation processes (León *et al*., 2013). Mussels are used as bioindicators because they are sedentary, plentiful filter feeders (Catharino *et al*., 2011; da Costa Filho *et al*., 2022). They also have the ability to bioaccumulate chemicals of interest (da Costa Filho *et al*., 2022).

The 4 R's (reduce, refine, replace, rehabilitation) concept has been implemented in monitoring studies to make them more ethically sound (Mandal and Parija, 2013). Alternative methods such as passive samplers are used to provide concentrations of contaminants (e.g. OCPs, PCBs, PAHs) in the environment (Schintu *et al*., 2014). Semi-permeable membrane devices (SPMD) are passive devices designed specifically to monitor lipophilic organic pollutants such as OCPs in marine environments (Richardson *et al*., 2001). A SPMD mimics the uptake patterns of a bioindicator organism (Schintu *et al*., 2014). The advantage of using SPMDs instead of mussels is because it removes biogenic hydrocarbons and lipids that interfere with the analysis and discount physical factors (variabilities in sex and age, biogenic hydrocarbon content etc.) that can alter the outcome of the results (Richardson *et al*., 2001). Numerous studies have been conducted using SPMDs to determine various compounds (OCPs, PCBs, PAHs) (e.g. Prest *et al*., 1995; Richardson *et al*. 2001; Degger *et al*., 2011b; Marrucci *et al*., 2013; Chang *et al*., 2015; Okay *et al*., 2017; Chiu *et al*., 2018). Prest *et al*. (1995) conducted

a study in Victoria, Australia, using SPMDs and mussels (*Mytilus edulis*) to determine both OCP and PCB concentrations at several sites. Another international study applied SPMDs to determine the levels of PAHs in coastal areas of China (Chiu *et al*., 2018). The potential of using passive monitoring tools, such as SPMDs to monitor OCPs, PAHs and PCBs along the South African coastline has been demonstrated by Degger *et al*. (2011b). Furthermore, as mentioned previously, the application of SPMDs as monitoring tools have been implemented in the United States of America, United Kingdom, and Czech Republic (Schintu *et al*., 2014).

The aims of this chapter were therefore to:

i) Conduct field studies comparing OCP and PCB concentrations in SPMDs and transplanted black mussels (*C. meridionalis*) from two research sites along the Namibian coast over a 6-week period.

ii) Compare the concentrations of OCP and PCB compounds in resident black mussels with the concentrations in transplanted black mussels at the two research sites.

3.2 Materials and methods

3.2.1 Site selection

As the deployment of both the transplanted mussels, AMs and SPMDs was carried out simultaneously, the same study sites described in the previous chapter (Chapter 2) were used.

3.2.2 Preparation of semi-permable membrane devices (SPMDs)

The SPMD (Figure 3.1) was developed by Huckins *et al*. (1990). The preparation of the SPMDs started by first measuring 106-110 cm of the polyethylene lay-flat tubing (5 cm wide and 0.05 cm thick) and then rinsing the tubing with 70% ethanol to ensure that it was clean. Once the tubing dried, one end of the tubing was heat sealed and 3 mL glyceryl trioleate (65%, Sigma) was pipetted into the lay-flat tubing and heat sealed at the other end. The SPMD was wrapped in aluminium foil and kept in a fridge until deployment. A blank SPMD sample was prepared prior to exposure to the air to detect any background contamination while working in the field and in the laboratory (Petty *et al*., 2000; Degger *et al*., 2011b). The SPMDs were woven through the steel prongs of the SPMD canister to make sure the tubing did not touch itself, thus optimising the surface area that was exposed to seawater (Richardson *et al*., 2002).

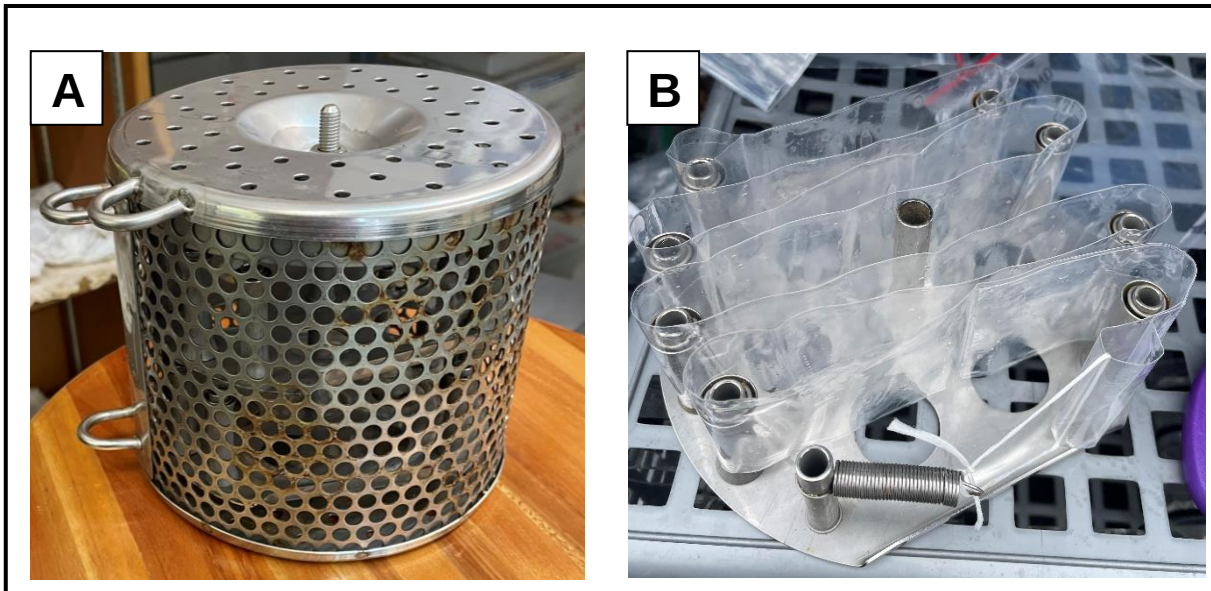

Figure 3.1: A - Semi-permeable membrane device stainless steel cannister. B – The polyethylene lay-flat tubing woven between the steel prongs of the semi-permeable membrane device.

3.2.3 Deployment and retrieval protocol

At each study site, 10 transplanted mussels (mussels from a reference site, Mile 17, see Chapter 2) with a shell length of approximately 4 to 5 cm were placed in plastic cages (35 x 26 x 20 cm, mesh size of 1.5 cm^2) and secured to structures at a depth of 2 m. Along with the transplanted mussels, two SPMD steel canisters containing two SPMDs membranes were deployed (Figure 3.2). The four SPMDs together with 10 transplanted mussels were retrieved from the baskets and canisters from each site after six weeks for analysis. At the end of the six week exposure period, 10 resident mussels were collected for comparison with the transplanted mussels. As stated in Chapter 2, both the transplanted and resident mussels shell lengths were measured in the field after retrieval from the deployment site. The tissue of each mussel was dissected out from the shell with a pre-cleaned scalpel and placed in aluminium foil and then in a falcon tube (approximately 3 g wet weight) and stored at -20°C for further analyses. The SPMDs were collected and individually wrapped in aluminium foil before being stored at -20°C for further analyses (Degger *et al*., 2011b). When deployed and retrieved the SPMDs field blanks were exposed to the air for background concentration correction (Schintu *et al*., 2014).

Figure 3.2: The deployment basket containing the artificial and transplanted mussels, as well as the semi-permeable membrane device attached to the side of the basket.

3.2.4 Biological tissue sample preparation for analyses

In the laboratory, the samples were defrosted and weighed to determine the wet weight of each sample, thereafter the samples were placed in a -80°C freezer for 24 hr, then transferred to a freeze drier (FreeZone 6, Labconco) for ±72 hr. The freeze-dried samples were again weighed to determine the dry weight. The samples were then homogenised before weighing approximately 0.5 g of the samples and placing it in 15 mL falcon tubes. At the same time approximately 0.2 g of rat liver was also weighed for quality control purposes (QC), 500 µL distilled water (DW) was used as a blank sample. All of the falcon tubes, with the exception of the DW, were then spiked with PCB (Table 3.3), PAH, OCP (Table 3.4) standard surrogates and internal standards (Table 3.1) (the PAH standards were added for future analysis and not for this study) and vortexed. The concentrations of the PCB, PAH, OCP standards are indicated in Table 3.2. A volume of 500 µL 0.1% formic acid (99%, Fujifilm Wako) in acetonitrile (99.9%, Fujifilm Wako) was added to the biological and quality control samples and thoroughly mixed before vortexed for 1 min. All samples were left to stand 1 – 2 hr after being spiked.

After 2 hr, 5 mL of 0.1% formic acid in acetonitrile was added to all the falcon tubes. A volume of 500 µL sodium acetate (Fujifilm Wako) was added to only the blanks and DW samples. This was done to separate the water from the solvent. All the samples were vortexed for 1 min, then ultra-sonicated for 5 min. Subsequently, the samples were placed in a mechanical shaker at the highest speed for 10 min. The samples were then centrifuged for 5 min at 10,000 rpm at 5˚C. The supernatant was decanted into activated EMP-Lipid tubes (Agilent Bond Elut) (EMP-Lipid salts in 15 mL EMP-Lipid tubes were activated by adding 5 mL DW and shaken vigorously). For blanks and DW samples, only 5 mL of the supernatant was added to the EMP-Lipid tube. All samples were vigorously shaken and vortexed. The samples were then placed into a mechanical shaker at the highest speed for 10 min. After this, they were centrifuged for 5 min at 10,000 rpm at 5˚C. The samples' entire supernatant was then decanted into pre-prepared EMP-Polish tubes (the tubes were prepared by weighing 1.4 g $MgSO_4$ (Fujifilm Wako) and 0.6 g NaCl (Kanto Chemical Co., Inc.) into 15 mL tube). These samples were then vortexed for 1 min in an upright position. The samples were then centrifuged again for 5 min at 10,000 rpm at 5˚C. A volume of 1 mL from the uppermost supernatant was pipetted into the corresponding GC vial.

Table 3.1: The volume of the standard surrogates and internal standards in µL used to spike the blanks, distilled water, quality control and biological samples.

Sample	Weight	Volume spiked µL								
		OCP	TMX	PCB #77	PCB #209	PCNB	PAH STD	PAH IS	PCB STD	PCB IS
Blank	500 µL	X	50	50	50	25	50	25	-	25
DW	500 µL	25	50	50	50	25	50	25	50	25
QC	0.2 g	25	50	50	50	25	50	25	50	25
Samples	0.3-0.5 g	-	50	50	50	25	50	25	-	25

Table 3.2: Concentrations of the standard surrogates and internal standards in µg/L.

Chemicals	OCP	TMX	PCB #77	PCB #209	PCNB	PAH STD	PAH IS	PCB STD	PCB IS
Stock concentration (µg/L)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Final concentration (µg/L)	5	10	10	10	5	10	5	50	5

Table 3.3: Polychlorinated Biphenyls standards used during analysis.

PCB Analyses			
PCB 981 Mix		**PCB Internal standards**	
PCB 1	2-chlorobiphenyl	PCB 77	3,3',4,4'-Tetrachlorobiphenyl-C13
PCB 5	1,3- dichlorobiphenyl	PCB 118	2,3',4,4',5-Pentachlorobiphenyl-C13
PCB 18	2,2',5-trichlorobiphenyl	PCB 126	3,3',4,4',5-Pentachlorobiphenyl-C13
PCB 31	2,4',5-trichlorobiphenyl	PCB 156	2,3,3',4,4',5-Hexachlorobiphenyl-C13
PCB 44	2,2',3,5'-tetrachlorobiphenyl	PCB 169	2,3',4,4',5',6-Hexachlorobiphenyl-C13
PCB 52	2,2',5,5'-tetrachlorobiphenyl		
PCB 66	2,3',4,4'-tetrachlorobiphenyl		
PCB 87	2,2',3,4,5'-pentachlorobiphenyl		
PCB 101	2,2',3,5,5'-pentachlorobiphenyl		
PCB 110	2,3,3',4',5-pentachlorobiphenyl		
PCB 138	2,2',3,4,4',5'-hexachlorobiphenyl		
PCB 141	2,2',3,4,5,5'-hexachlorobiphenyl		
PCB 151	2,2',3,5,5',6-hexachlorobiphenyl		
PCB 153	2,2',4,4',5,5'-hexachlorobiphenyl		
PCB 170	2,2',3,3',4,4',5-heptachlorobiphenyl		
PCB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl		
PCB 183	2,2',3,4,4'5',6-heptachlorobiphenyl		
PCB 187	2,2',3,4',5,5',6-heptachlorobiphenyl		
PCB 206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl		

Table 3.4: Organochlorine pesticide standards used during analysis.

OCP Analyses			
OCP Mix		**OCP Internal standards**	
α-HCH	α-Hexachlorocyclohexane	PCB 77	3,3',4,4'-Tetrachlorobiphenyl
β-HCH	β -Hexachlorocyclohexane	PCB 209	2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl
γ-HCH	γ -Hexachlorocyclohexane	PCNB/ Quintozene	Pentachloronitrobenzene
δ-HCH	δ -Hexachlorocyclohexane	TMX	
o,p'-DDD	1-chloro-4-[2,2-dichloro-1-(2-chlorophenyl)ethyl]benzene		
p,p'-DDD	p,p'-Dichlorodiphenyl dichloroethane		
HCB	Hexachlorobenzene		
Heptachlor			
trans-Heptachlor Epoxide			
cis-Heptachlor Epoxide			
Aldrin			
Dieldrin			
Endrin			
o,p'-DDE	1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene		
p,p'-DDE	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene		
o,p'-DDT	o, p' -dichlorodiphenyltrichloroethane		
p,p'-DDT	*p, p'* -dichlorodiphenyltrichloroethane		
Oxy-CHL	Oxy-chlordane		
trans-CHL	*trans*-Chlordane		
cis-CHL	*cis*-Chlordane		
trans-Nonachlor			
cis-Nonachlor			

3.2.5 Semi-permeable membrane device analysis

Prior to analysis the samples were removed from the freezer to defrost. The SPMDs were removed from the aluminium foil and gently cleaned using Milli-Q water. Following cleaning, the SPMDs were cut into smaller segments and placed in a 100 mL glass beaker with a total volume of 50 mL 0.1% formic acid (99%, Fujifilm Wako) in acetonitrile (99.9%, Fujifilm Wako). The sample extracts were spiked with 500 µL of 1 mg/L internal standard master mixture (TMX, PCB 209, PCNB), vortexed for 1 min and allowed to stand overnight to equilibrate in the fridge. The quality control samples consisted of DW (3 mL) spiked with 500 µL internal

standard with a concentration of 1 ppm, while the blank samples were not spiked and consisted only of DW. All the sample extracts were placed in an ultrasonic bath at ambient temperature (20˚C) for two hours to extract. A volume of 5 mL sample extract was taken and added to the activated EMR-lipid tube. The supernatant samples were then immediately vortexed for 1 min and placed in a mechanical shaker for 5 min. Subsequently, the samples were centrifuged at 10,000 rpm for 5 min. The supernatant was pipetted into a 15 mL falcon tube with 1.4 g $MgSO_4$ (Fujifilm Wako) and 0.6 g NaCl (Kanto Chemical Co., Inc.) and then vortexed for 1 min. Afterwards the samples were centrifuged at 10,000 rpm for 5 min. Lastly, 1 mL of the supernatant of the samples was pipetted into GC vials.

3.2.6 Instrument analyses

The OCP determination was performed with the aid of a GC-20230 Plus (Shimadzu) coupled with a GCMS-TQ8050NX triple quadrupole mass spectrometer (Shimadzu). The chromatographic separation was carried out using a Rxi-5Sil MS capillary column (30 m x 0.25 mm i.d., 0.25 µm df, Restek, Bellefonte, PA, USA).

OCPs analysis

The operating condition was splitless injection mode with an injection volume of 1 µl. Helium (≥99.999%) was used as the carrier gas with a linear velocity in flow control mode, 100.1 Kpa pressure, 30.0 mL/min total flow rate, 1.69 ml/min column flow, 47.2 cm/s linear velocity, 5 mL/min purge flow. The oven temperature program started at 50˚C (held for 1 min) and increased to 160˚C at 25˚C/min, and finally ramped to 280˚C at 5˚C/min, where it was held for 5 min. The total program duration was 25 min. The mass spectrometer system was operated in the electron ionsation (EI) mode at 70 eV. The ion source and interface temperatures were 230 and 250˚C, respectively. The regression line was determined using the internal standard method where the ratio of each analyte signal is taken to the internal standard signal and plotted against the analyte concentrations in the calibration solutions to obtain a regression line with a correlation factor of (r^2 = 0.9810) before analysing the samples.

PCBs analysis

The operating condition was splitless injection mode with injection volume of 1 µL at injector temperature of 270 °C. Helium (≥99.999%) was used as the carrier gas with linear velocity in flow control mode, 41.1 KPa pressure, 30.0 mL/min total flow rate, 0.75 mL/min column flow, 31.8 cm/s linear velocity, 5 mL/min purge flow. The oven temperature program started at 75°C (held for 2 min), increased to 180°C at 15 °C/min, and finally ramped to 300 °C at 4°C/min, where it was held for 6 min. The total program duration was 45 min. The mass spectrometer

system was operated in the electron ionisation (EI) mode at 70 eV. The ion source and interface temperatures were 230 and 290°C, respectively.

3.2.7 Quality control

The SPMD blanks exposed to air during sample deployment, the retrieval phase and during the preparation of the samples represented the airborne contaminants present. A similar analytical method was used in previous studies by Richardson *et al*. (2003) and Degger *et al*. (2011b). The reference material in the form of freeze-dried rat liver tissues was analysed, yielding recovery percentages of 33 - 93% for the 22 OCPs (Annexure Table E) and for the 25 PCBs (Annexure Table F).Only six OCPs were detected and no PCBs in the SPMDs and mussels (Table 3.5).

Table 3.5: Recovery rates (%), limit of detection (LOD) and limit of quantification (LOQ) of all of the different OCP compounds of interest that were detected.

Compound	Mussels			SPMDs		
	LOD	LOQ	Recovery (%)	LOD	LOQ	Recovery (%)
Aldrin	0.11	0.32	33.75	1.37	4.10	ND
Endrin	0.23	0.69	93.86	0.33	0.98	ND
beta-BHC	0.11	0.33	70.69	0.89	2.66	ND
gamma-BHC	0.19	0.56	58.32	1.81	5.42	ND
Oxy-Chlordane	0.16	0.47	56.42	1.05	3.15	ND
p,p'-DDD	1.78	5.34	75.53	5.49	16.48	ND
p,p'-DDE	0.77	2.31	51.68	5.98	17.93	71

ND, not detected

3.2.8 Statistical analyses

A range of uni- and multivariate statistical methods were used to compare the concentrations of the OCP compounds from the different biomonitoring tools (SPMD and mussels). All of the sample values that were below the limit of detection were changed to the limit of detection divided by two to make it possible to do statistical analysis (Kushner, 1976; Labuschange *et al*., 2020). The data was y=ln(2x+1) transformed before undertaking a two-way analysis of variance (ANOVA). The ANOVA was followed by Tukey's post-hoc and Šídák's multiple comparisons tests to test for significant differences in OCP compound concentrations between the SPMDs, transplanted mussels and resident mussels of the two study sites after the 6-week deployment period. The alpha was set at ($p < 0.05$) using GraphPad Prism version 9.

Results

The samples from this study were analysed for a total of 22 OCP and 25 PCB compounds. Only six of the OCP compounds (aldrin, endrin, p,p' DDD, p,p' DDE, total HCH and oxy-Chlordane) and none of the PCBs were detected in mussels (transplanted and resident) and SPMDs as they were under the LOD.

3.2.9 Spatial differences between transplanted mussels

Swakopmund's transplanted mussel's concentrations of p,p' DDD (Figure 3.3C) and total HCH (Figure 3.3E) were significantly ($p<0.05$) higher than the concentrations of Walvis Bay's transplanted mussels. The concentrations for Aldrin (Figure 3.3A) and p,p' DDE (Figure 3.3D) from Swakopmund's transplanted mussels were significantly ($p<0.05$) lower than Walvis Bay's. Walvis Bay's resident mussel's concentrations of oxy-Chlordane (Figure 3.3F) were significantly ($p<0.05$) higher than the resident mussels from Swakopmund. Only p,p' DDE in the SPMDs from Walvis Bay were significantly ($p< 0.05$) higher than in the SPMDs from Swakopmund (Figure 3.3D). None of the analysed PCBs were detected in either the transplanted or resident mussels from both study sites (Annexure Table H).

Swakopmund

Only Aldrin (Figure 3.3A), p,p' DDD (Figure 3.3C) and oxy-Chlordane (Figure 3.3F) were significantly ($p<0.05$) higher in the transplanted mussels than the resident mussels and the SPMDs. Most of the compounds were not detected in resident mussels except for Endrin (Figure 3.3B) and total HCH (Figure 3.3E). The total HCH concentration of resident mussels was significantly ($p<0.05$) higher than in transplanted mussels.

Walvis Bay

All the compound concentrations with the exception of Endrin (Figure 3.3B) and total HCH (Figure 3.3E) in the transplanted mussels were higher than in the resident mussels. Only Aldrin (Figure 3.3A); p,p' DDD (Figure 3.3C) and p,p' DDE (Figure 3.3D) were significantly ($p<0.05$) higher in the transplanted mussels than in resident mussels. The total HCH (Figure 3.3E) concentration in the resident mussels was significantly ($p<0.05$) higher than the transplanted mussels. Both Aldrin (Figure 3.3A), p,p' DDD (Figure 3.3C) and oxy-Chlordane (Figure 3.3F) were not detected in the SPMDs, while p,p' DDE (Figure 3.3D) concentrations in the SPMDs were significantly ($p<0.05$) higher than in the resident mussels. Only Endrin (Figure 3.3B) and total HCH (Figure 3.3E) were detected in resident mussels.

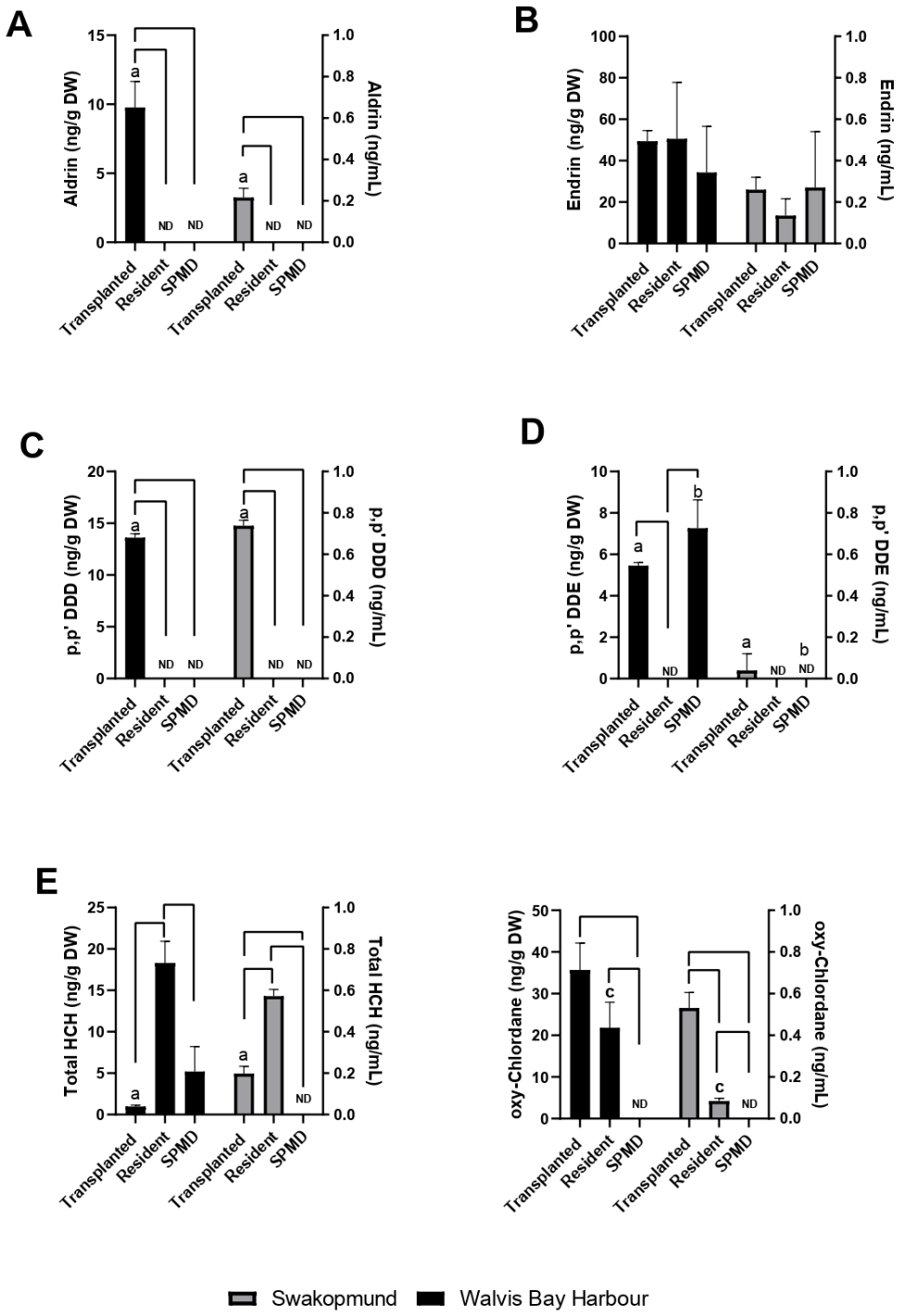

Figure 3.3: Spatial comparison of concentrations of Aldrin (A), Endrin (B), p,p' DDD (C), p,p' DDE (D), Total HCH (E) and oxy-Chlordane (F) (mean ± s.e.m) between transplanted mussels, resident mussels and the SPMDs for both Swakopmund and Walvis Bay Harbour. The mussels concentrations are in ng/g dry weight (Y1 axis) and the SPMDs in ng/mL lipids (Y2 axis).

Table 3.6: Two-way Analysis of Variance table comparing OCP concentrations of three different bioaccumulation indicators between two sites at week six. Asterisks (*) indicate interactions that are not significant.

Compounds	Bioaccumulation indicators (df=2)	Site (df=1)	Bioaccumulation indicators and site interaction (df=2)
Aldrin	F= 198.8; p< 0.0001	F= 0.7316; p=0.3979*	F= 10.07; p= 0.0003
Endrin	F= 4.785; p= 0.0178	F= 7.656; p= 0.0089	F= 8.526; p= 0.0009
p,p' DDD	F= 24003; p< 0.0001	F= 4.417; p= 0.0463	F= 0.3101; p= 0.7363*
p,p' DDE	F= 5.562; p= 0.0095	F= 21.75; p< 0.0001	F= 5.978; p= 0.0071
Total HCH	F= 45.18; p< 0.0001*	F= 22.36; p= 0.0002	F= 0.1390; p= 0.7139*
Oxy-Chlordane	F= 175.0; p< 0.0001	F= 4.839; p= 0.0328	F= 2.805; p= 0.0706*

3.2.10 Interaction between sampling sites and bioaccumulation indicators

The interaction between the sampling sites and the bioaccumulation indicators (Table 3.6) showed that for all compounds, except p,p' DDD, total HCH and oxy-Chlordane, there were significant ($p<0.05$) interactions in compound concentrations between the different bioaccumulation indicators (transplanted- and resident mussels and semipermeable membrane devices) and sampling sites. There were significant interactions ($p < 0.05$) for all compounds, except for Aldrin, when comparing the different sites (Table 3.6).

3.3 Discussion

Three different monitoring devices (SPMDs, transplanted and resident mussels) were used in this study to determine the OCP and PCB concentrations at two sites on the Namibian coast. The residues of these pesticides have been detected in food worldwide (Tesar, 2000). Vetter *et al*. (1999) stated that inland contaminants are possibly transported by the Orange River into the Atlantic Ocean. Contaminants that are released into the environment by various sources such as industries and agriculture can persist over years and be transported by ocean currents far away and even to remote areas (Strehse and Maser, 2020). These contaminants are then swept into the Benguela current and transported northwards along the Namibian coastline (Vetter *et al*., 1999). It can be assumed that different regions of the coastline will have different uptake patterns of OCP compounds, since their contaminant inputs (atmospheric transportation, localised sources) differ for each country (Teunen *et al*., 2021). Two factors (gametogenic cycle and lipid content) contribute to seasonal changes in OCP concentrations in bivalves (Firth *et al*., 2019a). The origin of OCPs in the ocean could be stormwater runoff or inefficiently treated waste water as there is no continuous input from a large river in the proximity, the same was observed at Saldanha Bay (Firth *et al*., 2019a).

3.3.1 Spatial and temporal analyses of semi-permeable membrane devices

Most of the results for the OCPs were not quantifiable for the SPMDs, this is attributed to the SPMDs measuring only the dissolved fraction of the compounds that were present in the water column, however, they are more prone to bind to the particle matter (Schintu *et al*., 2014). The SPMDs from Walvis Bay detected three compounds (Endrin, p,p' DDE and total HCH), while in the SPMDs from Swakopmund, only Endrin was detected. Levels of total HCHs from the Namibian sites were also lower than those detected in SPMDs from South Africa's Durban Harbour (Coetzee, 2015). There are limited studies on the use of SPMDs in southern Africa and no studies have been conducted in Namibia, making comparisons difficult. The OCP results of this study, compared to unpublished data by Coetzee (2015) on the east coast of South Africa, indicated that the p,p'DDE concentrations in Namibia's SPMD were lower than the concentrations in the SPMDs deployed in Durban Harbour (Annexure Table C). More than 95% of OCPs present in mussels can depurate in 10 days, with the exception of p,p'DDT (Richardson *et al*., 2008) and this is likely the reason for the findings of this study.

Some of the first studies that used SPMDs to determine organic compound concentrations were in 1995 in Australia with PCBs that ranged between 64–142 ng/g lipid (Prest *et al*., 1995) and in 2001 in Hong Kong ranging between 432–1844 ng/g lipids (Richardson *et al*., 2001). These devices successfully detected PCB concentrations in South African harbours (Degger *et al*., 2011b; Coetzee, 2015), however, and none were detected in Namibian sites (Annexure

Table D). Degger *et al*. (2011b) conducted a study using SPMDs to determine PCB and PAH concentrations between several harbours in South Africa. Another study was carried out in South Africa using SPMDs to determine OCP, PCB and PAH concentrations in two harbours (Coetzee, 2015). In Richards Bay Harbour, the total PCB concentrations ranged between 38 ng/g lipid (Coetzee, 2015) and 158 ng/g lipid (Degger *et al*., 2011b). The mussels from this site indicated the same decrease from 113.8 ng/g lipid in 2011 to 6.06 ng/g lipid in 2015.

3.3.2 Spatial and temporal analyses of transplanted and resident mussels

Of the six compounds that were detected, all were present in the transplanted mussels from both sites (Annexure Table G). The resident mussels from both study sites contained only three compounds, namely: endrin, total HCH and oxy-chlordane. There is limited information on the POPs concentrations along the Namibian coastline, as only a single study was conducted on OCP concentrations in seal blubber (Vetter *et al*., 1999). Many international transplantation studies have been conducted such as Young *et al*. (1974) who used *Mytilus californianus* as a bioindicator for DDT and PCBs while conducting a transplantation study in the Southern California Bight. Another study was conducted in the Baltic Sea to also determine DDT and PCBs by using *Mytilus edulis* (Lee *et al*., 1996).

Aldrin was only detected in the transplanted mussels from both study sites, suggesting potential adaptation by the resident mussels to regulate its concentrations. The aldrin concentrations of the transplanted mussels from Walvis Bay Harbour are similar to the aldrin concentrations from harbours in Hong Kong (Annexure Table C) (Richardson *et al*., 2001). Aldrin can have harmful effects on birds, fish and humans. Studies show that the average daily intake of aldrin in India is approximately 19 µg per person. Due to aldrin having hazardous characteristics, it has been banned in numerous countries (Tesar, 2000).

Both the transplanted and resident mussels from Walvis Bay Harbour had higher concentrations of endrin than the transplanted and resident mussels from Swakopmund. The two research sites depicted the same uptake pattern. Endrin is an insecticide that is used to suppress rodents - such as mice and voles and is sprayed on the leaves of grains and cotton (Tesar, 2000). This insecticide does not accumulate in animal fatty tissue to the same degree as structurally comparable compounds, since they are able to metabolise endrin (Tesar, 2000). It has a long half-life as it can persist in the soil for up to 12 years. Endrin is extremely toxic to fish. The general human population is exposed to endrin primarily through their diet (Tesar, 2000).

Both p,p'DDD and p,p'DDE were detected in the transplanted mussels. The concentrations of p,p'DDE in the SPMDs retrieved from Walvis Bay Harbour were higher than the concentrations

in the transplanted mussels from both sites. Comparing the results of this study to the OCP concentrations detected in previous studies indicates that the p,p'DDD levels in both the Walvis Bay Harbour and the resident mussels of Swakopmund are higher than what has been detected in Saldanha Bay, South Africa (0.7 ng/g dry weight) (Firth *et al*., 2019a). Levels of p,p'DDD (59.4 ng/g dry weight) and p,p'DDE (53.5 ng/g dry weight) in *M. edulis* collected from China (Fung *et al*., 2004) are much higher than in resident mussels from this study.

One of the most commonly used OCPs before 1972 was DDT, which was mainly used to control malaria and typhus outbreaks in various countries (Jayaraj *et al*., 2016). All OCP compounds undergo biotransformation processes, e.g. p,p' DDT can be transformed into p,p'DDD and p,p'DDE (Richardson *et al*., 2008; Skarphedinsdottir *et al*., 2010). The transformation of DDT into DDD occurs when there is an increase in the water depth and a decrease in the oxygen concentration in the environment (Yuan *et al*., 2013; Ahmed *et al*., 2015). Aerobic degradation of DDT produces DDE which is considered less toxic but more persistent (Zhang *et al*., 2003). Higher concentrations of the metabolite DDE can be expected to occur in higher trophic level organisms because of metabolic transformation (Fisk *et al*., 1998; Skarphedinsdottir *et al*., 2010). It is worth noting that DDE is an intermediate metabolite meaning that it can be further transformed into DDD (Lugo-Ibarra *et al*. 2011; Ahmed *et al*., 2015).

As p,p' DDT was not detected in this study but rather its metabolites: p,p' DDD and p,p' DDE, it indicates that there has not been a recent input of DDT into the marine environment of the study sites (Ding and Wu, 1995; Ramu *et al*., 2006; Richardson *et al*., 2008; Ahmed *et al*., 2015). This compound was one of 12 POPs banned by the Stockholm Convention due to the risks it poses to the environment and human health (Tesar, 2000; Batterman *et al*., 2008; Sibali, 2008; Sericano *et al*., 2014; Stockholm Convention, 2014; Firth *et al*., 2019a). Although DDT has been banned in various countries, it is still used in Namibia by local health departments to control the spread of malaria (Sadasivaiah *et al*., 2007). South Africa has banned most other OCP compounds, but DDT is also still applied as part of the malaria vector control programme (Mehlhorn *et al*., 2023). Although the use of DDT was banned by the USA and other countries, large quantities of this product and other pesticides are still provided to African countries (Horak *et al*., 2021).

The two bioindicators (transplanted and resident mussels) had the same accumulation trend for oxy-chlordane at both research sites. Walvis Bay Harbour Oxy-chlordane concentrations for both the transplanted and resident mussels in Walvis Bay Harbour were higher than in Swakopmund mussels. Chlordane can be transported in the atmosphere over long distances. If chlordane enters the water column it will be absorbed onto suspended sediments as well as

settle on the ocean floor (ATSDR, 2018). Chlordane bioaccumulates in marine and freshwater species, with complex biomagnification patterns across trophic levels and species (Zaroogian *et al*., 1985; Kawano *et al*., 1988; ATSDR, 2018). Most of the chlordane enters through runoff from urban and agricultural soils. Chlordane is extensively used to control termites and as a broad-spectrum insecticide in agriculture (Tesar, 2000; ATSDR, 2018). It persists in soil with a reported half-life of one year. Its lethal impact on fish and birds varies by species, posing risks to invertebrates such as pink shrimp. It has been classified as a possible carcinogen and can affect a human's immune system. It has been restricted or banned in many countries due to its health risks.

The uptake patterns of total HCHs for both the transplanted and resident mussels were similar at the two research sites. Total HCH concentrations of the Swakopmund transplanted mussels were higher than those of the Walvis Bay Harbour's transplanted mussels, while with resident mussels it was the opposite, with Walvis Bay Harbour having the highest total HCH concentrations. There are four HCH isomers, namely: α-HCH, β-HCH, γ-HCH and δ-HCH, all of these compounds have been added by the Stockholm Convention to the list of POPs that need to be controlled (Ahmed *et al*., 2015). The isomer β-HCH is known to be persistent and causes chronic toxicity, whereas γ-HCH also known as lindane, causes neurotoxicity (Ahmed *et al*., 2015). Hexachlorobenzene is a by-product that is produced during the manufacture of other industrial chemicals and is used to exterminate crop fungi (wheat bunt) globally (Tesar, 2000). High levels of HCH can be deadly, while lower levels of HCH decrease the success rate of reproduction of organisms and can be considered an environmental hormone (Tesar, 2000; Yang *et al.,* 2006; Ahmed *et al*., 2015). Biomagnification of the HCH concentration takes place throughout the trophic levels in the aquatic ecosystem (Kim *et al*., 2009; Ahmed *et al*., 2015).

According to Ramu *et al*. (2006), environmental levels of pesticides such as HCH are generally lower than those of DDT and PCBs. Sites with more anthropogenic influences show higher levels of DDT. Due to the volatile nature of HCH, it easily undergoes atmospheric transportation into the environment (Ramu *et al*., 2006). The reason for low levels of compounds such as lindane is that it has a lower bioaccumulation factor and a decrease in the use of that compound globally (Solé *et al*., 1993). As POPs are more soluble in lipid rich organic fluids than in water and easily bind to solid particles such as soil and sediment, it can be assumed that if the marine environment has high levels of organic fluids and particles present, there will be a higher concentration of POPs present (El-Shahawi *et al*., 2010).

The reported effects of extraneous factors on the uptake of such compounds by aquatic organisms have been argued to be determined by the presence of lipids that define the accumulation of organochlorines in the biota (Phillips, 1980; Phillips and Rainbow, 1990).

3.4 Conclusions

This study reported on the current state of organic contamination in both Walvis Bay Harbour and Swakopmund. The SPMD results were lower than previous studies conducted in South Africa. The lower concentrations of SPMDs could be due to the SPMDs only accumulating the compounds present in the water column. The SPMDs and bio-indicators (transplanted and resident mussels) depicted similar uptake patterns of the OCP compounds. No PCB compounds were detected in mussels from Walvis Bay Harbour and Swakopmund, thus showing that the Namibian marine environment is less contaminated than the South African coasts. As no DDT was detected and only its metabolites (p,p'DDD and p,p'DDE), it is clear that there has been no new input of DDT into the environment. It is important to note that the SPMDs and bio-indicators such as mussels need to be used together to get a more comprehensive assessment of the environments condition. This is because SPMDs accumulate the dissolved fraction of organic compounds such as OCPs and PCBs from the water column, whereas mussels accumulate these compounds from the water column as well as the particle matter they filter feed on, thus representing the metabolised fraction of these compounds (Richardson *et al*., 2001; Schintu *et al*., 2014).

Chapter 4: Conclusions and recommendations

4.1 Summary of the overall findings of the study.

As the human population in the world grows, so do the amount of anthropogenic activities that contaminate the environment (Wang, 2002; Vellemu and Omoregie, 2014). Thus, it is important to continuously monitor the state of the environment to ensure that it is well maintained for future generations. Therefore, studies have been conducted worldwide to establish baselines for contaminants to be able to monitor changes in the status of an environment. There is a lack of information on the Namibian coastline, specifically the concentrations of contaminants that are present in the marine environment.

As the Namibian coast is one of the most important resources for the country and plays a very important role in the economy, it is of great concern that there is no ongoing monitoring being conducted along the coastline (Namport, 2023). There has been a Namibian national marine pollution contingency plan in existence since April 2017, but this plan mainly focuses on what to do in the event of an oil spill. Therefore, it does not include the monitoring of other contaminants that may originate from the increase in urban and industrial development along the Namibian coastline.

As mentioned previously, there are limited published research that focus on the topic of contaminants in the Namibian marine environment available. To date only four articles have been published on element concentrations in mussels (Dahms *et al*., 2014; Vellemu and Omoregie, 2014; Omoregie *et al*., 2019; Nekhoroshkov *et al*., 2021) and only one on the organic contaminants in seal blubber (Vetter *et al*., 1999). Most of these studies were conducted almost a decade ago (Dahms *et al*., 2014; Vellemu and Omoregie, 2014; Omoregie *et al*., 2019), highlighting the importance of the present study and how it would be beneficial to determine the threats of metal elements and organic pollution to the Namibian coastline.

Therefore, this study was conducted to assess the application value of passive sampling devices as an alternative method to determine the organic (OCP and PCB) and inorganic elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, U and Zn) concentrations at two sites on the Namibian coastline. In Chapter 2, temporal and spatial element uptake patterns between the two research sites at Walvis Bay and Swakopmund, respectively, were assessed using three different indicator methods (artificial, transplanted and resident mussels). In addition a third site (Mile 17) was selected as reference site. There was a clear differentiation between the three sampling locations. The element concentrations for the three biomonitors were completely different to the element concentrations of Swakopmund and the reference site. The resident mussels collected at Swakopmund had element concentrations similar to

those of the control mussels collected at Mile 17. The results indicated that the element concentrations in resident mussels from both sites were lower than the concentrations measured in mussel species from other international and regional (South African) studies. Many elements decreased during the 6-week exposure time in the transplanted mussels, indicating that the mussels were able to regulate their element levels. The resident mussels also had higher concentrations than some of the transplanted mussels and this was attributed to long term exposure to these contaminants and the fact that they have adapted to be able to tolerate higher concentrations. Results further indicated that the resident black mussel (*C. meridionalis*) element concentrations have increased over the last decade compared to the concentrations reported by Dahms *et al*. (2014). High element concentrations can possibly be attributed to anthropogenic activities (shipping) or natural occurrences (upwelling events). The AM concentrations were much lower than those of the transplanted and resident mussels. This was attributed to the overall lower dissolved element concentrations at the two study sites. The data therefore support this study's first hypothesis that artificial and transplanted mussels would have similar element accumulation patterns after the 6-week exposure period, as well as the second hypothesis that element concentrations in resident mussels will be higher than transplanted mussels. The results in Chapter 3 indicated that only six OCPs were above detection limits in the three monitoring approaches and no PCBs were detected at the two research sites. Overall Walvis Bay had higher OCP concentrations than Swakopmund, with the exception of p,p'DDD and total HCH in Swakopmund transplanted mussels being higher. The OCP concentrations in the SPMDs were lower than those of other international studies in which they were used as a monitoring device. The uptake patterns of OCPs in the SPMDs and mussels were similar. Since only metabolites of DDT were detected at the research sites, it indicated that this was due to historical use and there have not been new inputs of the compound into the marine environment. Since PCBs were not detected in mussels or SPMDs, but these compounds were present in studies conducted along the neighbouring South African coastline, it was evident that the Namibian coastline is less exposed to these compounds than the more densely populated South African coastline. The data therefore support the hypothesis that the OCP concentrations in resident and transplanted mussels will be different from the concentrations present in the SPMDs.

The results from this study clearly demonstrated that passive monitoring devices (AM and SPMD) cannot be used in isolation to replace the traditional bioindicators (transplanted and resident mussels), as they only accumulate the dissolved fractions of the contaminants in the water column. The mussels accumulate not only the dissolved fractions in the water but also the fractions bound to the particulate matter, through filter feeding. However, the passive devices cannot metabolise or regulate the contaminants as the mussel bioindicators do. This

is beneficial as it gives a more accurate indication of the dissolved elements in the water column. Thus, an integrated approach that makes use of a combination of the three different monitoring approaches should be applied. Therefore, this study makes an important contribution to the development of an integrated marine monitoring program for Namibia.

This study has also contributed to providing baseline concentrations of organic contaminants present in the Namibian marine environment, as well as an updated status of element concentrations.

4.2 Recommendations for future studies

4.2.1 Recommendations to address study limitations

Based on the findings of this study, the following aspects that could be adapted for future studies were identified:

- Firstly, since the deployment time-frame was six weeks, the transplanted mussels started to die-off by the second week, the number of transplanted mussels should be increased to counter any loss of the transplanted mussels.
- To ensure that the exposure duration does not influence the results, both the transplanted and resident mussels should be collected from sites where they are continuously submerged (infratidal) so that the uptake is not influenced by tidal action. As the currents were extremely strong at the Swakopmund study site where the submerged resident mussels were located, a decision was made to collect resident mussels from the rocky shore that experiences tidal influence at the site. At the Walvis Bay study site it was possible to collect resident mussels that were continuously submerged with the help of some of the harbour divers. Future studies should take into consideration the environmental conditions for sampling and bring the require equipment for this instance it would be a boat.
- During the exposures the basket as well as the AMs were coated in biofouling material. To prevent this, it is recommended to line the basket with mesh, this will also keep the mussels safe from any other organisms and predators.
- As the AMs had to be prepared at the university in South Africa and transported to Namibia some did break during transportation. It is important to make extra just in case something like this occurs.
- As there were only four SPMD cannisters at our disposal it did limit our amount of study sites we could simultaneously conduct the experiment at as two cannisters are needed at a site. For the purpose of this dissertation the four cannister were adequate, but it is worth mentioning for future studies that want to used more than two study sites.

4.2.2 Other recommendations

- The transplanted mussel species that were selected for this study was *C. meridionalis,* which is not the only species found in this region. Comparative studies using the other species (*Perna perna*) would provide insight into potential sensitivity differences.
- During the exposures the basket as well as the AMs were coated in biofouling material. To prevent this, it is recommended to line the basket with mesh, this will also keep the mussels safe from any other organisms and predators.
- As the exposure was carried out over a 6-week period, the AMs had to be kept moist (in order for the gels to not dry out) until they could be returned to the laboratory, thus it is recommended to store them in a fridge to prevent fungal growth.
- This study found that the AMs reach element equilibrium at 4-weeks and it is thus recommended to shorten the exposure from six to four weeks.
- In future the inclusion of more study sites as well as additional reference sites along the coastline is recommended. The position of these sites should be close to river mouths or other areas that have increased human activity.
- The influence of season on element and OCP accumulation should be determined as undertaken in the South African marine environment by Degger *et al*. (2011a; 2011b).
- For this project the tissue samples for OCP and PCB analyses were freeze-dried. For future studies it is recommended to also use wet tissue since some of the compounds can be lost during the freeze drying process.
- Additional compounds such as PAHs should be included in future studies.

Chapter 5: References

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ANNEXURE – A

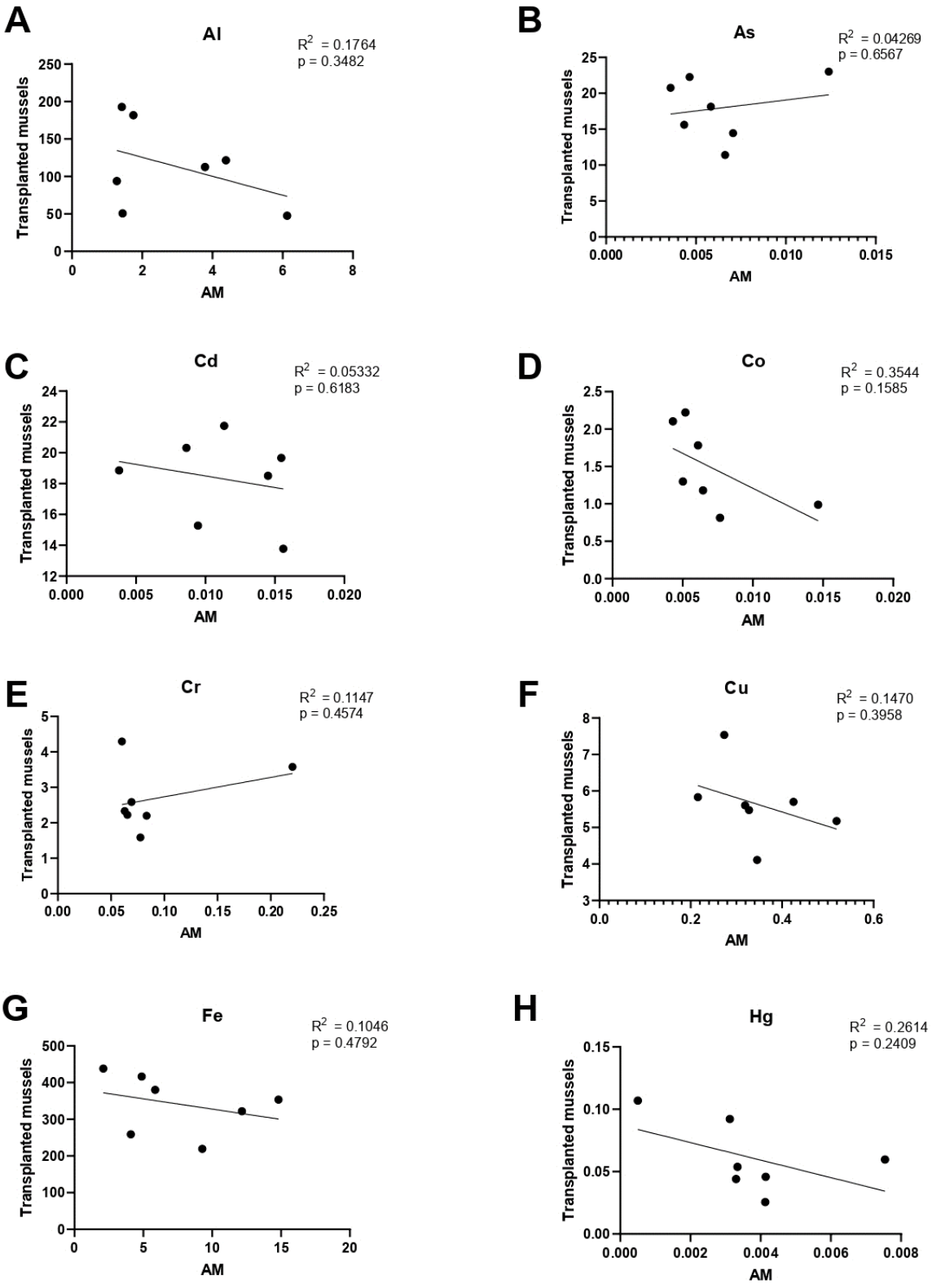

Figure A.1: Correlations and linear regressions between transplanted and artificial mussels element concentration averages for Swakopmund during the 6-week period.

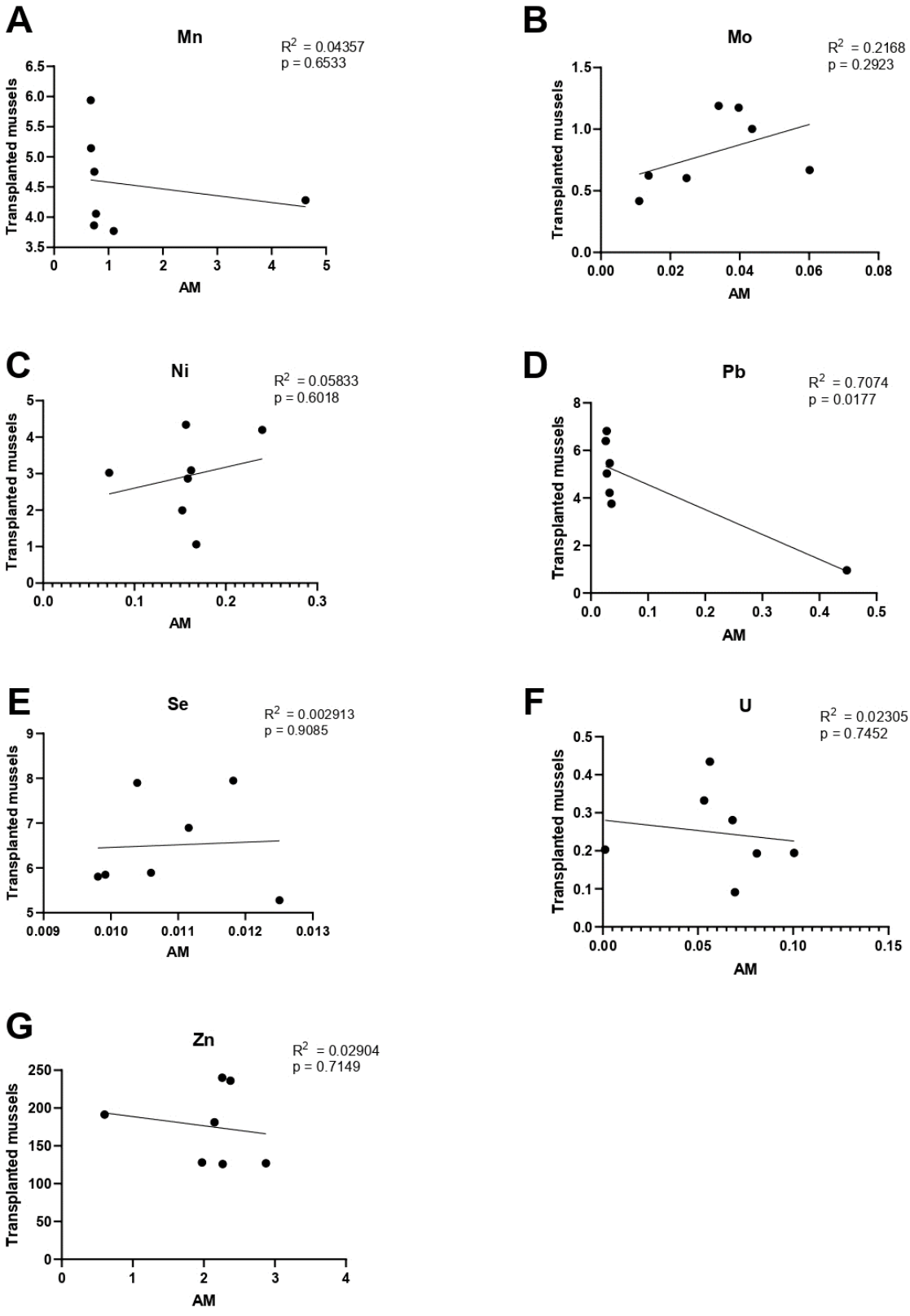

Figure A.2: Correlations and linear regressions between transplanted and artificial mussels element concentration averages for Swakopmund during the 6-week period.

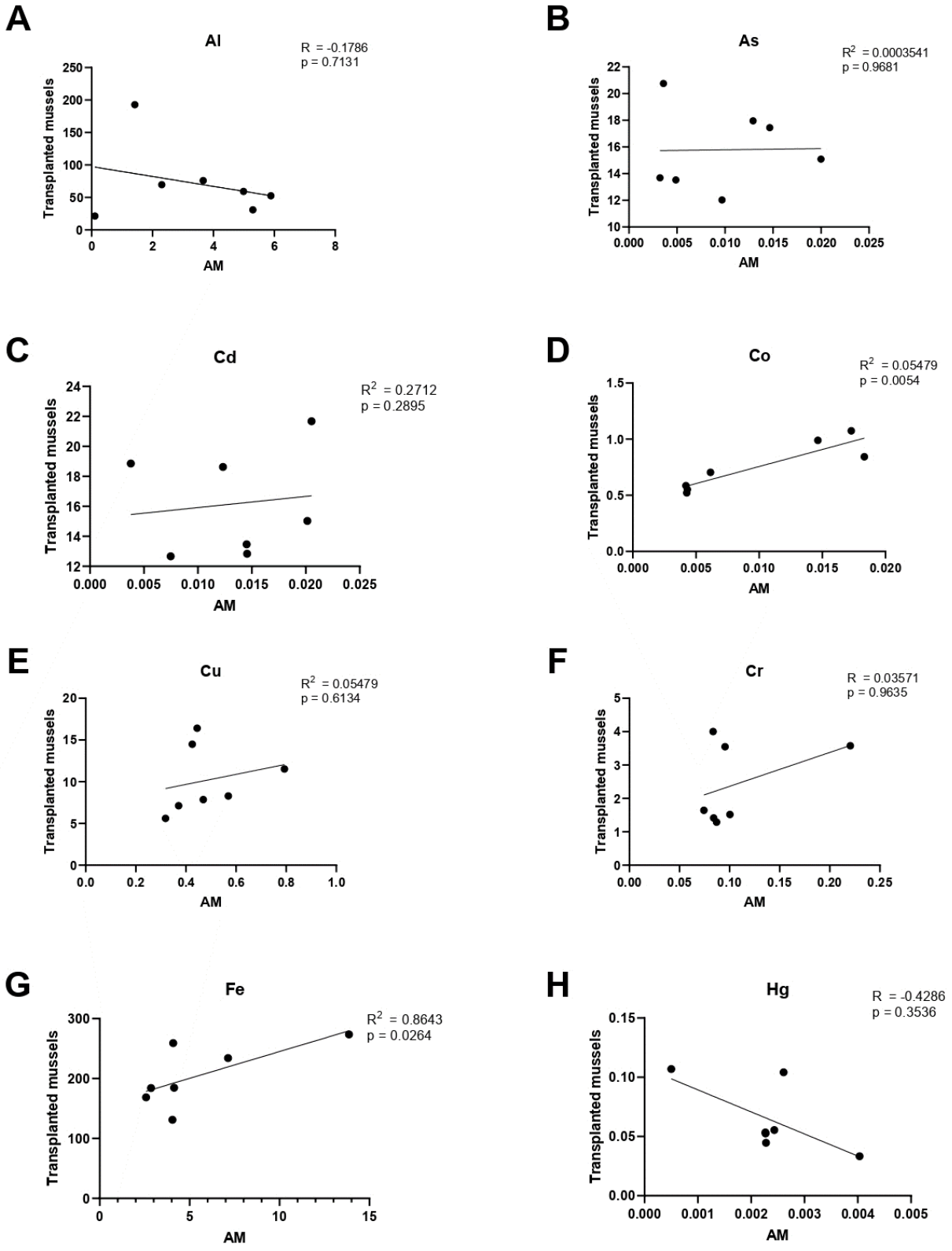

Figure A.3: Correlations and linear regressions between transplanted and artificial mussels element concentration averages for Walvis Bay Harbour during the 6-week period.

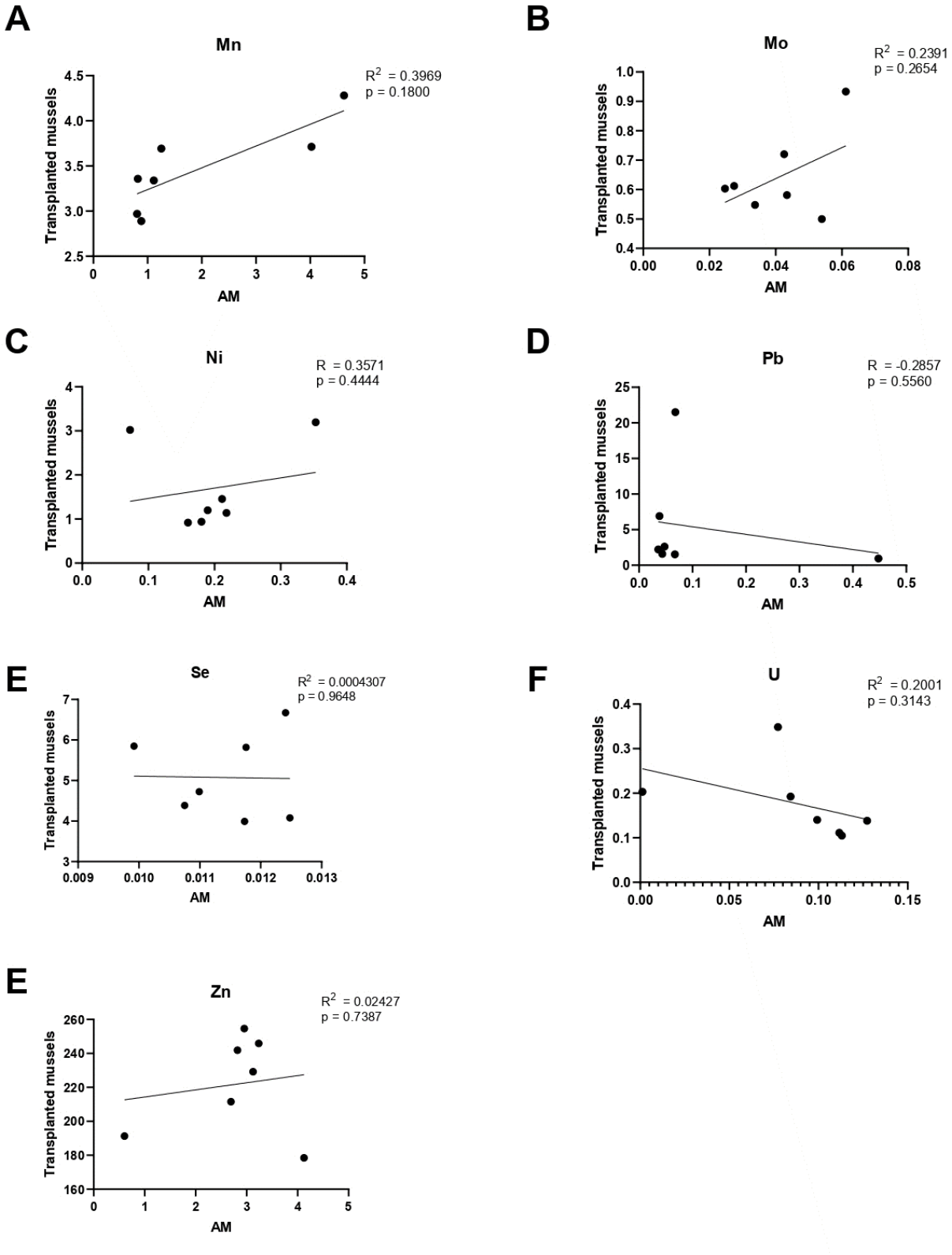

Figure A.4: Correlations and linear regressions between transplanted and artificial mussels element concentration averages for Walvis Bay Harbour during the 6-week period.

ANNEXURE – B

Table B: Comparison of element concentrations (µg/g dry weight) in various mussel species from previous studies to the resident mussel (*Choromytilus meridionalis*) used in this study.

	Elements															
Species	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**U**	**Zn**	**Location**	**Cited**
C. meridionalis	192.8 ± 66.11	20.76 ± 1.53	18.86 ± 2.81	0.99 ± 0.19	3.58 ± 0.14	5.61 ± 1.08	259.0 ± 35.17	0.11 ± 0.01	4.28 ± 0.54	3.02 ± 0.77	0.96 ± 0.28	5.85 ± 0.44	0.20 ± 0.04	191.3 ± 45.52	Mile 17, Namibia	This study, 2023
C. meridionalis	146.6 ± 83.68	11.88 ± 0.97	5.24 ± 0.79	0.23 ± 0.02	0.79 ± 0.09	23.94 ± 2.96	99.12 ± 6.24	0.07 ± 0.003	4.62 ± 0.33	0.41 ± 0.05	1.11 ± 0.15	3.54 ± 0.16	0.09 ± 0.02	207.7 ± 40.08	Walvis Bay, Namibia	This study, 2023
C. meridionalis	413.2 ± 138.9	20.8 ± 0.75	15.6 ± 1.28	1.33 ± 0.09	2.82 ± 0.35	5.77 ± 0.38	427.1 ± 28.2	0.07 ± 0.01	5.27 ± 0.44	2.95 ± 0.19	3.37 ± 1.2	6.61 ± 0.29	0.44 ± 0.05	297.8 ± 61.97	Swakopmund, Namibia	This study, 2023
M. galloprovincialis	560 ± 270	6.9 ± 1.3		1.37 ± 0.97	2.9± 1.9		550 ± 240		9± 4	9.4 ± 4.9		6 ± 1.3	0.19± 0.08	290 ± 170	Namibia	Nekhoroshkov *et al*., 2021
C. meridionalis	86±29	11.4 ± 3.4		0.21± 0.06	0.92± 0.88	12.7± 5.9	110± 50		9.1±3.0	0.81± 0.35		2.9±0.7	0.12± 0.09	93±28	Saldanha Bay, South Africa	Bezuidenhout *et al*., 2020
M. galloprovincialis	17.3 ± 0.82	1.8 ± 0.09	1.0 ± 0.03		0.2 ± 0.01	0.7 ± 0.02	25.1 ± 0.76	0.004 ± 0.0001	0.5 ± 0.01		0.5 ± 0.02			25.9 ± 0.52	Saldanha Bay, South Africa	Firth *et al*., 2020
M. galloprovincialis		±12.5	±1.05		±1.8	±20		±0.15			±2.9	±3		±400	Port Melbourne, Australian	Shen *et al*., 2020
P. perna	88.4 ± 17.8	18.4 ± 1.2	2.1 ± 0.3		8.1 ± 1.4	56.9 ± 7.5	290.2 ± 45.6		10.3 ± 1.6	2 ± 1.6	12.9 ± 1.5	8.6 ± 1.3	0.12 ± 0.01	334.4 ± 40.2	Cape Town, South Africa	Wepener and Degger, 2020
P. perna	335.8 ± 95.3	32.1 ± 2.0	2.9 ± 0.4		8.3 ± 1.7	28.3 ± 4.5	665.4 ± 156.7		39.3 ± 7.5	0.7 ± 0.4	BDL	9.7 ± 1.0	0.22 ± 0.03	556.8 ± 54.1	Mossel Bay, South Africa	Wepener and Degger, 2020
P. perna	236.8 ± 43.2	16.1 ± 4.5	2.4 ± 0.7		19.4 ± 13.2	24.4 ± 1.9	587.4 ± 163.0		169.2 ± 29.4	0.3 ± 0.03	BDL	10.1 ± 3.8	0.24 ± 0.03	618.9 ± 118.1	Port Elizabeth, South Africa	Wepener and Degger, 2020

	Elements															
Species	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**U**	**Zn**	**Location**	**Cited**
P. perna	249.9 ± 79.1	19.8 ± 3.2	1.6 ± 0.2		4.1 ± 0.8	16.9 ± 3.5	440 ± 105.8		86.1 ± 22.8	0.3 ± 0.2	9.1 ± 3.2	7.9 ± 0.8	0.31 ± 0.04	468.8 ± 71.9	East London, South Africa	Wepener and Degger, 2020
P. perna	153.1 ± 28.9	17.8 ± 1.6	1.3 ± 0.2		5.1 ± 0.7	8.9 ± 1.3	247.6 ± 34.8		71.6 ± 18.0	0.7 ± 0.3	11.4 ± 2.3	6.6 ± 0.9	0.14 ± 0.03	442.4 ± 73.1	Durban, South Africa	Wepener and Degger, 2020
P. perna	92.8 ± 19.7	13.3 ± 1.1	0.6 ± 0.1		3.9 ± 0.7	110.8 ± 8.1	200.5 ± 36.7		64.7 ± 9.1	0.9 ± 0.3	4.1 ± 0.8	6.8 ± 0.6	0.17 ± 0.05	229.7 ± 22.9	Richards Bay, South Africa	Wepener and Degger, 2020
P. perna	81.6 ± 13.7	0.9 ± 0.1	6.8 ± 0.8		2.1 ± 0.3		90.7 ± 15.5		4.5 ± 0.5	0.16 ± 0.02	1.8 ± 0.3	0.6 ± 0.04	0.05 ± 0.01	15.6 ± 3.0	Cape Town, South Africa	Wepener and Degger, 2020
P. perna	50 ± 12.6	1.7 ± 0.6	0.6 ± 0.2		2.9 ± 0.4		69.8 ± 17.6		4.9 ± 1.6	0.4 ± 0.1	0.9 ± 0.3	0.8 ± 0.2	0.04 ± 0.01	21.7 ± 6.2	Mossel Bay, South Africa	Wepener and Degger, 2020
P. perna	15.8 ± 5.7	0.2 ± 0.1	0.4 ± 0.1		2.4 ± 0.7		12.4 ± 4.8		3.6 ± 1.2	0.04 ± 0.01	0.4 ± 0.1	0.08 ± 0.02	0.01 ± 0.01	3.2 ± 0.5	Port Elizabeth, South Africa	Wepener and Degger, 2020
P. perna	70.9 ± 17.0	0.42 ± 0.1	0.05 ± 0.01		1.6 ± 0.2		78.9 ± 20.2		6.3 ± 1.9	0.3 ± 0.1	1 ± 0.3	0.4 ± 0.1	0.02 ± 0.01	9.8 ± 1.7	East London, South Africa	Wepener and Degger, 2020
P. perna	5 ± 1.4	0.08 ± 0.02	0.03 ± 0.02		1.3 ± 0.2		3.4 ± 1.6		1.4 ± 0.4	0.03 ± 0.01	0.09 ± 0.02	0.05 ± 0.01	0.002 ± 0.001	2.8 ± 0.6	Durban, South Africa	Wepener and Degger, 2020
P. perna	145.3 ± 27.1	0.4 ± 0.1	0.15 ± 0.02		0.6 ± 0.1		151.1 ± 28.0		11.4 ± 2.9	0.6 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.05 ± 0.01	16.1 ± 3.5	Richards Bay, South Africa	Wepener and Degger, 2020
C. meridionalis	6.4 ± 0.42	1.8 ± 0.09	0.4 ± 0.03		0.1 ± 0.01	1.2 ± 0.03	11.3 ± 0.42	0.003 ± 0.0001	1.6 ± 0.04		0.2 ± 0.01			15.5 ± 0.32	Saldanha Bay, South Africa	Firth *et al*., 2019b
C. meridionalis			5.25			2.06				5.61	10.76			156.7	Valsbaai, South Africa	Reinecke, et al., 2014
M. galloprovincialis	200± 150	31.2± 6.1	0.374 ±0.131	0.634 ±0.205	0.554 ±0.320	4.82± 1.50	177± 97		9.86± 3.87	1.41± 0.54	0.336 ±0.192	2.70± 0.78		72.6± 33.6	Urbino pond and the Diane pond, France	Richir and Gobert, 2014

	Elements															
Species	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**U**	**Zn**	**Location**	**Cited**
P. perna	4.475 ±0.8891		1.050 15±0.428	0.419 ±0.3052	0.662 77±0.3393	0.970 96±0.452	2.857 65±1.0424		1.188 7±0.4188	1.109 27±0.2276	1.887 75±0.6685			2.063 16±0.9341	Mile 17, Namibia	Dahms *et al*., 2014
P. perna	1.804 08±0.18684		0.927 71±0.09799	0.689 75±0.109103	0.733 93±0.112868	1.214 02±0.109483	2.374 11±0.17145		1.055 82±0.130841	0.933 8±0.099391	1.885 06±0.144655			2.293 43±0.200241	Walvis Bay Harbour, Namibia	Dahms *et al*., 2014
C. meridionalis	1.234 1±0.7927		0.555 5±0.3541	0.393 6±0.2433	0.442 3±0.277	0.534 9±0.3474	1.344 2±0.8736		0.538 6±0.3472	0.659 3±0.4423	0.975 7±0.6685			1.170 3±0.7514	Mile 17, Namibia	Dahms *et al*., 2014
C. meridionalis	4.475 ±0.8891		1.050 15±0.428	0.419 ±0.3052	0.662 77±0.3393	0.970 96±0.452	2.857 65±1.0424		1.188 7±0.4188	1.109 27±0.2276	1.887 75±0.6685			2.063 16±0.9341	Walvis Bay Harbour, Namibia	Dahms *et al*., 2014
C. meridionalis						2.76 ± 0.23	63 ± 12.34				0.12 ± 0.004			15.95 ± 1.60	Walvisbay Harbour, Namibia	Vellemu, 2014
C. meridionalis						0.9 ± 0.01	26 ± 3.47				0.06			15.91 ± 1.57	Swakopmund, Namibia	Vellemu, 2014
C. meridionalis						1.04 ± 0.02	121 ± 13.45							20.78 ± 2.90	Henties Bay, Namibia	Vellemu, 2014
C. meridionalis						2.01 ± 0.20	45 ± 4.41							10.95 ± 0.91	Cape Cross, Namibia	Vellemu, 2014
C. meridionalis			5.27 ± 4.3												False Bay, South Africa	Reinecke *et al*., 2012
P. perna		1.38 ± 0.03	0.064 ± 0.004	0.18 ± 0.01	<0.016		15 ± 1	0.016 ± 0.001			0.048 ± 0.003	0.34 ± 0.03		18.9 ± 0.5	Cocanha, São Paulo, Brazil	Catharino *et al*., 2011
P. perna		1.44 ± 0.03	0.050 ± 0.003	0.19 ± 0.01	<0.016		12 ± 1	0.012 ± 0.001			0.098 ± 0.006	0.30 ± 0.02		19.0 ± 0.6	Itaipu, São Paulo, Brazil	Catharino *et al*., 2011
P. perna		1.52 ± 0.03	0.089 ± 0.005	0.12 ± 0.01	0.068 ± 0.003		46 ± 3	0.023 ± 0.001			0.025 ± 0.001	0.58 ± 0.05		16.8 ± 0.8	I. Palmas, São Paulo, Brazil	Catharino *et al*., 2011

	Elements															
Species	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**U**	**Zn**	**Location**	**Cited**
P. viridis	774	10.6	0.46		2.1	43.8	786	0.15	37.4	2.3	5.5			118.0	Victoria Harbour Tsim Sha Tsui Hong Kong South	Liu and Kueh, 2005
M. galloprovincialis			0.24-0.49		0.32-7.27	2.44-5.49		0.11-0.15			0.84-2.41			75.9-201	Turkey Eastern Aegean Sea	Bilgin and Uluturhan-Suzer, 2017
M. galloprovincialis						0.7-12.9	24.3-82.0		0.4-4.8					43.8-133.5	N Aegean Sea (Strait of Canakkale)	Yigit *et al*., 2017
M. galloprovincialis						1.3-1.8	11.0-11.7							13.3-15.2	Island of Gossa (W coast of Norway)	Brooks *et al*., 2012
M. galloprovincialis						3.9-9.7								159-351	N Atlantic (Spanish Gallician coasts)	Besada *et al*., 2011
M. galloprovincialis						4.6-17.2	128-603		7.3-85.0					132-345	Adriatic Sea (Montenegro coasts)	Joksimovic *et al*., 2011
M. galloprovincialis		14.6-31.5	0.4-2.3	0.4-69.3	2.6-5.7	9.1-34.8			5.6-55.3	1.5-15.4	1.1-13.3	5.8-8.7		202.7-300.8	Spain Cantabrian Coast	Bartolome´ *et al*., 2010
M. galloprovincialis						3.5-5.3	48.6-49.9		2.6-4.7					17.8-28.5	Aegean Sea	Kucuksezgin *et al*., 2008
M. galloprovincialis						11.7-23.3			46.9-73.0					312-396	Black Sea (Turkish coasts)	Bakan and Ozko¸c, 2007
M. galloprovincialis			1.16-6.59		0.16-2.75	3.55-10.8					1.08-4.27			135-400	Italy Venice Lagoon	Nesto *et al*., 2007
P. viridis			0.24-3.49		BDL-0.46	BDL-1.84	BDL-235.6		1.91-8.77	BDL-2.89	BDL-1.95			BDL-17.36	India	Sasikumar *et al*., 2006
M. galloprovincialis		4--30			1-2.9	3.7-11.1	53.4-719		2--13	0.8-5	2--7			59.1-273	East Adriatic Sea, Croatia	Orescanin *et al*., 2006
M. galloprovincialis						6.7-9.5	120-415		4.5-11.7					208-320	Marmara Sea (NW coasts)	Topcuoglu *et al*., 2004
M. galloprovincialis			0.33-0.49		0.46-1.31	5.51-11.5					1.67-2.49			123-180	Italy Tyrrhenian coastal areas	Conti *et al*., 2003

	Elements														
Species	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**U**	**Zn**	**Location**	**Cited**
M. galloprovincialis						5.5-11.5								123-180	Tyrrhenian Sea (Gulf of Gaeta)	Conti *et al*., 2003
P. viridis			0.01-0.61		0.06-0.2	1.02-1.98		0.03-0.07		0.3-0.75				11.3-40.37	Trinidad	Astudillo *et al*., 2002
P. viridis			0.02-0.05		0.12-0.16	1.42-3.43		0.02-0.08		0.22-1.3				8.75-16.38	Venezuela	Astudillo *et al*., 2002

ANNEXURE – C

Table C: Comparison of OCP concentrations in various mussel species and semi-permeable membrane devices from previous studies.

Indicator	Unit	Contaminants															Study area	Reference
		OCPs																
		Alpha-HCH	Beta- HCH	Gamma-HCH	Delta-HCH	∑HCH	Aldrin	Dieldrin	Alpha-chlordane	Trans-Nonachlor	p,p'DDE	o,p'DDD	o,p'DDT	p,p'DDD	p,p'DDT	∑DDT		
Mussels																		
M. edulis	ng/g lipid	2.4		0.75			1.5		0.55	3.3	22	8.4	1.3	11	3.5		Port Richards, Victoria, Australia	Prest *et al*., 1995
M. edulis	ng/g lipid	1.2		0.49			2.4		ND	3.7	18	6.3	1.8	15	9.2		Corio Bay, Victoria, Australia	Prest *et al*., 1995
P. viridis	ng/g dry weight	2.7	0.6	4.7	0.2	8.2	6.6	3.8	3.3		11.7			13.7	5.9		Kat O, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight	8.7	2.1	8.1	0.4	19.3	10.0	2.9	1.1		11.7			19.3	7.9		Sai Wan Ho, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight	11.8	8.8	4.5	ND	25.1	5.7	3.8	0.6		18.7			15.0	12.9		Tsim Sha Tsui, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight	14.4	3.4	15.6	0.3	33.7	1.9	2.0	3.8		13.4			4.4	8.0		Tolo Harbour, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g lipid wt					<0.01										0.33	Cambodia	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					0.2										120	Hong Kong	Monirith *et al*., 2003

Indicator	Unit	Contaminants															Study area	Reference
		OCPs																
		Alpha-HCH	Beta- HCH	Gamma-HCH	Delta-HCH	∑HCH	Aldrin	Dieldrin	Alpha-chlordane	Trans-Nonachlor	p,p'DDE	o,p'DDD	o,p'DDT	p,p'DDD	p,p'DDT	∑DDT		
P. viridis	ng/g lipid wt					2										4.2	India	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					0.04										1	Indonesia	Monirith *et al*., 2003
M. galloprovincialis	ng/g lipid wt					0.32										3.5	Japan	Monirith *et al*., 2003
M. edulis	ng/g lipid wt					0.26										3.5	Korea	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					<0.05										1.4	Malaysia	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					0.03										0.4	Philippines	Monirith *et al*., 2003
C. grayamus	ng/g lipid wt					1										12	Russia	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					0.3										3	Singapore	Monirith *et al*., 2003
P. viridis	ng/g lipid wt					0.06										40	Vietnam	Monirith *et al*., 2003
M. edulis	(ng/g dry weight	0.22	4.03	0.13						0.51	53.93			59.44	159		China	Fang et al., 2004
M. edulis	ng/g lipid wt					6.27										143.7	Korea	Ramu *et al*., 2007

Indicator	Unit	Contaminants															Study area	Reference
		OCPs																
		Alpha-HCH	Beta- HCH	Gamma-HCH	Delta-HCH	∑HCH	Aldrin	Dieldrin	Alpha-chlordane	Trans-Nonachlor	p,p'DDE	o,p'DDD	o,p'DDT	p,p'DDD	p,p'DDT	∑DDT		
M. galloprovincialis	µg/g lipid										0.54				0.68		Mediterranean coast, Libya	Galgani *et al.*, 2014
M. edulis	ng/g lipid	11 ± 1	3 ± 0	3 ± 0		16 ± 1				13 ± 2	20 ± 5		3 ± 0	9 ± 2	4 ± 1	34 ± 7	Flatey, Iceland	Skarphedinsdottir et al., 2010
P. viridis	ng/g dry weight										8.23			5.51	2.84		Singapore	Bayen et al., 2003
P. perna	ng/g lipid	ND	ND	ND	ND						<LOD		ND	ND	ND		Richards Bay Harbour, South Africa	Coetzee *et al*., 2015
P. perna	ng/g lipid	ND	ND	ND	ND						<LOD		ND	ND	ND		Durban Harbour, South Africa	Coetzee *et al*., 2015
C. meridionalis	ng/g dry weight							4.4 ± 1.29	2.3 ± 0.39	1.2 ± 0.002				0.7 ± 0.009			Saldanha Bay, South Africa	Firth *et al*., 2019a
M. galloprovincialis	ng/g dry weight							3.2 ± 1.34	1.2 ± 0.16	1.2 ± 0.002				0.7 ± 0.009			Saldanha Bay, South Africa	Firth *et al*., 2019a
P. perna	µg/g lipid mass	2.19	20.9	ND	3.22		3.19			ND	12.8	11.5	ND	ND			Tsitskimma, South Africa	Erasmus *et al*., 2020

Indicator	Unit	Contaminants															Study area	Reference
		OCPs																
		Alpha-HCH	Beta- HCH	Gamma-HCH	Delta-HCH	∑HCH	Aldrin	Dieldrin	Alpha-chlordane	Trans-Nonachlor	p,p'DDE	o,p'DDD	o,p'DDT	p,p'DDD	p,p'DDT	∑DDT		
P. perna	µg/g lipid mass	ND	30.74		3.79		1.79				5.95			2.24			Sheffield Beach, South Africa	Greyling *et al*., 2021
SPMDs																		
SPMD	ng/g lipid	18		7.2			8.4		2.5	5.8	23	31	2.2	54	9.6		Port Richards, Victoria, Australia	Prest *et al*., 1995
SPMD	ng/g lipid	16		6.6			9.6		2.2	29	17	3.1	1.4	14	13		Corio Bay, Victoria, Australia	Prest *et al*., 1995
SPMD	ng/g lipid	ND	ND	12.1	1.6	13.7	15.8	ND	23.6		19.4			ND	9.4		Kat O, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid	15.9	9.5	23.0	4.9	53.3	38.9	19.5	31.9		51.4			24.4	17.8		Sai Wan Ho, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid	16.5	10.1	15.8	7.5	49.9	39	ND	22		30.8			ND	15.7		Tsim Sha Tsui, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid	36.2	19.3	20.3	9.7	85.5	44.1	ND	21.9		46.8			ND	29.4		Tolo Harbour, Hong Kong	Richardson *et al*., 2001

Indicator	Unit	Contaminants															Study area	Reference
		OCPs																
		Alpha-HCH	Beta- HCH	Gamma-HCH	Delta-HCH	∑HCH	Aldrin	Dieldrin	Alpha-chlordane	Trans-Nonachlor	p,p'DDE	o,p'DDD	o,p'DDT	p,p'DDD	p,p'DDT	∑DDT		
SPMD	ng/g lipid	48.2	ND	ND	2.7	50.9	ND	ND	ND		47.5			44.9	ND		Kwun Tong, Hong KOng	Richardson *et al*., 2001
SPMD	ng/SPMD triolein	<LOD	ND	ND	ND						<LOQ		ND	ND	ND		Richards Bay Harbour, South Africa	Coetzee *et al*., 2015
SPMD	ng/SPMD triolein	618 ± 81	ND	ND	ND						17		ND	ND	160 ± 46		Durban Harbour, South Africa	Coetzee *et al*., 2015

ANNEXURE – D

Table D: Comparison of PCB concentrations in various mussel species and semi-permeable membrane devices from previous studies.

Species	Unit	Contaminants															Study area	Reference
		PCBs																
		PCB18	PCB 26	PCB 28	PCB 29	PCB 44	PCB 52	PCB 77	PCB 87	PCB 101	PCB 104	PCB 110	PCB 118	PCB 138	PCB 153	PCB 180	∑PCB	
Mussels																		
M. edulis	ng/g lipid	ND		0.62	0.55	0.48	2.9		ND	1.8		2.2	1.7	5.7	3.1	0.48	36	Port Richards, Victoria, Australia	Prest *et al*., 1995
M. edulis	ng/g lipid	ND		1.4	0.55	1.4	5.2		3.7	13		13	11	19	19	0.69	152	Corio Bay, Victoria, Australia	Prest *et al*., 1995
P. viridis	ng/g dry weight																119	Kat O, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight																231	Sai Wan Ho, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight																415	Tsim Sha Tsui, Hong Kong	Richardson *et al*., 2001
P. viridis	ng/g dry weight																218	Tolo Harbour, Hong Kong	Richardson *et al*., 2001
P. perna	µg/g lipid		65.52	ND	ND		ND	ND	ND	ND	ND		ND	ND	ND	0.03	65.54	Cape Town, South Africa	Degger *et al*., 2011b

Species	Unit	Contaminants																Study area	Reference
		PCBs																	
		PCB18	PCB 26	PCB 28	PCB 29	PCB 44	PCB 52	PCB 77	PCB 87	PCB 101	PCB 104	PCB 110	PCB 118	PCB 138	PCB 153	PCB 180	∑PCB		
P. perna	µg/g lipid		39.02	ND	ND		ND	ND	ND	ND	ND		ND	ND	ND	0.02	39.04	Port Elizabeth, South Africa	Degger *et al*., 2011b
P. perna	µg/g lipid		50.4	ND	0		ND	36.7	26.6	ND	ND		ND	ND	ND	0.02	113.8	Richards Bay, South Africa	Degger *et al*., 2011b
P. perna	µg/g lipid		27.1	ND	22.3		ND	21.1	17.2	ND	25.2		18.4	ND	ND	0.01	131.4	Saldanha Bay, South Africa	Degger *et al*., 2011b
P. perna	µg/g lipid		0.01	ND	0.02		ND	ND	ND	ND	ND		ND	ND	ND	34.1	34.13	Tsitsikamma, South Africa	Degger *et al*., 2011b
M. galloprovincialis	µg/kg dry weight									0.54					0.82			Mediterranean coasts of Libya	Galgani *et al*., 2014
M. edulis	ng/g lipid			44 ± 24			10 ± 6			4 ± 0			18 ± 5	19 ± 5	35 ± 6	3 ± 0		Flatey, Iceland	Skarphedinsdottir et al., 2010
P. perna	ng/g lipid			<LOD			<LOD			2.58± 0[2]			3.48± 0[2]	<LOD	<LOD	<LOD	6.06 ± 0	Richards Bay Harbour, South Africa	Coetzee *et al*., 2015
P. perna	ng/g lipid			12±6			18±6			24±10[123]			26±6[12]	12±4	35±20	<LOQ	127 ± 40	Durban Harbour, South Africa	Coetzee *et al*., 2015
C. meridionalis	ng/g dry weight	0.5 ± 0.005		1.6±0.005		0.4 ± 0.009	1.2±0.003					1.5 ± 0.007	0.3±0.008	0.3±0.001	1.4±0.04	3.2±0.01		Saldanha Bay, South Africa	Firth *et al*., 2019a

Species	Unit	Contaminants																Study area	Reference
		PCBs																	
		PCB18	PCB 26	PCB 28	PCB 29	PCB 44	PCB 52	PCB 77	PCB 87	PCB 101	PCB 104	PCB 110	PCB 118	PCB 138	PCB 153	PCB 180	∑PCB		
M. galloprovincialis	ng/g dry weight	0.5 ± 0.005		1.6±0.007		0.5 ± 0.01	1.2±0.001					1.5 ± 0.005	0.3±0.006	0.3±0.016	1.4±0.05	3.2±0.004		Saldanha Bay, South Africa	Firth *et al*., 2019a
SPMDs																			
SPMD	ng/g lipid	ND		3.7	1.9	1.8	9.6		1.6	2.5		2.9	2.0	2.6	2.3	0.42	64	Port Richards, Victoria, Australia	Prest *et al*., 1995
SPMD	ng/g lipid	ND		6.6	1.1	3.2	13		3.4	7.8		9.0	5.2	7.8	6.6	1.4	142	Corio Bay, Victoria, Australia	Prest *et al*., 1995
SPMD	ng/g lipid																974	Kat O, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid																432	Sai Wan Ho, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid																1646	Tsim Sha Tsui, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid																1844	Tolo Harbour, Hong Kong	Richardson *et al*., 2001
SPMD	ng/g lipid																1509	Kwun Tong, Hong KOng	Richardson *et al*., 2001

Species	Unit	Contaminants																Study area	Reference
		PCBs																	
		PCB18	PCB 26	PCB 28	PCB 29	PCB 44	PCB 52	PCB 77	PCB 87	PCB 101	PCB 104	PCB 110	PCB 118	PCB 138	PCB 153	PCB 180	∑PCB		
SPMD	ng/g lipid	ND	ND	6.52	ND	ND	11.37	ND	ND	ND	7.38	ND	5.73	9.23	ND	ND	40.23	Cape Town, South Africa	Degger *et al*., 2011b
SPMD	ng/g lipid	ND	ND	18.62	10.10	6.82	4.42	11.14	ND	ND	ND	ND	0.001	43.21	ND	0.01	104.89	Port Elizabeth, South Africa	Degger *et al*., 2011b
SPMD	ng/g lipid	5.3	ND	2.7	ND	15.6	ND	ND	15.2	26.6	ND	ND	5.8	22.2	ND	ND	158.3	Richards Bay, South Africa	Degger *et al*., 2011b
SPMD	ng/g lipid	ND	ND	ND	3.55	ND	ND	1.99	ND	ND	ND	ND	ND	24.24	ND	0.01	29.79	Saldanha Bay, South Africa	Degger *et al*., 2011b
SPMD	ng/g lipid	ND	ND	4.82	0.11	3.19	ND	0.13	ND	57.72	ND	ND	ND	0.57	ND	ND	84.34	Tsitsikamma, South Africa	Degger *et al*., 2011b
SPMD	ng/SPMD triolein			<LOD			6.69±2			8±2			23±0.83	<LOQ	<LOD	ND	37.69±4.8	Richards Bay Harbour, South Africa	Coetzee *et al*., 2015
SPMD	ng/SPMD triolein			<LOQ			51±8			43±10			71±0.3[3]	46±6	44±8	<LOQ	255±32.3	Durban Harbour, South Africa	Coetzee *et al*., 2015

ANNEXURE – E

Table E: Recovery rates (%), limit of detection (LOD) in ng/mL and limit of quantification (LOQ) in ng/mL of all of the different OCP compounds of interest. The grey blocks represent the internal standards.

	Mussels		
Compounds	**LOD**	**LOQ**	**Recovery (%)**
Benzene, 1,2,3,5 tetrachloro-4,6-dimethyl-	0.72	2.15	99.80
alpha-HCH	0.54	1.62	63.95
beta-HCH	0.11	0.33	70.69
gamma-HCH (Lindane)	0.19	0.56	58.32
delta-HCH	0.51	1.53	82.13
Hexachlorobenzene	0.56	1.69	30.55
Heptachlor	0.41	1.23	42.36
Heptachlor-exo-epoxide	0.05	0.16	61.36
Heptachlor-endo-epoxide	0.10	0.30	51.30
Aldrin	0.11	0.32	33.75
Dieldrin			67.29
Endrin	0.23	0.69	93.86
cis-Chlordane	0.18	0.55	53.75
oxy-Chlordane	0.16	0.47	56.42
trans-Chlordane	0.07	0.20	50.40
o,p'-DDE			54.41
p,p'-DDE	0.77	2.31	51.68
o,p'-DDD	1.44	4.31	89.04
p,p'-DDD	1.78	5.34	75.53
o,p'-DDT	2.05	6.14	88.08
p,p'-DDT	1.31	3.92	83.59
cis-Nonachlor	0.06	0.18	81.25
trans-Nonachlor	0.10	0.29	52.19
Decachlorobiphenyl (#209)	0.34	1.01	32.68

ANNEXURE – F

Table F: Recovery rates (%), limit of detection (LOD) in ng/mL and limit of quantification (LOQ)in ng/mL of all of the different PCB compounds of interest.

	Mussels		
Compounds	**LOD**	**LOQ**	**Recovery (%)**
2-Chlorobiphenyl (#1)	32.45	97.35	284.02
2,3-Dichlorobiphenyl (#5)	32.54	97.63	337.26
2,2',5-Trichlorobiphenyl (#18)	15.01	45.04	292.18
2,4',5-Trichlorobiphenyl (#31)	13.29	39.86	242.28
2,2',5,5'-Tetrachlorobiphenyl (#52)	5.00	15.01	206.70
2,2',3,5'-Tetrachlorobiphenyl (#44)	3.05	9.15	208.61
2,3',4,4'-Tetrachlorobiphenyl (#66)	3.56	10.68	135.49
2,2',4,5,5'-Pentachlorobiphenyl (#101)	3.43	10.28	131.12
2,2',3,4,5'-Pentachlorobiphenyl (#87)	1.88	5.63	135.24
2,3,3',4',6-Pentachlorobiphenyl (#110)	2.58	7.74	119.19
3,3',4,4'-Tetrachlorobiphenyl-13C12 (#77L)			
2,2',3,5,5',6-Hexachlorobiphenyl (#151)	2.17	6.50	0.86
2,3',4,4',5-Pentachlorobiphenyl-13C12 (#118L)			
2,2',4,4',5,5'-Hexachlorobiphenyl (#153)	1.05	3.16	72.09
2,2',3,4,5,5'-Hexachlorobiphenyl (#141)	0.83	2.49	78.23
2,2',3,4,4',5'-Hexachlorobiphenyl (#138)	0.88	2.65	67.50
2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)	0.38	1.15	68.68
3,3',4,4',5-Pentachlorobiphenyl-13C12 (#126L)			
2,2',3,4,4',5',6-Heptachlorobiphenyl (#183)	0.40	1.19	63.63
2,3,3',4,4',5-Hexachlorobiphenyl-13C12 (#156L)			
2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)	0.49	1.46	63.23
3,3',4,4',5,5'-Hexachlorobiphenyl-13C12 (#169L)			
2,2',3,3',4,4',5-Heptachlorobiphenyl (#170)	0.57	1.72	67.75
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (#206)	0.28	0.84	52.14
Decachlorobiphenyl (#209)			

The grey blocks represent the internal standards.

ANNEXURE – G

Table G: Quantification of organochlorine pesticides (OCPs) concentrations in semi-permeable membrane devices (SPMDs ng/ml lipid, mean of four replicates), resident and transplanted *C. meridionalis* (ng/g dry weight).

	Walvis Bay Harbour			Swakopmund		
Compounds	**TM**	**RM**	**SPMD**	**TM**	**RM**	**SPMD**
alpha-BHC	ND	ND	ND	ND	ND	ND
Hexachlorobenzene	ND	ND	ND	ND	ND	ND
beta-BHC	13.45 ± 0.58	24.49 ± 3.87	ND	9.14 ± 0.81	20.36 ± 1.53	ND
gamma-BHC (Lindane)	0.96 ± 0.17	18.31 ± 2.62	10.41 ± 0.28	4.06 ± 0.94	14.3 ± 0.81	ND
delta-BHC	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND	ND	ND
Aldrin	9.77 ± 1.89	ND	ND	1.35 ± 0.55	ND	ND
oxy-Chlordane	35.68 ± 6.46	21.79 ± 6.14	ND	19.54 ± 3.86	2.05 ± 0.66	ND
Heptachlor-exo-epoxide	ND	ND	ND	ND	ND	ND
Heptachlor-endo-epoxide	ND	ND	ND	ND	ND	ND
trans-Chlordane	ND	ND	ND	ND	ND	ND
o,p'-DDE	ND	ND	ND	ND	ND	ND
cis-Chlordane	ND	ND	ND	ND	ND	ND
trans-Nonachlor	ND	ND	ND	ND	ND	ND
p,p'-DDE	5.46 ± 0.15	-3.27 ± 0.45	7.26 ± 1.37	1.2 ± 0.86	-3.06 ± 0.27	ND
Dieldrin	ND	ND	ND	ND	ND	ND
o,p'-DDD	ND	ND	ND	ND	ND	ND
Endrin	49.39 ± 5.15	33.7 ± 20.24	45.67 ± 20.9849	10.82 ± 4.51	8.07 ± 5.21	98.53
p,p'-DDD	ND	ND	ND	0.3 ±0.20	2.69	ND
cis-Nonachlor	ND	ND	ND	ND	ND	ND
o,p'-DDT	ND	ND	ND	ND	ND	ND
p,p'-DDT	ND	ND	ND	ND	ND	ND

ND, not detected

ANNEXURE – H

Table H: Quantification of polychlorinated biphenyl (PCBs) concentrations in semi-permeable membrane devices (SPMDs ng/ml lipid, mean of four replicates), resident and transplanted *C. meridionalis* (ng/g dry weight).

	Walvis Bay Harbour			Swakopmund		
Compounds	**TM**	**RM**	**SPMD**	**TM**	**RM**	**SPMD**
2-Chlorobiphenyl (#1)	ND	ND	ND	ND	ND	ND
2,3-Dichlorobiphenyl (#5)	ND	ND	ND	ND	ND	ND
2,2',5-Trichlorobiphenyl (#18)	ND	ND	ND	ND	ND	ND
2,4',5-Trichlorobiphenyl (#31)	ND	ND	ND	ND	ND	ND
2,2',5,5'-Tetrachlorobiphenyl (#52)	ND	ND	ND	ND	ND	ND
2,2',3,5'-Tetrachlorobiphenyl (#44)	ND	ND	ND	ND	ND	ND
2,3',4,4'-Tetrachlorobiphenyl (#66)	ND	ND	ND	ND	ND	ND
2,2',4,5,5'-Pentachlorobiphenyl (#101)	ND	ND	ND	ND	ND	ND
2,2',3,4,5'-Pentachlorobiphenyl (#87)	ND	ND	ND	ND	ND	ND
2,3,3',4',6-Pentachlorobiphenyl (#110)	ND	ND	ND	ND	ND	ND
3,3',4,4'-Tetrachlorobiphenyl-13C12 (#77L)	ND	ND	ND	ND	ND	ND
2,2',3,5,5',6-Hexachlorobiphenyl (#151)	ND	ND	ND	ND	ND	ND
2,3',4,4',5-Pentachlorobiphenyl-13C12 (#118L)	ND	ND	ND	ND	ND	ND
2,2',4,4',5,5'-Hexachlorobiphenyl (#153)	ND	ND	ND	ND	ND	ND
2,2',3,4,5,5'-Hexachlorobiphenyl (#141)	ND	ND	ND	ND	ND	ND
2,2',3,4,4',5'-Hexachlorobiphenyl (#138)	ND	ND	ND	ND	ND	ND
2,2',3,4',5,5',6-Heptachlorobiphenyl (#187)	ND	ND	ND	ND	ND	ND

3,3',4,4',5-Pentachlorobiphenyl-13C12 (#126L)	ND	ND	ND	ND	ND	ND
2,2',3,4,4',5',6-Heptachlorobiphenyl (#183)	ND	ND	ND	ND	ND	ND
2,3,3',4,4',5-Hexachlorobiphenyl-13C12 (#156L)	ND	ND	ND	ND	ND	ND
2,2',3,4,4',5,5'-Heptachlorobiphenyl (#180)	ND	ND	ND	ND	ND	ND
3,3',4,4',5,5'-Hexachlorobiphenyl-13C12 (#169L)	ND	ND	ND	ND	ND	ND
2,2',3,3',4,4',5-Heptachlorobiphenyl (#170)	ND	ND	ND	ND	ND	ND
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (#206)	ND	ND	ND	ND	ND	ND
Decachlorobiphenyl (#209)	ND	ND	ND	ND	ND	ND

ND, not detected