

# Presence, levels and distribution of pollutants in the estuarine food web-Swartkops River Estuary, South Africa

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"Man can hardly even recognize the devils of his own  
creation" ~ Albert Schweitzer

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## Abbreviations

Al:	Aluminium
As:	Arsenic
ATSDR:	Agency for Toxic Substances and Disease Registry
Cd:	Cadmium
Co:	Cobalt
Cu:	Copper
DDT:	Dichlorodiphenyl trichloroethane
dm:	Dry mass
EPA:	Environmental Protection Agency
ERL:	Effects range-low
ERM:	Effects range-median
FDA:	Food and Drug Administration
Fe:	Iron
GPS:	Global Positioning System
HDPE:	High-density polyethylene
Hg:	Mercury
ISQG:	Interim marine sediment quality guidelines
LOQ:	Level of quantification
Mg:	Magnesium
n:	Number of sampling sites
NA:	Not applicable
NMS:	Nonmetric Multidimensional Scaling
NMISA:	National Metrology Institute of South Africa
Ni:	Nickel
OCL:	Organochlorine
PAHs:	Polycyclic aromatic hydrocarbons

Pb:	Lead
PCA:	Principal component analysis
PCBs:	Polychlorinated biphenyls
PE:	Port Elizabeth
PEL:	Probable effects levels
POPs:	Persistent Organic Pollutants
SCPOPs:	Stockholm Convention on Persistent Organic Pollutants
SD:	Standard deviation
Sn:	Cyanide
Sr:	Strontium
SRE:	Swartkops River Estuary
Ti:	Titanium
USEPA:	United States Environmental Protection Agency
WHO:	World Health Organization
wm:	Wet mass
Zn:	Zinc

## Abstract

### **Presence, levels, and distribution of pollutants in the estuarine food web- Swartkops River Estuary, South Africa.**

Estuaries are among the most productive and diverse of aquatic habitats supporting a rich variety of plants and animals. They are nursery areas for many species of fish harvested by recreational and subsistence anglers. The Swartkops River Estuary (SRE) is situated approximately 10 km north-east of Port Elizabeth and the only major well-preserved estuary within a city, thus unique to South Africa. The SRE is surrounded by highly urbanized and industrialized regions in the Eastern Cape. The aim of this study was to determine and interpret the presence, levels, and distribution of selected priority pollutants in the food web of the SRE.

Different components within the SRE were analysed for the presence of environmental contaminants. Seven sites were selected, some coinciding with previous studies in the SRE. Three of these sites are major discharge points that discharge directly into the estuary. Sediment, mud prawn, sand gobies, bird eggs, and various fish species were analysed. Samples were collected in the middle and lower reaches of the estuary, the areas known to receive major pollution loads from neighbouring sources. Heavy metals found in the sediments were compared to previous studies.

Bottom sediments and organisms surrounding major discharge points showed higher concentrations of pollutants and compared to previous studies, these concentrations seem to be increasing. Due to biomagnification, higher concentrations were generally found in the top predators although certain elements did not show this trend. Some heavy metal concentrations found in the fish exceeded of the food guidelines and may in turn pose a threat for subsistence users of the SRE. There are indications of multiple different pollution sources. Bird eggs had detectable quantities of polychlorinated biphenyls, but its implications need more investigation.

**Keywords:** Swartkops Estuary, heavy metals, sediment, fish, mud prawn, bird eggs

## Opsomming

### **Teenwoordigheid, vlakke en verspreiding van besoedelstowwe in die voedselweb van die Swartkops Riviermond, Suid-Afrika.**

Riviermondings is van die mees diverse en produktiewe akwatiese habitats en ondersteun 'n ryke verskeidenheid van plante en diere. Hulle dien onder meer as broei-areas vir verskeie visspesies wat deur ontspanning- en bestaansvissers benut word. Die Swartkopsriviermonding (SRM) is ongeveer 10 km noord-oos van Port Elizabeth en word beskou as die enigste groot en bes-bewaarde riviermonding binne 'n stad, dus uniek in Suid-Afrika. Die SRM word omring deur van die digsbewoonde en mees geïndustrialiseerde gebiede in die Oos-Kaap. Die doel van hierdie studie was om die teenwoordigheid, vlakke, en die verspreiding van geselekteerde prioriteitsbesoedelstowwe in die voedselweb van die SRM te bepaal en te interpreteer.

Verskillende komponente binne die SRM was gebruik om te toets vir die teenwoordigheid van organiese en anorganiese besoedelstowwe. Sewe studie areas is gekies, met enkele daarvan wat ooreenstem met vorige studies in die SRM. Drie van hierdie studie areas was ook naby groot vrystellingspunte wat direk in die SRM vloei. Sediment, modder krewels, sand gobies, voëleiers, en verskeie vissoorte is ontleed. Monsters is ingesamel in die middel en laer dele van die riviermond, die gebied wat bekend is dat dit die meerderheid van besoedelstowwe ontvang. Swaarmetale in die sediment is ook vergelyk met vorige studies.

Bodemsedimente asook organismes wat rondom hierdie groot vrystellingspunte versamel is, toon hoër konsentrasies van besoedeling. Indien die vlakke vergelyk word met vorige studies, blyk dit dat die vlakke steeds toeneem. Weens die opbou en vermeerdering van gifstowwe deur die voedselketting, was hoër konsentrasies meer algemeen by die top-predatore, alhoewel sekere elemente nie hierdie eienskappe getoon het nie. Sommige swaarmetaalkonsentrasies in die vis oorskry ook voedsel riglyne en kan op sy beurt 'n bedreiging vir gereelde verbruikers van die SRM inhou. Daar is aanduidings dat daar meer as een bron van besoedeling is. Voëleiers het meetbare konsentrasies van poligechloreerde bifeniele getoon (PCB's), maar die implikasies hiervan moet verder bestudeer word.

Sleutelwoorde: Swartkopriviermond, swaar metale, grond, vis, modder krewels, voëleiers.

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# Chapter 1

## Introduction

### 1.1. Estuaries in South Africa

The South African coastline extends approximately 3000 km from the Orange River Mouth (28°38'S, 16°28'E) on the west coast, to Kosi Bay, Ponta Do Ouro (26°54'S, 32°53'E) on the east coast (Heydorn, 1989; Taljaard *et al.*, 2003; James & Harrison, 2010). The South African coastline has approximately 300 functional estuaries, ranging from small temporary open/closed estuaries, to large and permanently-open tidal estuaries (Fig 1.1; Turpie *et al.*, 2002; Taljaard *et al.*, 2003; Van Niekerk & Turpie, 2012). These systems cover approximately 70 000 ha (0.05% of South Africa's total surface area). Estuaries are considered one of the country's most productive habitat types (Morant & Quinn, 1999; Turpie *et al.*, 2002).

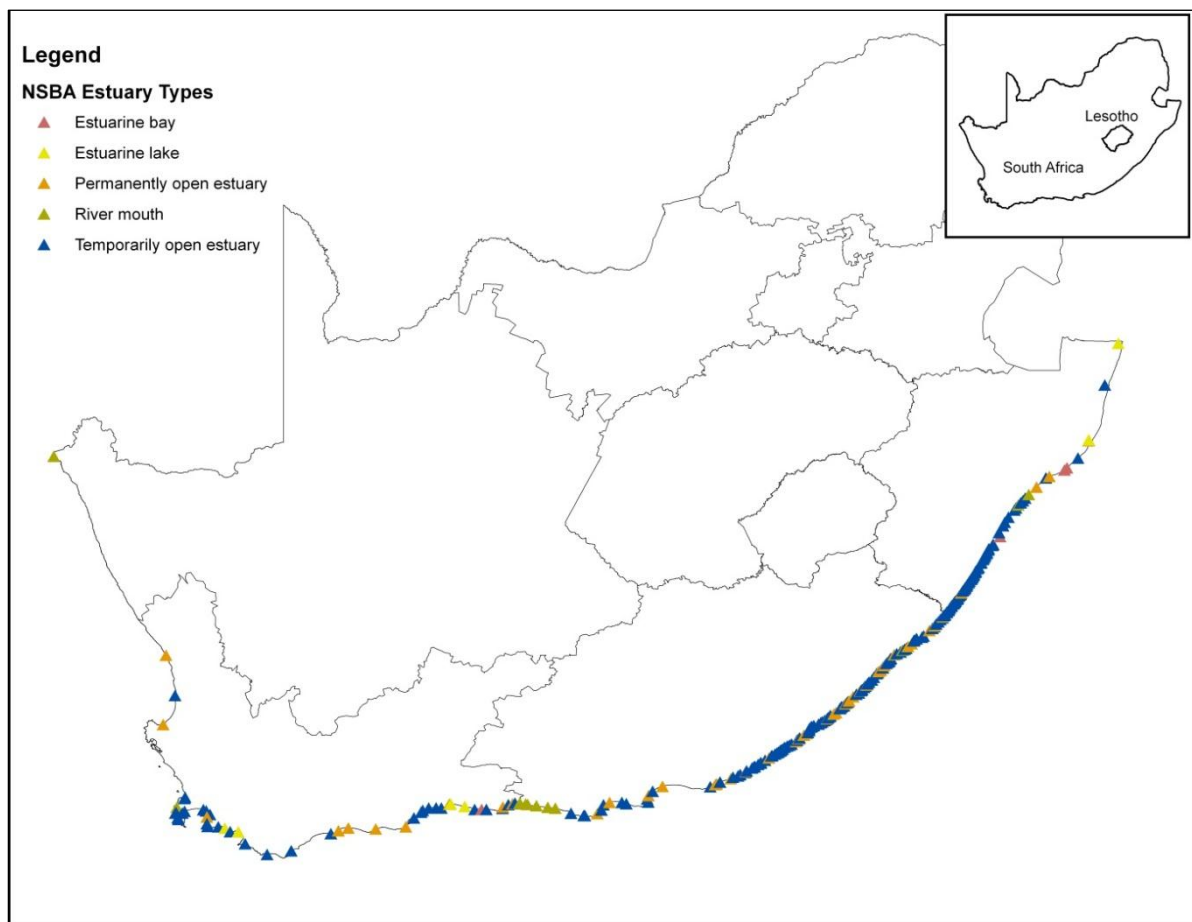


Figure 1.1. Map of South Africa, indicating all the estuarine types along the coastline. (With permission: BGIS, 2007).

## 1.2. Importance of estuaries

Estuarine ecosystems provide important refuge and feeding areas for many species. Their nursery role for fish, migration routes for fish species moving between oceans and rivers and feeding areas for fish and birds has been well described (Turpie, 2005; James & Harrison, 2010; Pattrick *et al.*, 2010). They also support a number of endemic and migratory species, many depending on estuaries for their survival and reproduction (Van Niekerk & Turpie, 2012).

Although estuaries constitute one of the most threatened habitats in South Africa, their current protection status is very poor (Van Niekerk & Turpie, 2012). A recent study on South African estuaries showed that a significant number (58%) are in good to excellent health, although these are generally smaller systems in rural areas with few anthropogenic pressures. Larger systems however, such as those of high economic and ecological importance and even more, great importance to fish nurseries, ranges from fair to poor due to pressures from the catchment, and degradation as a result of direct development in the estuarine functional zone. Most (85%) of estuarine habitats however, were in a fair to poor state, and there is a risk of this fraction increasing if appropriate management actions are further delayed (Van Niekerk *et al.*, 2013).

Estuaries have long been the focal point for human settlements and harvesting of marine resources (Heike *et al.*, 2006). During the 1970's, concerns grew about the conservation status of estuarine ecosystems in South Africa. It became clear that water abstraction, dam construction, coastal development, soil erosion, and agricultural and industrial pollution were affecting more and more estuaries (Morant & Quinn, 1999; Turpie, 2005; Newman, 2010; Van Niekerk *et al.*, 2013). Because of these pressures, many South African estuaries have become functionally degraded. Human impacts depleted >90% of formerly important species, destroyed >65% of surrounding wetland habitats, degraded the water quality, and contributed to the acceleration of alien species invasions, frequently accompanied by the loss of species (Turpie *et al.*, 2002; Heike *et al.*, 2006). Another pressure on estuaries is inadequate management (Morant & Quinn, 1999).

## 1.3. Management of estuaries

Rachel Carson, the author of *Silent Spring* (1962) said the following: “*Much of the necessary knowledge is now available but we do not use it. We train ecologists in our universities and even employ them in our governmental agencies but we seldom take their advice.*” One of the issues where the uptake of scientific advice has lagged behind the need to institute sustainable practices is in the management of estuaries. The framework for the

sustainable management of an estuary needs a legally accepted definition (Morant & Quinn, 1999). An internationally accepted definition for an estuary is a semi-enclosed coastal body of water which has a free and open connection with the sea and within which seawater is measurably diluted with freshwater from a terrestrial source (Fig 1.2; Van Niekerk, 2007). Since environmental managers need to operate within a legal framework, they require a 'watertight' definition. Ecologists can still function within the indistinctness in boundaries of a given ecosystem. However, from a management point of view, a definition of an estuary is required, which is legally unambiguous (Morant & Quinn, 1999).

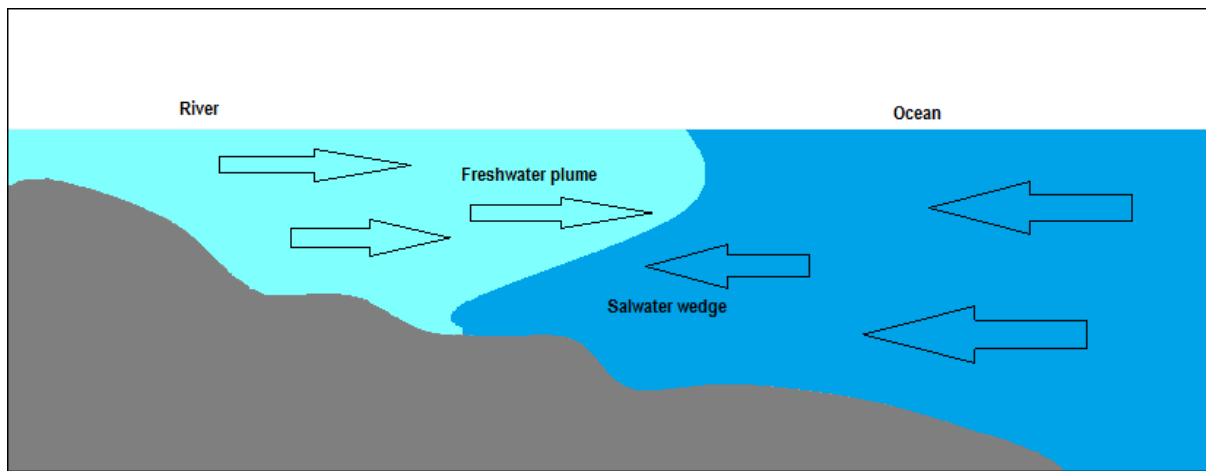


Figure 1.2. A schematic representation on the functioning of an estuary. The dilution of freshwater from the land and saltwater from the sea creates a dynamic and very productive system (Adapted from Biodiversity BC, 2009).

For effective management of estuaries, our knowledge needs to be focused on developing simple, well-motivated, and cost-effective strategies to monitor the condition of estuaries and predict the results of management actions (Morant & Quinn, 1999; Robertson *et al.*, 2002; Taljaard *et al.*, 2003). According to Morant & Quinn (1999), there are two important components necessary to achieve effective estuarine management, namely reference frameworks and predictive tools. Reference frameworks are required to rank estuaries in terms of their importance to certain biota; e.g. birds, fishes, vegetation, etc., or the ranking of estuaries themselves as complete systems. Predictive tools provide insight to the response of various components in the estuaries; they usually take the form of computer-based models (Morant & Quinn, 1999). Finally, management decisions should be converted into an implementation programme that can be cost-effectively monitored (Morant & Quinn, 1999). The collection and interpretation of monitoring data often proves to be time consuming and

requires scientific expertise (Taljaard *et al.*, 2003). Methods for these management components are discussed in Morant & Quinn (1999).

A complication of the above is fragmented communication and collaborative processes between researchers and policymakers, which often leads to 'gaps' between practice and management needs. An important aspect in the environmental policymaking, therefore, is learning. Learning is a key concept needed for monitoring and evaluation, and supports a broader policy-relevant science, in order to provide feedback and evidence on the effects of past policy decisions (Hermans *et al.*, 2013).

#### *1.4. Pollution of estuaries*

South Africa's National Programme of Action for Protection of the Marine Environment from Land-based Activities listed four key sources of pollution in estuaries (Van Niekerk *et al.*, 2013):

1. Municipal wastewater
2. Industrial wastewater
3. Storm water runoff
4. Agricultural runoff (herbicides, pesticides, suspended solids)

In South Africa, estuaries are subjected to major anthropogenic pressures that include freshwater abstraction, the overexploitation of living resources, coastal development, and agricultural and industrial pollution. Increasing human populations and the growing demand for freshwater constitutes a major threat to estuaries, not only on a national, but a global scale as well (Dafforn *et al.*, 2012; Van Niekerk *et al.*, 2013). The recent study of Van Niekerk *et al.* (2013) showed that 15% of South African estuaries are under severe pollution pressure and less than 1% (three systems) are subject to minimal pollution pressures, with most of these being fed by small local catchments located in national or provincial protected areas. The continuation of overfishing and the destruction of water qualities due to land-based activities on marine invertebrate populations may lead to strong and negative consequences on the well-being of local communities (Bodin *et al.*, 2013).

## Literature Review

### 1.5. Introduction to contaminants

*“The rapidity of change and the speed with which new situations are created follow the impetuous and heedless pace of man rather than the deliberate pace of nature”* and the time it would require to adjust to these situations would require a lifetime of generations (Carson, 1962). Concerns regarding environmental contaminants and their possible ecological effects have been the centre of attention for many decades, dating as far back as the 1950's and 1960's, where some agricultural pesticides were found to affect wildlife (Moriarty, 1999; Newman, 2010).

In 1999, Moriarty defined a contaminant as a substance released by means of man's doings, and unless it shows any biological effect on individuals this can be referred to as a pollutant. The term *“pollutant”* according to the National Water Act means *“the direct or indirect alteration of the physical, chemical or biological properties of a water resource so as to make it (a) less fit for any beneficial purpose for which it may reasonably be expected to be used; or (b) harmful or potentially harmful;*

- *to the welfare, health or safety of human beings;*
- *to any aquatic or non-aquatic organisms;*
- *to the resource quality; or*
- *to property”* (NWA, 1998)

Contaminants are often subdivided as organic or inorganic. This distinction is clearest when one focuses on the organic compounds; these are composed of carbon chains or rings (Newman, 2010). Organic contaminants are often divided into two categories; those intentionally released such as pesticides, insecticides, fungicides, and wood preservatives, while others are classified as unintentional such as degreasers, solvents, and industrial by-products. Organic pollutants released because of social requirements such as pharmaceuticals (antibiotics, drugs, and birth control products) and personal care products (detergents and perfumes) designed to benefit human needs remains a problem in the environment. Organic contaminants such as these become a problem when non-target species are exposed (Newman, 2010). Acute exposure can lead to the reduction of the organisms' abundance through mortality, whereas sub-lethal effects may cause reproductive impairment, behavioural impairment, or physiological stress (Fleeger *et al.*, 2003).

Inorganic contaminants, as for organic contaminants, are divided into intentional and unintentional; some released via specific purposes such as pesticide usage, while others are released through neglect or unintentionally due to human activities like those in the industrial

sector. These activities ultimately lead to above normal concentrations of certain elements, which can be harmful (Newman, 2010).

## *1.6. Organic contaminants*

### *1.6.1. Persistent Organic Pollutants*

#### *1.6.1.1. What are POPs?*

Persistent organic pollutants (POPs) are toxic organic compounds of natural and/or anthropogenic origin, that are resistant to photolytic, chemical, and biological degradation, and therefore persist in the environment (Bouwman, 2004). They have a low solubility in water, and accumulate in the food web and fatty tissues of living organisms (Lichtinger, 1997; Newman, 2010). These chemicals also have the ability to be transported over long distances, sometimes reaching areas where they have never been used before. Due to their toxicity (toxic, mutagenic, carcinogenic, etc.), they pose a threat to humans and the environment (WHO, 2003; Bouwman, 2004; Doong *et al.*, 2008).

POP chemicals that are produced and released into the environment due to human activities in the form of pesticides or industrial chemicals continue to pose risks to human health and ecosystems (WHO, 2003; Hanlon, 2009). The deliberate production and use of most POPs have been banned around the world; however, unintended production and releases of some POPs from anthropogenic and natural processes persist. Properties such as their stability are responsible for the difficulty in reducing their concentrations in the environment (Doong *et al.*, 2008; Hanlon, 2009).

#### *1.6.1.2. POPs and the Stockholm Convention*

The Stockholm Convention on Persistent Organic Pollutants (SCPOPs) is a global treaty focusing on protecting human health and the environment from listed persistent organic pollutants. This Convention was established with the aim of reducing the use and emission and ultimately eliminating the production of these POPs from anthropogenic sources worldwide (IPEP, 2006; Hanlon, 2009; SCPOPs, 2013). The Stockholm Convention on Persistent Organic Pollutants was adopted on 22 May 2001 in Stockholm, Sweden and entered into force as part of international law on 17 May 2004. As of January 2014, the Convention had 179 Parties (SCPOPs, 2014). These Parties are obliged to follow the actions set out by the Convention, to reduce and where feasible eliminate the production and/or release of these POP chemicals through a number of interventions (IPEP, 2006). This is mainly informed through the development of source inventories and release estimates as well as plans for release reductions, and taking actions based on this information. The Convention also requires making use of the best available techniques to reduce the release



of unintentionally produced POPs from major industrial sources (DEFRA, 2013). Assistance is offered to developing countries and countries with economies in transition to help implement the Stockholm Convention, as these actions would have low priority within the development agenda of developing states (IPEP, 2006).

The Stockholm Convention on Persistent Organic Pollutants originally listed twelve POPs, collectively known as the "dirty dozen" that can be placed into three categories (Bouwman, 2004; IPEP, 2006; Roos, 2010; SCPOPs, 2013).

- ✎ Pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene;
- ✎ Industrial chemicals: hexachlorobenzene, polychlorinated biphenyls (PCBs);
- ✎ By-products: hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs.

There are currently 22 POPs listed in the Convention, that includes 10 new chemicals added between 2009 and 2011 (DEFRA, 2013). The following chemicals have been added: chlordecone, alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, gamma-hexachlorocyclohexane (lindane), pentachlorobenzene (PeCB), endosulfan, hexabromobiphenyl, hexa- and hepta-bromodiphenylether, perfluorooctanesulfonic acid (PFOS; including its salts and perfluorooctane sulfonyl fluoride PFOS-F), and tetra- and penta-bromodiphenyl ether (DEFRA, 2013).

Parties may submit their proposals to list new chemicals in Annex A, B, or C of the Convention. This will be reviewed by the POPs Review Committee and recommendations are made to the Conference of the Parties on such listing in accordance with Article 8 of the Convention. The following chemicals are currently under review (SCPOPs, 2013):

- ✎ Hexabromocyclododecane (to enter into force on 26 November 2014);
- ✎ Short-chained chlorinated paraffins;
- ✎ Chlorinated naphthalenes;
- ✎ Hexachlorobutadiene and
- ✎ Pentachlorophenol

#### *1.6.1.3. How POPs are transported over long distances?*

Apart from trade in POPs and POPs-containing products, POPs can be transported through natural means over long distances often reaching the atmosphere, rivers and oceanic currents that consequently results in its widespread distribution across the earth, sometimes reaching areas where POPs have never been used or produced before (WHO, 2003; Bouwman, 2004). These compounds may be generated by anthropogenic processes

such as combustion, incineration, and industrial processes and introduced to the environment through various routes (Doong *et al.*, 2008). In aquatic environments, they are generally introduced through “point” and “nonpoint” sources. A point source refers to a specific outlet, such as a discharge pipe or smokestack from a factory. Nonpoint sources are usually many and diffuse sources of pollutants, such as urban and agricultural runoff (Gilkeson *et al.*, 2005; Oren *et al.*, 2006; Doong *et al.*, 2008). A point source is a situation where pollutants are discharged directly into coastal waters, seas, rivers, or canals that carry these contaminants to marine environments (Gilkeson *et al.*, 2005). These inputs are of particular importance to this study as estuaries are the major interface between land and ocean, receiving, accumulating (thereby increasing exposure to residential organisms), and releasing pollutants to air and water, and via assisted transport (suspended particles) and contaminated biota (Bhattacharyaa *et al.*, 2003).

Contaminants may also enter aquatic and snow (polar and alpine) environments in vapour form via gas exchanges. The chemicals accumulate in the ice or snow until they are released back into the ecosystem by means of snow melting and spring runoff. An example of this can be seen during spring in the Rocky Mountains, where glacial runoff feeds into many of the surrounding lakes and rivers and often contains high enough concentrations of POPs to affect wildlife at the top of most food webs (Gilkeson *et al.*, 2005). The conditions under which POPs are released into the South African environment are similar to those in developed countries, but there are additional sources and exposure pathways not normally found in developed countries, such as use of dichlorodiphenyl trichloroethane (DDT) in malaria control and subsistence activities (Bouwman, 2004).

#### 1.6.1.4. *The important POPs*

Since the majority of POPs analysed for in this study was below the level of quantification, only polychlorinated biphenyls (PCBs) will be discussed in more detail.

##### 1.6.1.4.1. *Polychlorinated biphenyls*

PCBs are a class of chemicals that has up to 209 individual chlorinated compounds known as congeners (Toaspern, 2003; ATSDR, 2011). They belong to a broad family of synthetic organic chemicals deliberately manufactured since 1929 and in the 1960s their increasing usage resulted in environmental and health concerns and by 1979 their manufacturing was banned (Pieters, 2007; USEPA, 2013).

PCBs are characterized by their low solubility in water and high lipid solubility that increases as the number of chlorine atoms increases (Perugini *et al.*, 2004). They have a strong tendency to bio-accumulate higher up in trophic levels and their persistence often leads to negative biological effects at both biological and cellular level (Toaspern, 2004).

They are not flammable which make them good insulators; they have a high electrical resistance, and remain stable even when exposed to heat (Barbalace, 2003). The introduction of PCBs to the environment are the result of extensive industrial uses such as additives in dyes, plastics, cable insulation, caulking, adhesives and tapes, carbonless copy paper and rubber products to name a few. Natural sources include forest and-vegetation fires (the result of chloride ions found in wood, soils and minerals), and volcanic eruptions (Gribble, 1994; Martínez *et al.*, 2000). PCBs also occur in range of phases: oily liquids, colourless solids, and vapour (Quinn, 2010; ATSDR, 2011; USEPA, 2013).

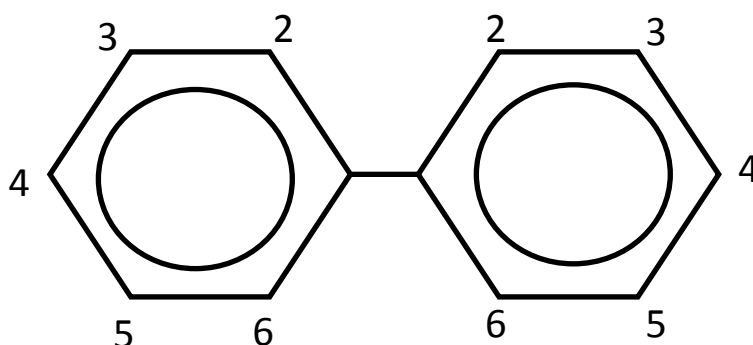


Figure 1.3. The basic structure of a PCB. It consists of two benzene rings with a carbon-carbon bond between carbon 1 on the first ring and carbon 1' on the second ring. It also indicates numbering of the potential chlorine positions (Barbalace, 2003).

As the number of chlorine atoms in a PCB mixture increases, the flash point increases, making the substance less combustible, less likely to volatilize, and more resistant to biodegradation (Fig 1.3; Barbalace, 2003). Their toxicity depends not only on the number of chlorines on the biphenyl but also their position. For instance, congeners with chlorines in both *para* positions (4 & 4') with at least two chlorines in the *meta* position (3, 5, 3', 5') are considered "dioxin-like" and are particularly toxic. When there is no or one chlorine substitutions in the *ortho* positions (2, 6, 2', 6'), the atoms of the congener are able to line up in a single plane, known as a "flat" configuration and are also particularly toxic (Fig 1.3). Specific PCBs are often given names, but more often numbers (Barbalace, 2003). The most common numbering system to refer to specific congeners was developed by Ballschmiter and Zell (1980). "It correlates to the structural arrangements of the PCB congener in an ascending order of number of chlorine substitutions within each sequential homologue." Unprimed numbers are considered a higher numbering priority than the corresponding primed number, and as a result, congeners are numbered from CB-1 to CB-209 (Ballschmiter & Zell, 1980). High-numbered congeners turned out to be those presenting the

greatest environmental and health risk (Barbalace, 2003). Due to a congener's physiochemical properties, their individual potential for inducing toxicity varies (Ashley *et al.*, 2009). The 12 most toxic congeners are CB-77, CB-105, CB-114, CB-118, CB-123, CB-126, CB-156, CB-157, CB-167, CB-169, and CB-189 (Pieters, 2007).

Considerable research has focussed on the presence, levels, and composition of PCBs in estuaries, such as known sources and how they are transported, and their fate within these systems. They enter estuarine systems via point and non-point sources, industrial and urban runoff, and atmospheric deposition (Barbalace, 2003).

## 1.7. Inorganic pollutants

### 1.7.1. Heavy Metals

#### 1.7.1.1. What are heavy metals?

Ecotoxicologists and environmental scientists use the informal term “heavy metals” to refer to those metals that may result in environmental problems if exceeding certain concentrations (Valavanidis & Vlachogianni, 2010). Heavy metals are persistent, stable (Fianko *et al.*, 2006), indestructible as an element, occurs naturally at locally normal background concentrations (Öztürk *et al.*, 2009), but are also widespread contaminants (if the concentrations are above background concentrations) in almost all aquatic environments (Jackson, 2005). They are chemicals with high densities and are generally toxic at low concentrations (Banjo *et al.*, 2010). Metals such as Co, Cu, Fe, Zn, and Mg are essential for the metabolic activities, but have the potential to become toxic at elevated concentrations, while some metals such as Cd, Pb, Hg, and Sn are toxic at low concentrations and have no known role in biological systems (Öztürk *et al.*, 2009; Adu, 2010; Kamaruzzaman *et al.*, 2010).

#### 1.7.1.2. Priority hazardous heavy metals

The Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) put together a list of all the priority hazardous substances, collectively known as the substance priority list. This list prioritizes substances based on their frequency of occurrence at National Priority List (NPL) sites, and their toxicity and potential for human exposure to these substances. This list is revised and published every two years with yearly informal reviews and revisions. Four of the priority substances in the top 10 of this list is heavy metals with arsenic (As) listed as number 1, followed by lead (Pb), mercury (Hg), and in the seventh place is cadmium (Cd) (ATSDR, 2011a). These four elements will be discussed in more detail below, together with four other metals of

environmental importance - they are chromium (Cr), copper (Cu), aluminium (Al) and zinc (Zn).

#### 1.7.1.2.1. Arsenic (As)

The metalloid arsenic is the first on the ATSDR list of toxic substances ATSDR, 2011). The toxicity of this metal has been known from ancient times and is commonly associated with accidental or deliberate poisoning, which may eventually lead to death (Cooksey, 2012). Both anthropogenic and natural sources contribute to the exposure of arsenic e.g. domestic wastewater, the most important source of arsenic to aquatic environments. Other sources include mining and smelting, fertilizers, chemical production, paint pigments, and wood preservatives (Redfern, 2006; Cooksey, 2012).

Arsenic is not a very abundant element in the earth's crust (1.8 mg/kg) (Erasmus, 2004) and relatively clean environments are considered to have concentrations of <10 µg/g, whereas grossly contaminated sites have been found to reach concentrations as high as 3732 mg/kg (Redfern, 2006). Exposure to high concentrations of inorganic arsenic (> 60 000 µg/kg in food or water) may be fatal while lower concentrations (300 – 30 000 µg/kg in food or water) may cause abdominal irritations with associated symptoms such as stomachache, nausea, vomiting, and diarrhoea (Erasmus, 2004; Redfern, 2006). Marine animals tend to accumulate arsenic in very high concentrations, which becomes a problem for people consuming contaminated fish on a regular basis (Erasmus, 2004). Long-term oral exposure can also lead to skin cancer (Erasmus, 2004; Redfern, 2006; Cooksey, 2012).

#### 1.7.1.2.2. Lead (Pb)

Lead is the 36<sup>th</sup> most abundant element in the earth's crust (13 mg/kg) (Erasmus, 2004) and defined as potentially hazardous to most forms of life according to USEPA. Although not biologically essential, Pb is considered toxic and relatively accessible to aquatic organisms (Erasmus, 2004; Adu, 2010). The accumulation of lead through marine animals may be up to 1400 times the environmental concentration (Erasmus, 2004).

For humans, blood concentrations above 0.8 mg/ℓ are associated with lead poisoning (average in humans is 0.25 mg/ℓ) (Erasmus, 2004). For fish, lethal Pb concentrations range from 1- 500 mg/ℓ, while prolonged exposure of 0.007-0.02 mg/ℓ may have adverse effects on their growth and biochemical responses (Erasmus, 2004). In coastal and estuarine sediments, Pb is reported to be in the 15-50 mg/kg

range worldwide and < 25 mg/kg for clean coastal sediments (Redfern, 2006; Erdoğan, 2009). Lead may enter natural waters via manufacturing processes and atmospheric deposition (e.g. metal production, burning of wood and coal, and refuse incineration). Other sources include domestic wastewaters, and sewage (Redfern, 2006; Erdoğan, 2009).

In humans, Pb has two distinct toxic effects; physiological and neurological. Exposure occurs through breathing or swallowing (Redfern, 2006; Erdoğan, 2009). Immediate effects of lead poisoning include nausea, vomiting, abdominal pains, mood disturbances, coordination loss, and anaemia. Neurological effects such as memory impairment, restlessness, and hyperactivity are examples of more severe situations (Erdoğan, 2009). Exposure to lead may also lead to miscarriages for pregnant women (Redfern, 2006; Adu, 2010).

#### 1.7.1.2.3. Mercury (Hg)

Mercury pollution is a growing concern worldwide and in the last 150 years Hg pollution has increased as much as twenty times (Haghighat *et al.*, 2011). Mercury enters the environment via anthropogenic and natural sources, but predominantly from coal fired power plant emissions (Chen *et al.*, 2009). When Hg is released into freshwater and marine ecosystems, it changes to its organic form methyl mercury (MeHg) and is biomagnified in the food web (Chen *et al.*, 2009). Volcanoes are the most common natural source, whereas anthropogenic sources include, chemical manufacturing, burning of fossil fuels, gold mining, and the discharge of domestic waste (Redfern, 2006; UNEP, 2013). Globally it is estimated that 30% of the annual mercury discharges are due to anthropogenic discharges, another 10% are due to natural geological sources, and the final 60% are due to 're-emissions' of previously released mercury that has been build up over decades in ocean floors and surface soils (UNEP, 2013).

This metal has three forms with its organic form, methylmercury, as the most toxic (Howard, 2002; Adu, 2010). Methylmercury is the result of mercury that has been transformed in aquatic systems, which is then accumulated through the food web (UNEP, 2013). This compound is of great concern due to its accumulation ability in edible freshwater and marine fish, with concentrations being much higher than that of the surrounding matrix (Redfern, 2006). The ingestion of Hg-contaminated fish is of particular concern for the local human populations living near oceans, rivers and estuaries, especially subsistence fishers (UNEP, 2013).

The trophic position of fish often determine the concentrations of accumulated metals such as Hg, for instance predatory fish such as the Spotted Grunter would be

expected to have higher Hg concentrations than their prey, which is the mud prawn (Haghighat *et al.*, 2011). Mercury is considered as a non-essential metal with no required function in any living organism, and is toxic even at low concentrations (Adu, 2010). Organic and inorganic mercury each presents different toxicities, with different effects on the human body. Organic mercury, such as methyl mercury affects the nervous system. Earlier symptoms of poisoning are numbness in hands and feet, whereas later symptoms include memory loss, insomnia, timidity, and delirium (also known as the mad-hatter disease<sup>1</sup>) (Adu, 2010; Haghighat *et al.*, 2011). Inorganic mercury may cause neurological and physiological symptoms such as depression, anxiety, personality changes, and kidney damage (Adu, 2010).

Global pattern models and measurements show that South Africa, along with North America, Europe, and South and East Asia has the highest concentrations of elemental mercury in the air and is highest in major industrial regions. For the past 15 years, continuous high air quality monitoring has been performed in Europe and North America, but only recently started in East Asia and South Africa as part of a global effort to expand coverage provided by long-term monitoring sites (UNEP, 2013).

#### 1.7.1.2.4. Cadmium (Cd)

Contrary to As, Hg and Pb, Cd is a more recently discovered toxic (1817) metal (Cooksey, 2012). Cd is considered as a priority pollutant, and listed as number seven on the ATSDR priority substance list (Erasmus, 2004; ATSDR, 2011a). This metal usually exists as complex oxides, sulphides, and carbonates in zinc, lead, and copper ores and because of its atomic similarity, it is often associated with zinc (Redfern, 2006; Erdoğan, 2009; Cooksey, 2012). The predominant dissolved form of Cd in freshwater is ionic, while in seawater, the chloride salt dominates. After entering aquatic systems, Cd accumulates in sediments, presenting a risk to benthic biota, and, under certain conditions, cadmium may re-enter the water column (Wright & Welbourn, 1994).

While Cd serves no biological function, it too, like Hg, Pb, and Sn is toxic at low concentrations, and extremely toxic to most plant and animal species (Erasmus, 2004; Cooksey, 2012). Cd is a rare element in the earth's crust (0.15- 0.2 mg/kg) and high concentrations found in the environment are attributed to anthropogenic activities such as metallurgical industries, municipal effluents, sewage sludge, pigments and plastics (Erasmus, 2004; Redfern, 2006). They can be found in water,

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<sup>1</sup> Mad hatter, like in Alice in Wonderland, refers to the use of mercury to make felt for hats and affected the mental health of the 'hatters'.

meats, grains, vegetables, and even cigarettes. Exposure is usually through breathing or consumption of contaminated foodstuffs (Howard, 2002; Erasmus, 2004).

The exposure of Cd can lead to several health implications. Profound exposures to Cd may cause severe respiratory irritation while occupational exposures may lead to chronic lung diseases or testicular degeneration. Lower concentrations of Cd exposure may damage the functional units of the kidney resulting in kidney damage or failure. Cadmium also affects the loss of calcium that can lead to the weakening of the bones generally known as the Itai-Itai disease (Howard, 2002). Two phases are associated with Cd poisoning; the first is the yellowing of teeth, loss of the ability to smell and a dry mouth, and secondly, a decrease of red blood cells which results in the impairment of bone marrow. Associated features of this disease include lumbar (lower back) pains, leg myalgia (muscle pains), and urinary excretion of aluminous substances (Erasmus, 2004).

#### 1.7.1.2.5. Chromium (Cr)

Chromium is the 21st most abundant element in the earth's crust, with a mean concentration of 122 mg/kg, and its main source is chromite ( $\text{FeO} \cdot \text{Cr}_2\text{O}_3$ ) (Erasmus, 2004; Erdoğan, 2009).

The main sources of Cr into marine environments apart from natural sources are industrial and domestic runoff and sewage sludge (Redfern, 2006). In the environment, Cr usually occurs in two valence states, trivalent chromium (Cr III) and hexavalent chromium (VI), with the latter being more harmful. Chromium (III) occurs naturally and is an essential nutrient whereas Chromium (VI) is most commonly produced by industrial processes (USEPA, 2000). Nowadays, chromium is extensively used in industries such as leather processing, and as a result, is becoming a global trend as a major factory run-off pollutant (Howard, 2002). Other sources of input include electroplating, metal finishing industries, and wood preservatives (Redfern, 2006; Newman, 2010).

Cr is carcinogenic, has a tendency to be corrosive, causes allergic reactions, and long-term exposures has been associated with lung cancer (Howard, 2002; Redfern, 2006). It is moderately toxic to aquatic organisms, although its hexavalent form is regarded as the most toxic type of chromium.

According to the United States Environmental Protection Agency (USEPA) sediment quality guidelines for Cr, marine sediment concentrations less than 25 mg/kg are considered non-polluted, 25-75 mg/kg moderately polluted and higher than 75 mg/kg heavily polluted (Redfern, 2006; Erdoğan, 2009).



#### 1.7.1.2.6. Copper (Cu)

Copper is an essential element that is toxic at high concentrations (Newman, 2010). It is a moderately abundant element in the earth's crust with a mean concentration of 68 mg/kg (Erasmus, 2004; Redfern, 2006). This metal have a strong tendency to accumulate in organisms and extreme accumulation of Cu, as seen in some bivalves, may cause their flesh to become green and develop a metallic smell (Erasmus, 2004). In nature, this metal may be present in its elemental form or as oxides, complex sulphates, and carbonates (Sekwele, 2008). It is widely used in agriculture, industries, municipalities and domestically, and is extensively used for wiring, electronics, plumbing and algaecides (Erasmus, 2004; Newman, 2010). Their introduction in marine environments is by means of atmospheric discharges, domestic and industrial wastewaters, incineration emissions and the dumping of sewage sludge (Erasmus, 2004; Redfern, 2006).

Cu deposits are often elevated in the sediments near the source of input where it strongly absorbs to organic material, clay, and carbonates, reducing their bioavailability (Redfern, 2006; Sekwele, 2008). Although copper is essential for the growth of most aquatic organisms, it becomes toxic at concentrations as low as 10 mg/kg. For sediments, Cu concentrations exceeding 200 mg/kg have been classified as heavily polluted (Redfern, 2006).

Cu is essential for good human health although exposure to high concentrations can be fatal. Long-term exposure may lead to irritation in the nose, mouth and eyes, and cause headaches, dizziness, nausea and diarrhea (Redfern, 2006).

#### 1.7.1.2.7. Aluminium (Al)

Aluminium is a naturally abundant element in the environment and listed under the top 10 metals of earth's abundant elements. It represents 8.3% of the earth's crust and is a major component of many indigenous minerals such as feldspars and mica (Erasmus, 2004). Under low pH conditions, for instance those resulting from acid precipitation or acid mine drainage can increase to unusually high dissolved concentrations that can substantially affect and even eradicate aquatic species (Erasmus, 2004; Newman, 2010). High concentrations of aluminium contribute to brain dysfunction in patients with severe kidney disease while undergoing dialysis. High concentrations of aluminium have been found in neurofibrillary tangles and furthermore in drinking areas and soil of areas with unusually high incidences of Alzheimer's disease (Howard, 2002).

In its purest form, aluminium is not toxic and is used extensively in the construction industry in materials for screens and doors, and the aerospace industry

in fuels (Erasmus, 2004). Aluminium has a variety of compounds used for different purposes such as water treatment, papermaking, fire retardants, fillers, food additives, and pharmaceuticals (EFSA, 2008). Consumption is the major exposure route in humans, primarily due to the increasing use of aluminium cookware and food packaging (Howard, 2002; EFSA, 2008).

The database on aluminium's carcinogenetic is generally limited with the majority of the studies being old and contains little to no experimental studies. Under normal and typical conditions, the accumulation of Al from food materials would represent a small fraction of the total dietary intake (EFSA, 2008).

#### *1.7.1.2.8. Zinc (Zn)*

Zinc is an essential element in biological systems and forms the building blocks of several proteins and structural components (Erasmus, 2004). It is a very widespread environmental element, constituting 76 mg/kg of the earth's crust and often found in association with lead and cadmium (Erasmus, 2004; Redfern, 2006). Although this is considered as an essential metal, it can be toxic in high concentrations and continuous exposure to high concentrations may cause anaemia, pancreatic damage, and decreased concentrations of high-density lipoprotein (HDL) cholesterol (Redfern, 2006; Adu, 2010).

It is widely used in modern societies in anticorrosion coatings, roof claddings, and manufacturing of dry batteries. The major anthropogenic sources of zinc to the environment include the discharge of domestic wastewaters, atmospheric fallout and particles released from vehicle tyres (Erasmus, 2004; Redfern, 2006; Adu, 2010).

Sediments are major sinks for zinc in aquatic environments and concentrations as high as 3000 µg/g have been reported close to mines and smelters (Redfern, 2006). In marine fish, zinc concentrations in major organs such as the kidney or liver will be higher compared to the concentration found in the muscle, and higher concentrations are even more common with increasing age or length of the fish (Erasmus, 2004).

#### *1.7.1.3. Sources and environmental transport of heavy metals*

Since industries utilize a number of these metals, it is almost impossible to determine the source of pollution in aquatic environments (Erasmus, 2004). Table 1.1 summarizes the industrial and agricultural sources of heavy metals in aquatic environments for the above-mentioned metals.

*Table 1.1. Industrial and agricultural sources of heavy metals in aquatic environments (compiled from Duffus, 1980; Denton et al., 1997; Erasmus, 2004; Orr, 2007; Newman, 2010).*

	As	Pb	Hg	Cd	Cr	Cu	Al	Zn
Alloys	X	X			X	X		X
Anti-fouling paint			X	X		X		
Cement, asbestos, glass		X			X			
Cosmetics	X	X	X	X	X	X	X	X
Electroplating				X	X		X	X
Inks					X	X		X
Leather tanning					X			
Motor vehicles	X	X			X		X	X
Organic chemicals, petro-chemicals, detergents	X	X		X	X			X
Paints	X	X	X		X			X
Pesticides	X	X	X	X		X		X
Petroleum refining		X		X	X	X		X
Pigments		X	X	X	X	X	X	X
Steel works		X		X	X	X	X	X
Water treatment						X		X

The occurrence of heavy metals is natural and universal. They are components of the lithosphere that is released into the environment through volcanism and the weathering of rocks. Anthropogenic sources (Table 1.1), more often than not, are associated with large-scale heavy metal releases (Redfern, 2006; Orr, 2007; Erdoğan, 2009). Metals released from human intervention include runoff from municipal wastewater treatments, industrial processes, combustion of fossil fuels, mining wastes, increasing urbanization, and recreational activities (Redfern, 2006; Shuping, 2008; Erdoğan, 2009; Bahnasawy *et al.*, 2011). An estimated 90% of particulate matter carried by rivers, streams, and canals eventually settles in estuaries and coastal environments, causing concentrations to be much higher. Increasing rainfall may also elevate the amount of pollutants entering these aquatic systems (Binning & Baird, 2001; Redfern, 2006; Orr, 2007). In the past 20 years, metal contamination within rivers and estuaries received much attention, due to the persistence and toxicity for many of these metals and their wider impacts on sustainability, ecosystem health, and concomitant implications for human health (Orr, 2007). Although not many of these metals serve any biological functions, they may act synergistically with other chemical pollutants to increase toxic impact (Binning and Baird, 2001).

#### *1.7.1.4. Heavy metals in water and sediment*

Understanding of the presence, levels, and distribution of heavy metals in natural waters are of extreme importance. There is a need to establish their influence on various ecosystems, and potentially reduce the manner in which they reach aquatic environments (Fianko *et al.*, 2006; Orr, 2007). This is often a difficult task to achieve since elemental metals are non-biodegradable and gets concentrated in the food web, producing their toxic effects sometimes very far from the original point of pollution (Bahnasawy *et al.*, 2011). Once heavy metals enter aquatic ecosystems, they tend to bind to the fine-grained sediment, such as mud and organic matter and deposit on the bottom (top sediment), increasing their tendency for contamination and accumulation of aquatic biota (Binning & Baird, 2001; Orr, 2007; Lin & Harichund, 2011). Because heavy metals are not indefinitely bound to the sediment, they may be re-mobilized and return to solution or become more bio-available. The concentrations of heavy metals in the sediment are typically higher in magnitude compared with the water column, therefore metal concentrations within the water column may meet the water quality guidelines, but the sediment may not (Binning & Baird, 2001; Orr, 2007). Contaminated sediments continue to threaten creatures in these marine environments and certain toxic sediments may kill the benthic organisms, thus reducing the food availability for larger animals such as fish (Erdoğan, 2009).

### *1.8. Effect of pollutants on vertebrates*

#### *1.8.1. Fish*

Fish consumption continues to increase worldwide, serving as an important human food source. Fish provides essential nutrition with fatty acids such as omega-3, proteins, vitamins and minerals (Kamaruzzaman *et al.*, 2010; Ozuni *et al.*, 2010). Fish form the largest and most important group of vertebrates in aquatic systems (Zhang *et al.*, 2007). However, increasing pollutant concentrations with their strong tendency to accumulate in these fish has concerns about hazard to humans (Ozuni *et al.*, 2010).

Various studies found that heavy metals have toxic effects in fish such as mutations, disruption of immune reactions, changing of blood parameters, reduction in adaption qualities, and resistance to diseases (Staniskiene *et al.*, 2006; Al-Weher *et al.*, 2008). Since fish species have the ability to occupy different trophic levels at different life stages, they are considered as good indicators for heavy metal contamination (Zhang *et al.*, 2007).

Multiple factors play a significant role in the accumulation of metals in different fish tissues e.g. different rates of accumulation, feeding habits, environmental factors (temperature & salinity), and size and age of fish (Indrajith *et al.*, 2008; Ozuni *et al.*, 2010; Murtala *et al.*, 2012). Since fish are generally at the highest point of the food web, they can potentially accumulate large amounts of these toxic pollutants throughout its lifetime (Suiçmez *et al.*, 2006). Various studies indicate that the accumulation of metals in fish occupying polluted waters may be considerable but does not seem to cause mortalities. The level of metal accumulation varies throughout the organs and may be attributed to different uptake, deposition, and excretion rates. The following metal level ranking is usually seen in fish, from highest to lowest: Fe > Zn > Pb > Cu > Cd > Hg (Jezierska & Witeska, 2006).

PAHs (polycyclic aromatic hydrocarbons) in wild fish have been well documented. Although they do not bioaccumulate in vertebrate tissues, they are known to cause adverse health effects (Newman, 2010). PCBs may cause neurological effects that may have serious effects on the immune system and which makes them susceptible for many other diseases and infections such as pneumonia and viral infections (Nieuwoudt, 2006).

### 1.8.2. Birds

There are increasing concerns about organic pollutants and their possible threats, not only to humans consuming contaminated seafood, but also the possible toxic effects on top predators such as birds (Leat *et al.*, 2011). By 1971, it was clear that any bird dependent on the marine food web anywhere in the world was unlikely to be free of organochlorine contamination (Ohlendorf *et al.*, 1978). This was recently confirmed by a study of bird eggs from an isolated oceanic island in the Indian Ocean (Bouwman *et al.*, 2012). Marine birds are continuously exposed to several types of environmental pollutants such as heavy metals, organohalogens, POPs, and others (Ohlendorf *et al.*, 1978; Leat *et al.*, 2011).

Numerous studies have shown that some PCBs and OCPs (organochlorine pesticides) have negative influences on the reproduction of wildlife and as a result, their populations. An example of this was seen in the 1950s and 1960s where bird populations in Europe and North America have decreased because of eggshell thinning that lead to increased embryo mortality (Quinn, 2010). Eggshell thinning is still observed due to the exposures of historical and modern releases of POPs. The concentrations of organochlorines in marine bird eggs reflect the diet of females at the time eggs was laid and possible concentrations in body reserves such as fat, showing birds to be good indicators of contamination (Goutner *et al.*, 2005; Quinn, 2010; Bouwman *et al.*, 2012).

Bird eggs are particularly useful for the analysis of pollutants because the egg contents can remain stable for a long time and they can be easily handled. In certain marine bird species, additional clutches will be laid should the first have been removed, thus having a minimal effect on the population. This is an important characteristic since studies are often only initiated once a population decline is observed (Ohlendorf *et al.*, 1978). The accumulation of highly toxic chemicals may lead to serious deformities or even death in birds. This is usually seen in top predators such as predatory fish (e.g. Dusky Kob) or fish-eating gulls (Nieuwoudt, 2006).

The exposure of heavy metals to birds is mainly via their food, water, respiratory exposure to airborne contaminants and the cleaning of their feathers. Numerous studies are focussed on the effects of certain pollutants such as DDT, PCBs, and mercury in bird populations, but less attention has been given to heavy metals. As with aquatic organisms, heavy metals may have profound consequences on bird populations, where they may weaken the immune system, change normal behavioural patterns, decrease reproductive success, and even death (Pickard, 2010).

Methylmercury (MeHg) is the toxic form of mercury and biologically, the most available. MeHg is also the molecular form of mercury found in most organisms. It can act as a powerful teratogen, neurotoxin and endocrine disruptor in vertebrates. A more recent study showed that experimental exposure to environmentally relevant dietary MeHg concentrations (0.05-0.3 ppm wm) resulted in homosexual pairing of male White Ibises (*Eudocimus albus*). It showed a decrease in egg productivity and dosed males were less approached by courting females compared to control males. Although male-male pairing behaviour has been reported on extensively, this mechanism has not been reported as the effect of MeHg exposure (Frederick & Jayasena, 2011). Environmentally relevant concentrations of pollutants such as mercury could have subtle but important effects on biota, such as on behaviour.

### 1.9. Effect of pollutants on human consumers

Metals exceeding normal concentrations may have detrimental long-term effects on human health (Jackson *et al.*, 2005) like in the case of the Minamata disease. In the 1950's, nearly 1000 people fell victim to the Minamata disease (Newman, 2010). The Minamata disease is a toxic nervous disease and major symptoms include sensory disturbance, ataxia, numbness in hands and feet, concentric constriction of the visual field and auditory disorders. Symptoms reported for extreme cases include insanity, paralysis, coma and death. In young it may cause neurological damage, which result in mental retardation,

seizures, vision and hearing loss, delayed development, language disorders and memory loss (UNEP, 2013a). The Minamata disease in the town of Minimata, Japan, is regarded as the most serious Mercury (Hg) poisoning incident. This disease was the result of consuming Hg-contaminated fish and other seafood. This disease was the first recognised pollution-derived disease, and led to the search of Hg in ecosystems around the world. As a result, Hg concentrations in fish have been recorded all over the world (Olivero-Verbel *et al.*, 2008). According to the World Health Organization (1990), Hg is an environmentally and toxicologically important element occurring naturally in ecosystems (Olivero-Verbel *et al.*, 2008). Fish samples are considered one of the most significant indicators for the estimation of heavy metal pollution concentrations in aquatic environments. Commercial and edible species have been widely investigated in order to determine those hazardous to human health (Öztürk *et al.*, 2009; Adu, 2010). There is much concern about the presence and concentrations of heavy metals in aquatic environments and their influence on plant and animal life (Fianko *et al.*, 2006). From an environmental pollution point of view, the removal of toxic heavy metals from industrial wastewaters is essential (Lin & Harichund, 2011).

The organochlorine family is the most important and persistent groups of all the halogenated hydrocarbons that includes the PCBs and DDTs. Due to the persistence of PCBs, they have a strong tendency to bio-accumulate in fatty tissues and are usually associated with the sediment of aquatic systems. The main route of PCB exposure in humans is through eating of fish, shellfish, or animal fats (Perugini *et al.*, 2004). Their slow natural breakdown is a characteristic that adds to the concerns about their potential health effects. Two events have been related with direct overexposure of PCBs in humans - the first of these poisonings occurred in Japan in 1968, and the second in Taiwan in 1979. In both circumstances, rice-oil that has been contaminated during processing (with thermally degraded PCB-containing heat transfer fluid from leaky equipment) was ingested, and many of the individuals consuming the oil (including children) became ill. Symptoms associated with this illness included decreased birth rates, somatic complaints, chloracne (toxic exposure to dioxins), and hyperpigmentation (Ross, 2004).

One significant difference between heavy metals and PCBs is their toxicity in the environment. Metals such as copper and mercury are toxic in its own right that also varies greatly with their molecular forms, whereas the toxicity of PCBs is dependent on the structure of the whole molecule. Should the molecule of a PCB be broken down, it loses its ability to cause pollution, whereas a metal such as copper does not disappear or change its elemental form (Moriarty, 1999).

## 1.10. Effects of pollutants on ecosystems

### 1.10.1. The movement of pollutants through the food web

Concerns' regarding the continuous increase of pollutants in marine ecosystems is a topic regularly talked about. The wide dispersion and accumulation of pollutants in wildlife are continuously effecting the composition and diversity of infaunal communities (Gyedu-Ababio *et al.*, 1999; Binning & Baird, 2001; Newman, 2010).

Contaminants become available to aquatic organisms via food ingestion which includes active (facilitated transport) or passive (water dissolved) diffusion (Eggleton & Thomas, 2004). These contaminants are taken up by benthic organisms in a process called bioaccumulation. When predators feed on these contaminated organisms, pollutants are accumulated in higher trophic levels. As a result, fish, shellfish, birds, and many other freshwater and marine animals may accumulate these toxic chemicals (Erdoğan, 2009).

Biomagnification is the increase of a contaminant's concentration from one trophic level to the next (Newman, 2010). Biomagnification is a possibility that must be considered in any assessment of ecological and/or human risk. Often predators may be larger than their prey and the allometric effects of bioaccumulation may result in higher concentrations of certain contaminants. An example of the latter can be seen in harbour seals, where the concentration of PCBs may be as much as five times higher than the fish they consume and a thousand times higher than the PCBs in surrounding waters. Organisms at the lowest levels of the food web tend to grow faster at higher levels. Therefore, growth dilution may be more prominent at the lower levels than those of the higher levels. Difficulties arise when one should define the trophic status of certain species since feeding habits tend to change with age (Newman, 2010). Through bioaccumulation, these compounds become more concentrated as living organisms take them up storing them in body tissues at a rate faster than they can be broken down or excreted (Gilkeson *et al.*, 2005).

### 1.10.2. Bioconcentration

The term bioconcentration describes the process by which chemicals or pollutants enter organisms directly from water through the gills or through epithelial tissues and in which the chemical concentrations exceeds that in the water (Gobas & Morrison, 2000; Katagi, 2010). This process is generally controlled by the physico-chemical properties of the chemical involved, the physiological deposition of each organism, and the surrounding environmental conditions (Katagi, 2010). In order to determine the environmental fate of chemicals released from industrial, agricultural or residential sources, it is essential to determine their bioconcentration in aquatic species (Ivanciuc *et al.*, 2006). This can be determined with a bioconcentration factor (BCF), which is the ratio of the chemical



concentration in an organism ( $C_B$ ) to the concentration in water ( $C_W$ ) (Gobas & Morrison, 2000). A BCF can be described as a parameter that represents the net effects of all bioaccumulation and elimination processes which affects the transfer of an element or molecule from food, water and particles to an organism (Templeton *et al.*, 1997).

$$BCF = C_B/C_W$$

### 1.10.3. Accumulation of pollutants by aquatic plants

Aquatic plants have long been the focus for water quality studies in order to monitor the presence of heavy metals and other pollutants. Aquatic plants can accumulate pollutants much greater than their associated surroundings, some as great as 100 000 times (Stottmeister *et al.*, 2006; Ebrahimi *et al.*, 2011; Alfadul & Al-Fredan, 2013). In wetlands, natural and constructed, aquatic plants are an important component and several studies reported a significant contribution of pollutant removal (Lee & Scholz, 2007).

The Common Reed (*Phragmites australis*) is one of the most ubiquitous plant species and has one of the largest geographical distributions in the world (Ye *et al.*, 1997; Lee & Scholz, 2007). This robust and-reed like perennial grass can grow as tall as three meters, has the ability to withstand extreme environmental conditions and can tolerate high concentrations of toxic heavy metal contaminants such as Zn, Cd and Pb (Ye *et al.*, 1997; Ma, 2005; Taban *et al.*, 2012; Alfadul & Al-Fredan, 2013). With its fibrous root systems covering vast areas, pollutants can be absorbed through roots and rhizomes. Because these plants are stationary and constantly exposed to their associated surroundings, they are considered as suitable bio-indicators for these pollutants (Taban *et al.*, 2012; Alfadul & Al-Fredan, 2013).

The common reed, although a very invasive and problematic plant, can be seen as effective and an alternative low-cost method to treat wastewater whilst promoting the reuse and recovery of wastewater (Saltonstall, 2002; Ebrahimi, 2011; Home *et al.*, 2012). In the last two decades, the common reed has been widely used in constructed wetlands for the treatment of mine- and industrial wastewaters (Ye *et al.*, 1997; Ma, 2005; Stottmeister *et al.*, 2006). Constructed wetlands are engineered systems designed and constructed to simulate the natural processes associated with wetlands to treat wastewaters. Their purpose is to take advantage of these natural processes provided by wetland vegetation, soils, and associated microbial assemblages, but in a more controlled environment (Kivaisi, 2001; Vymazal, 2010). Constructed wetlands may differ in their design characteristics as well as the processes responsible for pollutant removal (Vyzamal, 2010). In February 2010, the SRE opened its first constructed wetland below the Motherwell Canal. This is discussed in Section 2.1.3.

From historic images of the Chatty River, it is clear that the common reed have increased tremendously. This increasing reed beds are evident throughout the Chatty River and although they are considered as a threat to the natural ecosystems, they may well be considered an advantage from a pollution abatement point of view.

### *1.11. Rationale*

Estuaries are among the most productive and diverse of aquatic habitats. They support a rich variety of plants and animals (Eddy, 2005), and are known to be often more productive than neighbouring freshwater and marine environments. Estuaries are sheltered environments and often serve as important nursery areas for various fauna and flora (Strydom, 2008; James & Harrison, 2010). Estuaries also support various economic and recreational activities but are also subject to large amounts of wastewater discharges (Eddy, 2005). The SRE (Swartkops River Estuary) is a prime example of an urban system subject to countless forms of anthropogenic impact, especially pollution. Little research is available on the chemical aspects of this type of environmental degradation in South Africa. Sewage discharge and agricultural runoff has been linked to eutrophication of estuaries while industrial effluent results in organic and inorganic pollution of ecosystems (Schlacher & Wooldridge, 1996). Despite this knowledge, very little information exists on the effects of chemical pollutants on ecosystem components in estuaries of South Africa. The urban SRE was selected as an ideal candidate to assess the presence, levels, distribution and potential threats of anthropogenically derived organic chemicals and metal pollutants in a heavy polluted South African estuary.

### *1.12. Aim and Objectives*

#### *Aim of research*

The aim of this project is to determine and interpret the presence, levels, distribution, and potential threats of pollutants in the estuarine food web using candidates from the Swartkops River Estuary, South Africa.

Key objectives in support of achieving the aim of this study:

1. Identify priority pollutant categories that need further investigation given the anthropogenic activities in the SRE.
2. Identify key biological and physical elements associated with the identified priority pollutants.

3. Identify areas of potential concern in the ecosystems and provide links between pollution levels and known industrial activity.

### *1.13. Hypotheses*

Hypotheses 1: Bottom sediment concentrations will be higher at discharge points. The corresponding null-hypothesis would be that the bottom sediment concentrations would not be higher at the discharge points.

Hypothesis 2: Pollutant concentrations will be higher within organisms in the lower and middle reaches of the estuary since they are exposed to direct discharges. The corresponding null-hypothesis would be that the pollutant concentrations would not be higher in organisms located in the middle and lower reaches of the estuary.

Hypothesis 3: Toxic pollutants will bio-accumulate from lower to higher trophic levels. The corresponding null-hypothesis would be that toxic pollutants will not bio-accumulate from lower to higher trophic levels.

These hypotheses were tested by measuring concentrations of pollutants, comparisons with historic data and comparisons between matrices

## Chapter 2

### Study area and methods

#### *2.1. Study area*

The study area selected for this project is on the south-eastern coast of South Africa. The Swartkops River Estuary (SRE) is situated approximately 10 km northeast of Port Elizabeth and has been selected for this study since it is a unique and important wetland system (also having the country's third largest intertidal saltmarsh) in South Africa (SA). It is also the only major well-preserved estuary within a city in SA (Winter, 1979; Enviro-Fish Africa, 2009). The Swartkops River is surrounded by a highly urbanized and industrialized region in the Eastern Cape that forms an essential part of Nelson Mandela Metropolitan and the surrounding areas (Fig 2.1; Binning & Baird, 2001).



*Figure 2.1. An aerial view of the Swartkops River Estuary (With Permission: Spearmon, 2008)*

### 2.1.1. Swartkops River

The Swartkops River was first colonized in the 1700's by farmers migrating eastward from the Western Cape (Baird *et al.*, 1986). Since then, urban development rapidly increased and the Swartkops catchment now holds the largest number of inhabitants of the larger Port Elizabeth metropolitan (Binning & Baird, 2001).

The Swartkops catchment surface area is approximately 1360 km<sup>2</sup>, and can be divided into three sections (Fig 2.2). The main catchment area lies in the Groot Winterhoek Mountains, west of Uitenhage, where the lower river receives its water mainly from the Swartkops and Elands rivers. These two rivers meander alongside one another before feeding into the lower floodplain and the estuary, contributing to frequent floods. Further to the south is the Chatty River, the largest tributary flowing directly into the estuary, joining the Swartkops River where they flow out into the open sea (Baird *et al.*, 1986; Enviro-Fish Africa, 2009). The Swartkops catchment covers half of Port Elizabeth's municipal area including Uitenhage and Kwanobuhle, Despatch, and Ibhayi (Binning & Baird, 2001). These areas have a large variety of land uses that includes residential townships, industrial estates, and railway systems. Adjacent to the estuary are three major residential areas, Swartkops Village, Redhouse and Amsterdamhoek / Bluewater Bay (Baird *et al.*, 1986; Enviro-Fish Africa, 2009).

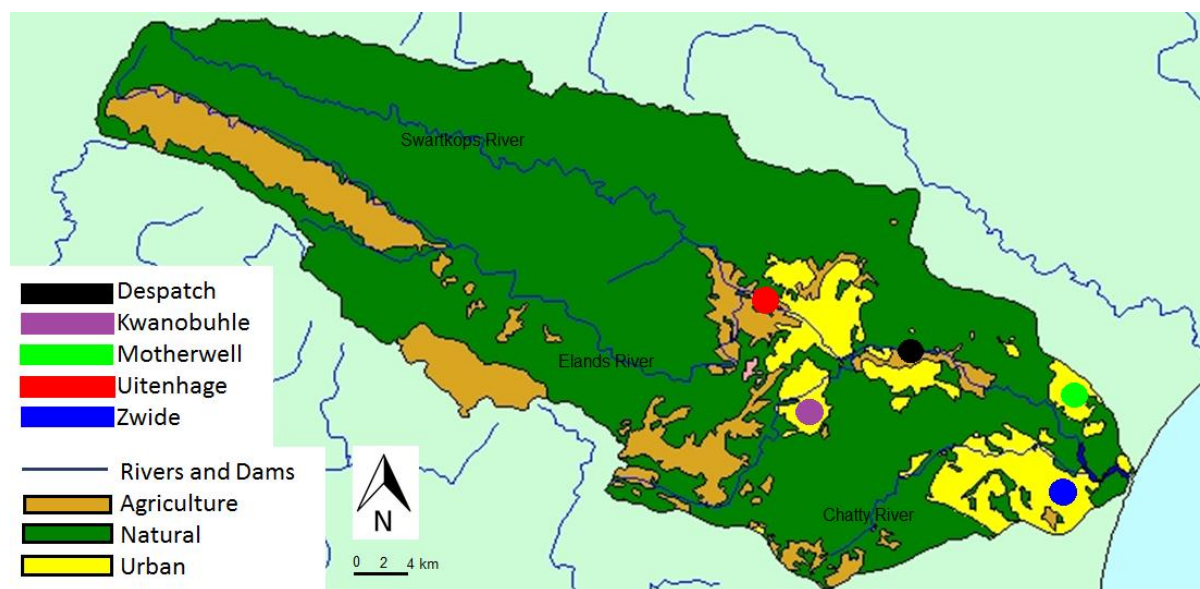


Figure 2.2. Generalised Land-cover Map for the Swartkops Catchment (Adapted from Department of Environmental Affairs, 2013)

The primary form of land-use around the estuary is industrial. Activities include the Algorax (previously Philips Carbon Black manufacturing carbon black from oil), Fishwater Flats sewerage works, Swartkops power station (now closed-down), brick manufacturing, motor

manufacturing and parts industries, saltpans, tanneries, railway yards and depots, and the Marksman Industrial area on the northern plateau. Located in the middle and lower reaches of the estuary is the Swartkops Aloes Nature Reserve (Enviro-Fish Africa, 2009). Agricultural activities around the estuary are restricted due to the saline soil in most areas, but do take place higher up the river around Uitenhage, Despatch, and Kwanobuhle (Baird *et al.*, 1986).

### 2.1.2. Swartkops River Estuary (SRE)

The SRE is an essential aquatic ecosystem and a valuable recreational and ecological asset for the region resulting in high-density development (Binning & Baird, 2001; Snow *et al.*, 2012). It is estimated that approximately one million people live and work in the Swartkops River catchment (Binning & Baird, 2001). The Swartkops River catchment contains not only the largest metropolitan population (Port Elizabeth or the Nelson Mandela Metropolitan area), but is also the area where the largest diversity of urban users can be found, and where urban growth is most rapid (Binning & Baird, 2001).

### 2.1.3. Ecological value of the estuary

Key invertebrate species like *Upogebia africana* (mud prawn) and *Callinassa krausii* (sand prawn) play an important role in the transfer of energy to higher trophic levels such as fish and birds (Hodgson *et al.*, 2002; Jooste, 2003). Mud prawn is the most abundant macrobenthic species, comprising up to 82% of the total macrofaunal biomass (Nel & Branch, 2013). In the SRE, mud prawn is prey to five of the major bird species, where it consists 58% of the Kelp Gull (*Larus dominicanus*) diet. It is also an important prey for the Spotted Grunter (*Pomadasys commersonnii*) (Cretchley, 1996; Enviro-Fish Africa, 2009).

The majority of the fish in SRE are marine migrants, using these systems as nursery areas from as early as the postflexion larval stage and return to the marine environment after substantial grow out (Strydom, 2008; Enviro-Fish Africa, 2009; Wasserman, 2010). Many of South Africa's important recreational and commercial line fish make use of these systems for survival and growth. These fish include Spotted Grunter, Dusky Kob (*Argyrosomus japonicus*), Garrick (*Lichia amia*), White Steenbras (*Lithognathus lithognathus*) Cape Stumpnose (*Rhabdosargus holubi*), and Riverbream (*Acanthopagrus vagus*) (Enviro-Fish Africa, 2009; Wasserman, 2010).

The SRE is also one of the most important estuaries for birds in South Africa. On average, this system holds up to 14 500 birds per year and up to 4000 birds are regularly present at the Redhouse Saltpans and SRE (Enviro-Fish Africa, 2009). The two islands at the Redhouse Saltpans are considered as the most important breeding sites for birds in the Eastern Cape, and host some of the largest breeding colonies for several species namely, White-Breasted Cormorants (*Phalacrocorax lucidus*), Sacred Ibis (*Threskiornis aethiopicus*),

Kelp Gull (*L. dominicanus*) and Grey-headed Gull (*Larus cirrocephalus*) in the province (Enviro-Fish Africa, 2009). These birds also contribute to the biological functioning of the estuary as both consumers and recyclers (Hockey & Turpie, 1999).

#### *2.1.4. Pollution in the Swartkops Estuary*

Pollution in the SRE has been an on-going problem, recognised since the 1950's. The Swartkops Conservancy was started by citizens in response to the continued decline in water quality and the increasing threat of pollution in the SRE on 19 June 1968 (Rump, 2013). Pollutants from industrial and sewerage discharges enters the estuary on a regular basis via storm water canals and contributory rivers and streams (Baird *et al.*, 1986). The first heavy metal survey in the SRE was conducted some 30 years ago by Watling & Watling (1982), measuring heavy metal concentrations in water and sediment. A more recent, but similar study was done by Binning & Baird (2001). The results indicated that the SRE showed clear indication of anthropogenic heavy metal contributions which have substantially increased in the last two decades (1982-2001).

On 19 February 2010, the Nelson Mandela Bay Municipality in the Eastern Cape opened its first constructed artificial wetland system (Fig 2.3). Believed to be the first of its kind in South Africa, it was designed as a pilot scheme for the Motherwell Canal (Steward *et al.*, 2010). The primary function of the Motherwell Canal is to discharge storm water into the Swartkops Estuary, but due to continuous sewerage spills from the Motherwell residential area, the water became increasingly contaminated (Steward *et al.*, 2010). After the opening of the constructed wetland, water quality monitoring was performed (although pollutant concentrations were not examined during this time), and the concentration of faecal coliforms and total coliforms showed a reduction (Steward *et al.*, 2010).





*Figure 2.3. The constructed wetland below the Motherwell Canal. This canal originates from the low-income residential area, Motherwell (Steward et al., 2010).*

The environmentally unsustainable urbanisation and development along the Swartkops catchment raises reasons for concern (Fig 2.5) (Enviro-Fish Africa, 2011). Canals, like the one leading straight from the Motherwell residential area are continuously polluted with alleged illegal dumping in spite of it being designed for the discharge of storm water (image top left and right, bottom right, own observations). The bottom left image is standing rainwater in the Markman Industrial Area with accumulated waste products.





Figure 2.4. Images depicting the pollution around the Motherwell Canal (see text for detail; own observations).

#### 2.1.5. Human influences around Swartkops River Estuary

The estuary's proximity to a major city and number of urban and informal settlements bordering it makes it a very popular angling site. Numerous boats and shore anglers can be seen fishing in the system on any day, with the majority being subsistence fishers. The exact number of the subsistence fishers is not known (Enviro-Fish Africa, 2009).

During the week, approximately 20 boats can be seen on the estuary, and over weekend and holidays, this number can more than double. There are also a number of jetties and slipways along the estuary, especially at Amsterdamhoek/Bluewater Bay, adding to the pressure on the system.

Subsistence bait collectors are also seen on a regular basis collecting bait for selling to recreational anglers. A number of these collectors are licensed to collect bait for selling, but

unlicensed collecting does take place, probably leading to the overexploitation of the food resources necessary for wading birds and fish such as Spotted Grunter.

Melville-Smith and Baird (1980) conducted a fishery survey between 1972 and 1978; the Spotted Grunter was by far the most dominant catch by anglers, comprising 87% of the total catch, approximately 127 Grunters a month. This was followed by Garrick, and White Steenbras, each comprising 3% of the total catch. The Dusky Kob and White Steenbras only became a more popular catch species in the early 20<sup>th</sup> century, when Spotted Grunter comprised only 2% of the total catch. The current fishery is not much different from the 1980's, with Spotted Grunter still dominating most of the catches from the estuary (Enviro-Fish Africa, 2009).

## *2.2 Methods*

### *2.2.1. Sampling*

Samples were collected in the SRE over a period of a month from mid-September to mid-October in 2012, coinciding with high fish abundance and bird breeding. All samples were collected from the lower to middle reaches of the SRE. This region is known to receive the largest portion of domestic and industrial discharge. Figure 2.5 shows a map of the SRE and each of the sampling points. The GPS points for each of the sampling points are shown in Table 2.1. The map also indicates the three major discharging points. All discharging points that end in the estuary have meandered through informal settlements, industrial areas, sewerage farms, and so forth, collecting pollutants from discharge and run-off. Four of these sites also corresponded to the study of Binning & Baird, 2001.

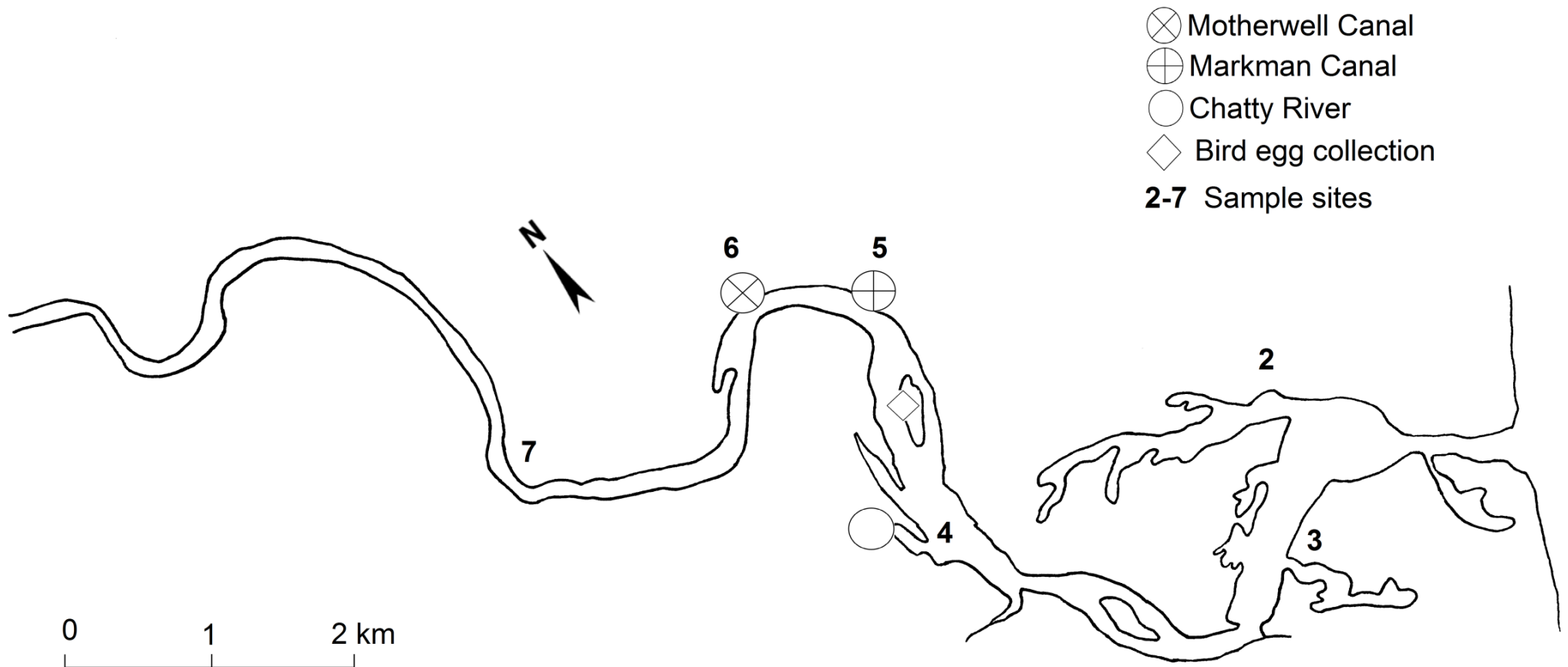


Figure 2.5. Map of the Swartkops River Estuary showing the sampling sites and the main discharge points. Sediment collection (Sites 2-7), mud prawn (Sites 3-6), bird egg collection (isolated island within the estuary).

Table 2.1. GPS points for each of the sampling points

	Latitude	Longitude
2	-33.853619	25.614124
3	-33.868158	25.612098
4	-33.85306	25.593856
5	-33.84305	25.600435
6	-33.836424	25.594709
7	-33.837384	25.570025

The necessary collection permits were obtained. The permit for the bird eggs was obtained from the Department of Economic Development, Environmental Affairs and Tourism, Eastern Cape (PERMIT NOS. CRO 124/12CR). The permit for sediment, fish and invertebrates was an integrated research permit jointly issued by the National Department of Environmental Affairs and the National Department of Agriculture, Forestry and Fisheries (Permit Reference Number: RES2012/92). The applications were based on ethical clearance from the NWU (NWU-00055-07-A3).

Sampling involved different components of the food web that includes sediment, invertebrates, and vertebrates. Mud prawn represented the invertebrates where organic pollutants and heavy metals have been analysed. Vertebrates included six fish and one bird species, the Kelp Gull. Organic pollutants was analysed in both fish and bird samples, and heavy metals was only measured in the fish samples.

The fish that were sampled were:

- ✎ *Gilchristella aestuaria* (Estuarine Round Herring),
- ✎ *Psammogobius knysnaensis* (Knysna Sand Goby),
- ✎ *Mugil cephalus* (Flathead Mullet),
- ✎ *Lichia amia* (Garrick/Leervis),
- ✎ *Argyrosomus japonicus* (Dusky Kob),
- ✎ *Pomadasys commersonnii* (Spotted Grunter)

Sample analysis for the heavy metals was performed at the Eco Analitica Laboratory at the North-West University, Potchefstroom<sup>2</sup>, and analysis for organic pollutants was performed at RPS Mountainheath<sup>3</sup>, United Kingdom.

<sup>2</sup> Thabo Mbeki Drive (R501) - Out on the Carletonville Road Potchefstroom 2531

<sup>3</sup> 2 Shaftesbury Industrial Centre, Icknieldway, Letchworth, Hertfordshire SG 6 1 HE

### *2.2.2. Pre-cleaning of equipment*

Before any equipment was used, they were washed three times with acetone and hexane according to the EPA Method 1668B (USEPA, 2008). This removes all polar and non-polar bindings and any other trace elements that may still be present.

### *2.2.3. Sediment sampling*

Sediment samples were collected from the six sampling points as indicated in Fig 2.5. Samples were collected with a standard core borer (Fig 2.7 a); five scoops were taken a few meters apart and placed in a large container where it was thoroughly mixed. From this mixture, six sub-samples of 200 grams each were placed in pre-cleaned 250 ml polypropylene Nalgene bottles. These six samples were furthermore divided into two groups, one for heavy metals, and the other for organic pollutants. The bottles were then placed on crushed ice until it could be frozen. It was kept frozen until analysis could be performed.

### *2.2.4. Invertebrate sampling*

Mud prawn was collected from the four sampling points indicated in Fig 2.5. Mud prawn was collected by using a stainless steel prawn pump to pull prawns from their U-shaped burrows. Again, six samples from each site were collected. Three sample sets were collected for heavy metals, which were placed in Zip-lock bags. Another three sample sets were collected for the analysis of organic pollutants and these samples were placed in pre-cleaned (using acetone and hexane) aluminium foil. For each sample, an average of 30 grams was collected, and the samples were labelled accordingly. After collection, they were placed on ice to slow down their metabolism, until they could be frozen.

### *2.2.5. Vertebrate sampling*

#### *2.2.5.1. Ichthyofuana sampling*

All fish species were collected from the SRE. Larger fish such as the Spotted Grunter, Dusky Kob, and Garrick (Fig 2.6 b-d) was caught with fishing rods and artificial bait whilst smaller species were caught with seine nets. Since fish are very mobile and not restricted to one place in the river, they were collected from sites distributed all over the estuarine region. Larger fish were caught from a boat where they were also immediately processed. First, they were euthanized by administering a forceful blow to the head with a fish club. They were then weighed and measured. Three of the species (Garrick, Spotted Grunter & Flathead Mullet) lengths were recorded to the fork of the tail, whereas the total length was recorded for Dusky Kob. Liver, muscle, and fat samples were collected for each fish. The tissue samples analysed for heavy metals were placed in Ziploc type bags or pre-cleaned Falcon tubes and those for organic pollutants in pre-cleaned aluminium foil. Each sample had an average

mass of 30 g, except less for the liver because of its small size. Samples were placed on ice until they could be frozen on the same day. Before every fish were sampled, all apparatus were cleaned three times with acetone and hexane to eliminate the effect of contamination. All fish remains were returned to the estuary for crabs and other scavengers.

The smaller fish (smaller than 20 cm) such as the Flathead Mullet (Fig 2.5 a) was collected with a small cast net from the boat. They were euthanized in the same way as larger fish and placed on ice until they could be frozen. Since this species was too small for individual sampling of most organs, only muscle tissue was sampled. The removal of muscle was done at the North-West University Laboratory.

The two smallest fish species, the Estuarine Round Herring and Knysna Sand Goby , (smaller than 8 cm), was collected by using seine nets. They were collected in different parts of the estuary, where these were most likely to occur. Because of their size, they were collected in mass and was analysed as such. The fish were also placed on ice until it could be frozen.

#### 2.2.5.1.1. Fish species sampled



a. *M. cephalus* (Flathead Mullet)

Nine fish were collected with a mean length of 24 cm. Because of their small size, only muscle was sampled.



b. *L. amia* (Garrick)

Six fish were collected with a mean length of 37 cm. Liver, muscle and fat were sampled.



c. *P. commersonnii* (Spotted Grunter)

Six fish were collected with a mean length of 50 cm. Liver, muscle and fat were sampled.



d. *A. japonicus* (Dusky Kob)

Six fish were collected with a mean of 39 cm. Liver, muscle and fat were sampled.

Figure 2.6. Fish species sampled in the Swartkops River Estuary



#### 2.2.5.2. Sampling of avifauna

Bird eggs were collected from an island within the estuary (Fig 2.5). Only one species, Kelp Gull (*L. dominicanus*) was targeted (Fig 2.7b). The Kelp Gull is the dominant avifaunal species around the SRE and lies between 2-3 eggs per clutch (Fig 2.7c). Only two eggs were collected from each of the eight nests sampled. The nests were randomly selected, and in the case where more than two eggs were present, the two “oldest” eggs were selected. The eggs were selected based on size, where the smallest egg was expected to be last laid (Quinn, 2010). Where the distinction between the eggs could not be observed, eggs were randomly selected. After eggs were collected, they were wrapped in pre-cleaned (acetone and hexane) aluminium foil, covered in ice, and immediately frozen. They were kept frozen until further treatment could be performed at the North-West University, Potchefstroom.

At the NWU laboratory, the egg contents were homogenized and eggshell thickness was measured (further discussed in next section).





*a. Sediment samples was collected by using a core borer and cores were deposited into a container to be mixed*



*b. Kelp Gulls on the island where they are breeding*



*c. Kelp Gull eggs- from each nest two eggs were collected*

*Figure 2.7. Images that represents the sampling around the Swartkops River Estuary*

## *2.2.6. Laboratory analysis*

### *2.2.6.1. Preparation and homogenization of bird eggs*

Eggs were removed from the freezer and immediately weighed and measured. They were weighed by using a digital scale and dimensions were then measured. The circumference of the eggs was measured at the widest and longest points, using a piece of string and measuring tape. The maximum length and width of the eggs were also measured, using a digital calliper. All measurements were done in triplicate.

For the rest of the homogenization procedure, lights were switched off to prevent the degradation of brominated flame-retardants in the samples. For thawing, eggs were placed in pre-cleaned tin foil cups; this ensured that any of the egg contents leaking through the cracked shells into the foil container would not be lost. When the eggs were completely thawed, the contents (albumin and yolk) were placed in pre-cleaned HDPE bottles by means of a funnel. The pre-cleaning procedure involves all apparatus to be triple-washed with acetone, followed by hexane. The HDPE bottles were weighed thrice before and after the egg contents were placed in the bottles. All bottles were clearly marked for each of the samples. Eggs were then homogenized using an ultrasonic liquid processor (ULP) while taking care that minimum foam is produced. Between homogenising each sample, the homogenizer was cleaned with warm water and soap followed by a double-distilled water rinse, a 96% ethanol rinse, followed by acetone and hexane. All other apparatus was cleaned in warm water, rinsed with distilled water and triple-washed with acetone, followed by hexane. Egg contents were placed into a -20°C freezer until further analysis could be performed. The remaining eggshells were gently washed with soap and water with a soft brush to remove the remaining membrane. Shells were left for three weeks to completely dry until further analysis was performed.

Eggshell measurements: The thickness of eggshells was measured at the apex of each shell, and each measurement was repeated three times for the average of nine measurements with an accuracy of 0.01 mm. This was done using an electronic digital calliper.

#### 2.2.6.2. Heavy metal analysis

All samples were analysed for the presence of heavy metals, except bird eggs.

Before samples were sent for the heavy metal analysis, they were placed in a -60° C freezer for two hours before being freeze-dried. This was only applicable to the fish and invertebrate samples. Sediment samples were dried prior to analysis at Eco-Analitica. For the analysis of heavy metals, the USEPA 3050b method was used and measured with an inductively coupled plasma mass spectrometer (ICP-MS) as follows:

1. 2 grams of material was weighed and placed into a 150 ml beaker.
2. 15 ml concentrated nitric acid ( $\text{HNO}_3$ ) was added to the material and the beaker was covered with a watch glass.
3. The mixture was placed on a sand stove at medium heat ( $\pm 95^\circ\text{C}$ ).
4. The mixture was allowed to reflux for at least an hour (but not boil).
5. After the fumes have settled and the sample appeared digested, the watch glass was removed in order to allow the acid to evaporate.
6. The mixture remained on the stove uncovered until the volume was reduced to  $\pm 5$  ml. Care was taken to ensure the mixture did not dry.
7. The mixture was removed from the stove and allowed to cool completely.
8. 3 ml 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added and the mixture was again allowed to cool down.
9. A further 2 ml  $\text{H}_2\text{O}_2$  was added and the mixture was allowed to cool once again. If the reaction was still intense, more peroxide was added (but no more than 10 ml) until foaming was reduced.
10. Once the mixture had cooled, 10 ml 3 N hydrochloric acid was added and the beaker was again covered with a watch glass.
11. The sample was again placed on the sand stove and allowed to reflux for one hour
12. It was then removed from the heat and allowed to cool down.
13. The mixture was filtered through a Whatman 40 filter paper into a 50 ml volumetric flask.
14. The filter paper was washed with deionised water and the flask brought to volume.

After the extract was received, a mixture was made of 1:1 65% Suprapure nitric acid and distilled water. Three millilitre of this was added to a litre of distilled water. A final mixture was made, 9 ml of last mentioned and 1 ml of the extract (i.e. a 10 X dilution) already taken into account by the ICP-MS. The mixture was then read on the inductively coupled plasma-mass spectrometry (ICP-MS).

### *2.2.7. Organic Pollutants analysis.*

All samples were sent to RPS Mountainheath, UK for the analysis of organic pollutants including PCBs, PAHs (Polycyclic aromatic hydrocarbons), and OCs (Organochlorines). Prior to being shipped, contents were made up with a 50% Methanol solution, in order to be preserved.

The method used at RPS Mountainheath for the analysis of organic pollutants was developed in-house, the details of which is propriety. PCBs, PAHs and OCs were extracted from the solid samples into hexane:acetone (1:1 solid/liquid extraction). Prior to extraction, samples were ground and homogenised then dried with sodium sulphate. Extracts were analysed by gas chromatography- mass spectrometry (GC-MS) against appropriate calibration standards.

### *2.2.8. Statistical analysis*

#### *2.2.8.1. Heavy metals*

A distribution map for the concentrations of heavy metals in the sediment was designed in ArcGIS and visually graphed using Microsoft Excel for comparison. All values are given in mg/kg for comparison studies.

Visual representations for the invertebrates and vertebrates, and how they compare to one another were done with Prism 4 and Microsoft Excel. Additionally, linear regressions for fish lengths were done using Prism 4. Means and standard deviations were calculated in Microsoft Excel. NMS (Nonmetric Multidimensional Scaling) bi-plots for the heavy metals in fish were done in PCORD 5.

#### *2.2.8.2. Organic pollutants*

Visual representations for PCB concentrations in the bird eggs and eggshell thickness were drawn in Microsoft Excel and scatter plots in Prism 4. Linear regressions for the eggshell thickness were done in Prism 5. A principal component analysis (PCA) was done on elemental concentrations in the gull egg contents using PCORD 5. All PCAs were done using the correlation method and the scores for the compound were calculated for distance-based bi-plot.

## Chapter 3

### Results

#### 3.1. Heavy metals

##### 3.1.1. Sediment

Table 3.1. Elemental concentrations in the sediments from the six sampling sites. (Concentrations are given in mg/kg dm). The asterisks (\*) indicate the eight priority metals discussed in the text. The limits of quantification were not reported.

	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Be	0.3	0.3	0.4	0.3	1	0.3
B	33	18	17	20	20	10
Na	6000	5000	3000	4000	4000	3000
Mg	2000	3000	3000	4000	5000	2000
*Al	7000	6000	6700	7000	9800	5000
P	5000	5000	5000	5000	5000	5000
K	2600	2000	2300	2400	3000	2000
Ca	9000	130000	5000	11000	50000	3000
Ti	200	170	160	140	1500	92
V	10	10	10	10	30	9
*Cr	20	20	30	20	30	20
Mn	55	60	80	70	180	70
Fe	7000	6000	7000	8000	10000	6000
Co	3	2	3	3	5	3
Ni	5	6	7	7	12	6
*Cu	5	4	6	5.5	11	6
*Zn	25	20	30	25	40	30
*As	5	4	6	4	7	2
Se	1	1	1	1	1	0.4
Rb	14	11	14	14	19	9
Sr	44	560	200	500	200	20
Mo	3	1	1	2	2	1
Pd	1	2	1	2	1	0.4
Ag	0.001	0.01	0.004	0.01	0.004	0.002
*Cd	0.2	0.2	0.13	0.2	0.2	0.1
Sb	0.3	0.2	0.2	0.2	0.2	0.3
Ba	18	18	22	22	67	22
Pt	0.1	0.1	0.1	0.07	0.1	0.1
Au	0.3	0.2	0.2	0.2	0.3	0.2
*Hg	0.003	0.002	0.002	0.002	0.03	0.002
Tl	0.1	0.1	0.1	0.1	0.2	0.1
*Pb	10	9	10	9	16	10
Bi	0.4	0.4	1	0.4	1	0.4
Th	2	2	3	3	5	2
U	1	1	1	1	1	1

*Table 3.2. The heavy metal concentrations in the sediments from the six sampling sites combined (Concentrations are given in mg/kg dm).*

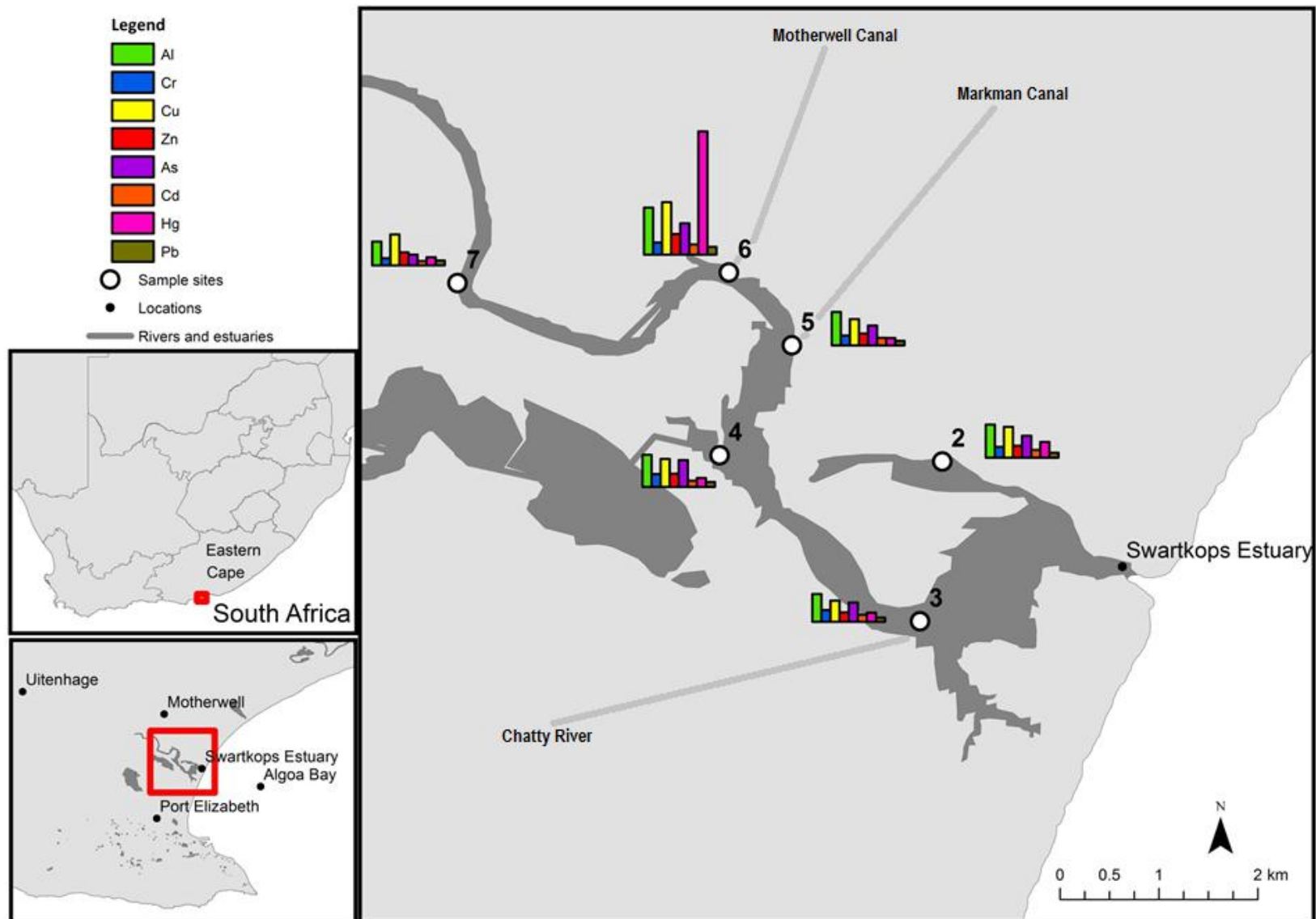
	<b>MEAN</b>	<b>SD</b>	<b>MEDIAN</b>	<b>MIN</b>	<b>MAX</b>
<b>Be</b>	0.4	0.1	0.3	0.3	0.6
<b>B</b>	200	7	18	12	33
<b>Na</b>	4000	1000	4000	3000	5900
<b>Mg</b>	3000	1000	3000	2000	5000
<b>Al</b>	7000	2000	7000	5000	10000
<b>K</b>	2000	600	2000	2000	3000
<b>Ca</b>	60000	50000	50000	3000	130000
<b>Ti</b>	160	39	160	90	200
<b>V</b>	10	4	10	9	20
<b>Cr</b>	22	4	23	15	27
<b>Mn</b>	84	47	700	50	180
<b>Fe</b>	8000	3000	7000	6000	14000
<b>Co</b>	3	1.1	3	2	5
<b>Ni</b>	7	2.4	7	6	12
<b>Cu</b>	7	2.3	6	4	11
<b>Zn</b>	28	9	26	20	43
<b>As</b>	5	2	4	2	7
<b>Se</b>	1	0.2	1	0.4	1
<b>Rb</b>	14	3	14	9	19
<b>Sr</b>	300	200	200	29	600
<b>Mo</b>	1	1	1	0.4	3
<b>Pd</b>	1	0.6	1	0.4	2
<b>Ag</b>	0.003	0.002	0.004	0.001	0.01
<b>Cd</b>	0.1	0.04	0.2	0.1	0.2
<b>Sb</b>	0.2	0.02	0.2	0.2	0.3
<b>Ba</b>	28	20	22	18	67
<b>Pt</b>	0.1	0.02	0.07	0.1	0.1
<b>Au</b>	0.2	0.02	0.2	0.2	0.3
<b>Hg</b>	0.01	0.01	0.002	0.002	0.03
<b>Tl</b>	0.1	0.03	0.1	0.1	0.2
<b>Pb</b>	11	3	10	9	16
<b>Bi</b>	0.4	0.04	0.4	0.4	1
<b>Th</b>	3	1	2	2	5
<b>U</b>	1	0.3	1	1	1

The heavy metal concentrations found in the sediment at each of the six sites is indicated in Table 3.1. All data are presented for archival purposes, although only some will be discussed in more detail.

Table 3.2 indicates the mean, standard deviation, median, minimum and maximum concentrations for the six sediment samples, combined.

Selected heavy metal concentrations at each site are indicated in Fig 3.1. Sites 2 and 6 showed the highest metal concentration, whereas Site 7 showed the lowest overall metal concentrations.





ary, South Africa

Figure 3.1. A map displaying the concentrations of heavy metals in the sediment at each sampling site. For display purposes, the values of the heavy metals concentrations were scaled as follows, for visual interpretation:  $[Cr] \times 100$ ;  $[Cu] \times 1000$ ;  $[Zn] \times 100$ ;  $[As] \times 1000$ ;  $[Cd] \times 10\,000$ ;  $[Hg] \times 1000\,000$ ;  $[Pb] \times 100$ .  $[Al]$  remains the same. Comparisons should therefore only be done per element and not between elements.



In Chapter 1, it was mentioned that previous heavy metal surveys have been done in the SRE. This was the work of Watling & Watling (1982) and Binning & Baird (2001). These sites also correspond to the current study.

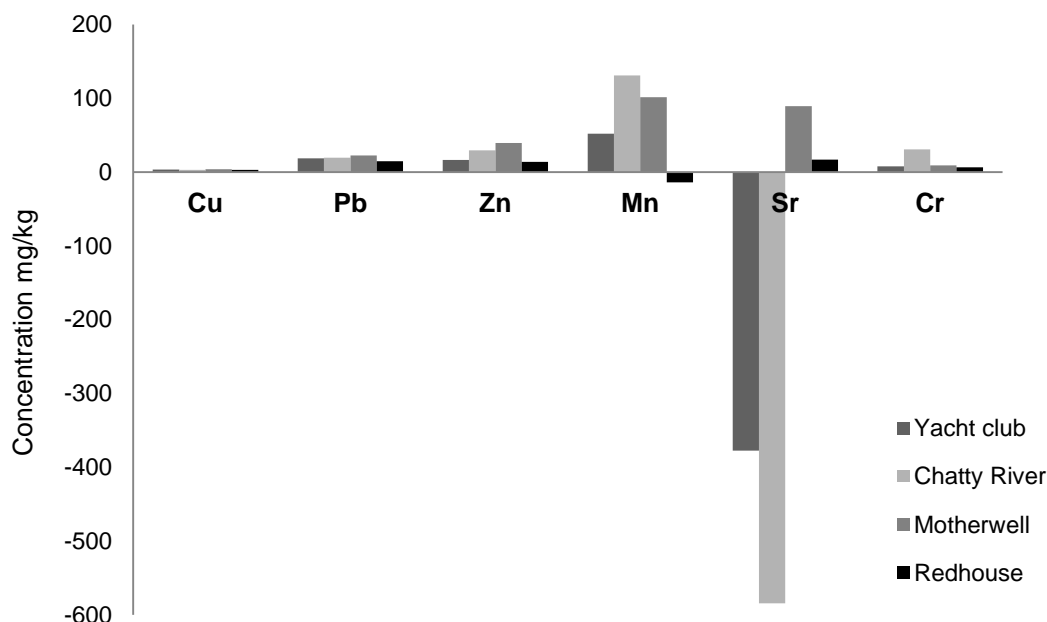


Figure 3.2. The difference in metal concentrations in the sediment from previous studies in the SRE, Port Elizabeth: Watling & Watling (1982) & Binning & Baird (2001). The latter concentrations were subtracted from the earlier values and the differences indicated. Bars above the 0-line = increase from 1982 to 2001, bars below the 0-line = decrease over the same period.

The changes in mean heavy metal concentrations between two previous studies: Watling & Watling (1982) and Binning & Baird (2001) are shown in Fig 3.2. Because this study followed a sediment sampling protocol different from the present study, the results cannot be directly compared with that of the present study. Bars above the 0-line indicate where the mean heavy metal concentrations have increased since the Watling & Watling (1982) study, whereas bars below this line indicate decrease. Only strontium (Sr) and manganese (Mn) showed a decrease. Strontium showed a decrease of 400 mg/kg dm at the Yacht club and 600 mg/kg dm at the Chatty River.

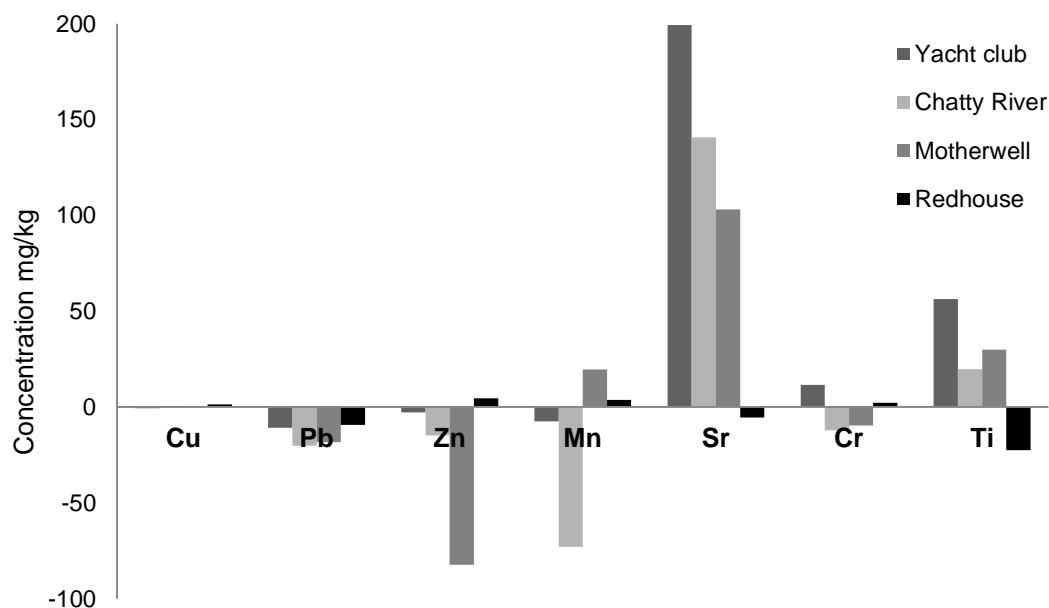


Figure 3.3. Change in mean heavy metal concentrations in the sediments in the SRE, Port Elizabeth based on the results of a study conducted by Binning & Baird (2001). The concentrations from 2012 were subtracted from the earlier values and the differences indicated. Bars above 0-line= increase from 2001 to 2012, bars below 0-line = decrease over the same period.

The changes in sediment concentrations for the present study were also compared to the study of Binning & Baird (2001). Strontium (Sr) and Titanium (Ti) concentrations showed an increase at three of the sites (Yacht club, Chatty River & Motherwell). Fig 3.3 is merely an indication of comparisons. Concentrations in the surface sediments determined by the present study were mostly lower compared historical concentrations.

Table 3.3. Information on heavy metals in sediments from international studies, compared with the present. Guidelines indicated are the Canadian Sediment Quality Guidelines (incl. ISQG & PEL), ERL and ERM (Long *et al.*, 1995) for marine and estuarine sediments. All metal concentrations, including guidelines are given in mg/kg dm.

	n*	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Reference
Canadian SQG										
ISQG <sup>a</sup>		7.24	0.7	52.3	18.7	0.13	50	30.2	124	CEQG, 1999
PEL <sup>b</sup>		41.6	4.2	160	108	0.7	-	112	271	
ERL <sup>c</sup>		205	30	2025	850	3.75	522.5	1167.5	3750	Long <i>et al.</i> , 1995
ERM <sup>d</sup>		1750	240	9250	6750	4.25	1290	5450	10250	
Ría de Heulva (Spain)	3	211.98-343.86	1.50-5.41	NA**	1412.51-2505.34	NA	NA	269.64-404.33	966.92-1337.25	Vicente-Martorell <i>et al.</i> , 2009
Negombo Estuary (Sri Lanka)	12	NA	0.03-0.22	19.2-73.5	5.3-24.2	0.3-2.2	9.2-34.7	4.8-20.1	119.5-207.4	Indrajith <i>et al.</i> , 2008
Hugli Estuary (SE India)	3	NA	NA	12.91-84.74	4.30-45.29	NA	6.86-52.45	?-44.47	22.96-204.99	Chatterjee <i>et al.</i> , 2006
Pearl River Estuary (China)	27	NA	0.29-155	84.1-138	52.7-105	NA	39.2-76.6	31.9-88.5	99.9-274	Liu <i>et al.</i> , 2011
Passaic River (New Jersey)	12	5.0-16	1.5-8.5	59-202	79-273	0.91-5.8	NA	101-385	NA	Armstrong <i>et al.</i> , 2005
Ha Long Bay (Vietnam)	36	13-62	0.02-0.20	10-49	13-30	NA	3-20	4-41	12-93	Ho <i>et al.</i> , 2010
Loukkos Estuary (Morocco)	3	7.96-18.5	<0.3	33-137	6.99-25	0.004-0.055	3.17-24	<2-3.15	42-116	Palma <i>et al.</i> , 2012
Dom João Estuary (Brazil)	5	NA	<0.1-0.56	5.60-24.60	16.09-27.33	NA	5.65-19.66	<0.1-20.3	27.99-52.22	Celino <i>et al.</i> , 2008
Cochin Estuary (India)	17	NA	0.594-14.940	15-121	5.4-53.2	NA	16.0-66.5	19.3-71.3	92-1266	Balachandran <i>et al.</i> , 2006
Han River Estuary (Korea)	14	2.11-7.30	0.06-0.14	33.7-59.8	7.64-21.3	0.0128-0.0843	11.3-20.4	14.2-23.8	42.6-89.2	Kim <i>et al.</i> , 2011
<b>Swartkops Estuary (SA)</b>	<b>6</b>	<b>2.3-7</b>	<b>0.09-0.2</b>	<b>15-27</b>	<b>4-11</b>	<b>0.002-0.03</b>	<b>6-12</b>	<b>0.4-2</b>	<b>20-43</b>	<b>Present study</b>

\*n= number of sampling sites

\*\*NA= not applicable

ALL VALUES INCLUDING GUIDELINES ARE GIVEN IN MG/KG

<sup>a</sup>ISQG: interim (marine) sediment quality guidelines

<sup>b</sup>PEL: probable effects levels

<sup>c</sup>ERL: Effects-range low

<sup>d</sup>ERM: Effects-range median

The sediment concentrations from other estuaries are indicated in Table 3.3. Two additional sediment guidelines are indicated; the Canadian Quality Guidelines and the guidelines discussed in Long *et al.*, 1995 (ERM and ERL). The SRE showed the lowest heavy metal sediment concentrations when compared to international estuaries (Table 3.3) and SRE was the only estuary where all metal concentrations were below any of the given guidelines. Four of the international estuaries showed very high metal concentrations and maximum concentrations often exceeded the ISQG. Ría de Heulva was the only estuary where all of the metals analysed exceeded the ISQG; the maximum Cu concentrations exceeded almost 23 times. The Pearl and Passaic river estuaries and the Cochin Estuary highest metal concentrations exceeded nearly all of the ISQGs. The Dom João Estuary and the SRE was the only two estuaries where all metals concentrations were below that of the Canadian ISQG, except the maximum Cu concentration in the Brazilian estuary.

### 3.1.2. Vertebrates and invertebrates

#### 3.1.2.1. Invertebrates

This study looked at the crustacean mud prawn, which is frequently used as bait and also serves as food for other animals. The mean, standard deviation, median, minimum and maximum concentrations found in mud prawn at the four sampling sites are indicated in Table 3.5.

Table 3.5. Heavy metal concentrations found in mud prawn at four sampling sites (3, 4, 5 & 6) of the SRE, Port Elizabeth (Concentrations are given in mg/kg dm).

	MEAN	SD	MEDIAN	MIN	MAX
Be	0.04	0.01	0.04	0.03	0.1
B	12	1.4	13	10	14
Na	13000	2000	13000	11000	15000
Mg	9000	600	9000	8000	9200
Al	1000	100	1000	900	1200
K	12000	3000	12000	8000	20000
Ca	78000	19000	80000	50000	90000
Ti	41	5	40	35	45
V	2	0.4	3	2	3
Cr	8	2	7	7	10
Mn	100	90	70	27	200
Fe	2000	200	1500	1000	2000
Co	1	0.1	1	0.7	1
Ni	2	1	2	2	3
Cu	150	40	150	100	180
Zn	77	24	70	54	110
As	7	2	7	5	8
Se	4	1	4	2	5
Rb	5	1	5	4	6
Sr	700	180	800	500	900
Mo	1	0.2	0.1	0.5	1
Pd	2	1	2	2	3
Ag	2	0.3	1	1	2
Cd	0.2	0.02	0.2	0.2	0.2
Sb	0.2	0.1	0.2	0.2	0.3
Ba	28	27	20	7	70
Pt	0.2	0.2	0.1	0.1	0.4
Au	3	5	0.4	0.2	10
Hg	0.2	0.3	0.02	0.01	1
Tl	0.04	0.01	0.04	0.03	0.1
Pb	2	0.04	2	2	2
Bi	0.4	0.1	0.4	0.3	1
Th	0.3	0.1	0.3	0.3	0.4
U	0.1	0.1	0.1	0.1	0.2

*Table 3.6. The heavy metal concentrations found in pooled mud prawn samples collected at each sample site in the Swartkops Estuary, Port Elizabeth. Metals listed are the eight priority metals discussed in the text. (Concentrations are given in mg/kg dm).*

	<b>Site 3</b>	<b>Site 4</b>	<b>Site 5</b>	<b>Site 6</b>
	<b>(n=3)</b>	<b>(n=3)</b>	<b>(n=9)</b>	<b>(n=9)</b>
<b>Description</b>	3 large	3 large	1 large 8 small	1 large 8 small
<b>Al</b>	900	1200	200	1000
<b>Cr</b>	7	7	10	7
<b>Cu</b>	100	180	120	180
<b>Zn</b>	50	100	70	72
<b>As</b>	8	8	6	5
<b>Cd</b>	0.2	0.2	0.2	0.2
<b>Hg</b>	0.01	0.01	1	0.02
<b>Pb</b>	1.6	1.6	1.5	1.6

The heavy metal concentrations for mud prawn pools are shown in Table 3.6. Site 4 showed the highest values for Al and Zn, and higher concentrations were found in Site 6 compared to Site 3, however there were not enough data to conduct any meaningful statistics.

Fig 3.4 shows the association between the heavy metals present in mud prawn and that of the sediment. Higher metal concentrations could be observed in mud prawn for the majority of the metals (Cd, Cu, Zn, As & Hg) whereas Al, Cr and Pb showed no increase towards the mud prawn. The sediment is not the only factor attributing to higher concentrations in the mud prawn, external factors such as water and feeding may also contribute.

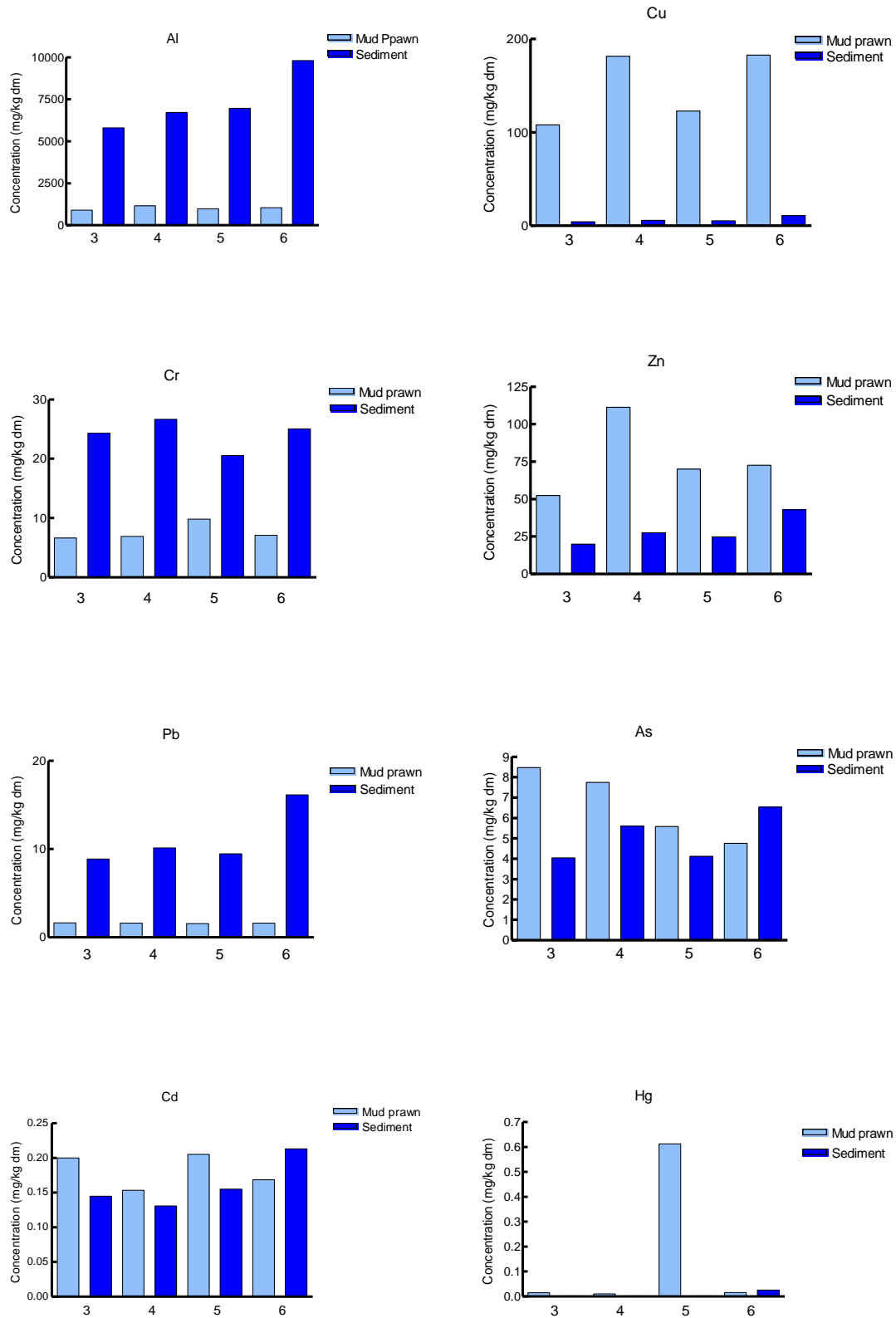


Figure 3.4. Heavy metal concentrations for mud prawn and the sediment at Sites 3-6 in the SRE, Port Elizabeth.

### 3.1.3. Vertebrates

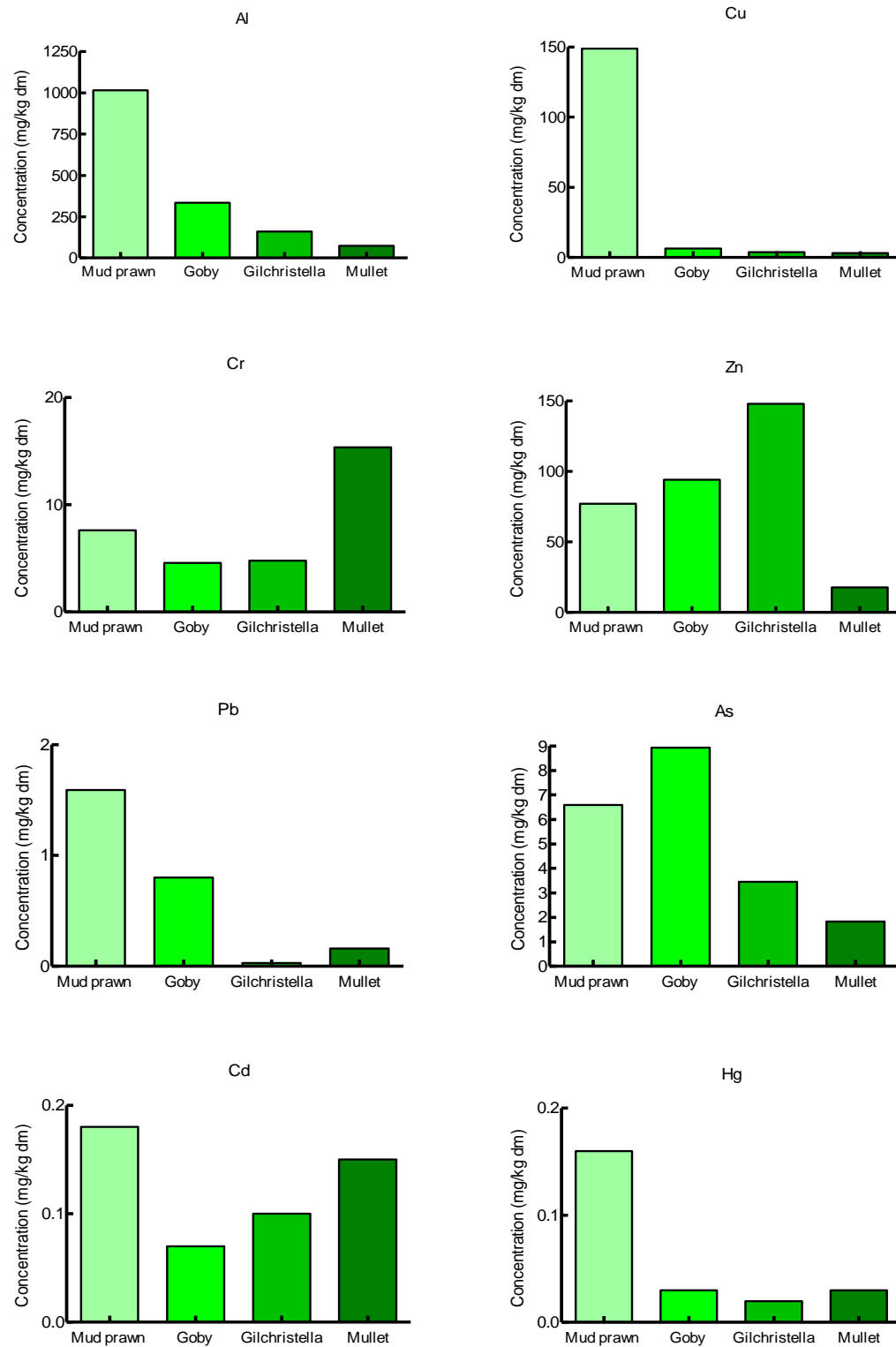


Figure 3.5. Heavy metal concentrations for the smaller aquatic organisms (smaller than 20 cm) in the SRE, Port Elizabeth. This shows the degree of accumulation for the eight priority pollutants discussed in the text.



Table 3.7. Mean and SD for metal concentrations in the liver, muscle and fat for each of the vertebrates. Each mean is for six specimens (Concentrations are given in mg/kg dm).

<b>Spotted Grunter (<i>P. commersonnii</i>)</b>							<b>Garrick (<i>L. amia</i>)</b>						<b>Dusky Kob (<i>A. japonicus</i>)</b>					
	<i>Liver</i>		<i>Muscle</i>		<i>Fat</i>		<i>Liver</i>		<i>Muscle</i>		<i>Fat</i>		<i>Liver</i>		<i>Muscle</i>		<i>Fat</i>	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Na	5900	1000	4000	700	4400	640	9000	3000	2800	700	6000	2300	7100	3000	2600	1060	4600	2200
Mg	770	200	1000	200	1700	200	800	170	1600	65	1600	260	800	300	1600	170	2000	350
Al	80	80	10	2	12	3	40	25	8	3	12	7	120	260	8	2	11	3
K	12000	4000	27000	4900	3000	2300	12000	1700	29000	2600	23000	3500	12400	2800	70000	2000	26000	3800
Ca	500	200	1600	1900	18000	12000	690	330	600	280	16000	4000	800	700	1000	900	85000	5300
Ti	27	20	20	4	27	8	46	40	20	2	27	9	37	40	17	1	25	5
Cr	26	15	4	1	6	2	55	37	6	2	8	2	36	34	5	0.2	7	4
Mn	5	2	1	0.3	2	1	10	4	1	0.3	1	1	8	4	1	0.2	3	1
Fe	2700	1600	80	15	120	70	930	300	50	30	100	30	760	400	35	18	68	23
Co	2	1	0.1	0.02	0.2	0.1	1	0.4	0.1	0.02	0.2	0.03	1	1	0.1	0.01	0.1	0.03
Ni	2	1	0.3	0.04	1	0.1	3	2	0.3	0.1	1	0.2	2	2	0.3	0.1	1	0.2
Cu	2000	1300	3	1	2	0.5	170	130	2	0.4	2	0.6	62	55	1	0.4	2	0.5
Zn	200	180	20	2	20	4	100	20	17	3	26	15	130	56	17	2.3	16	2
As	10	4	8	3	8	5	10	2	4	1	4	1	7	5	5	4	5	3
Se	100	80	6	1	3	1	37	18	2	0.4	2	0.4	10	5	3	1	3	1
Sr	3	1	8	9	66	45	3	1	2	1	45	13	3	2	3	3	26	15
Cd	1	1	0.04	0.01	0.1	0.02	1	0.4	0.1	0.02	0.1	0.02	0.6	0.4	0.1	0.01	0.1	0.03
Hg	1	1	0.1	0.03	0.1	0.03	0.3	1	0.1	0.02	0.1	0.1	0.1	0.1	0.02	0.01	0.1	0.1
Tl	0.1	0.1	0.02	0.0003	0.02	0.01	0.2	0.1	0.02	0.01	0.03	0.01	0.2	0.1	0.02	0.001	0.03	0.02
Pb	1	1	0.1	0.02	0.1	0.1	0.6	0.3	0.1	0.04	0.1	0.1	0.3	0.3	0.1	0.02	0.1	0.1

The heavy metal concentrations for aquatic organisms smaller than 20 cm is illustrated in Fig 3.5. Mud prawn showed higher concentrations than the others for five of the eight priority metals; Al, Cu, Pb, Cd and Hg. The Prison Goby had the second highest metal concentrations, followed by Estuarine Round Herring (indicated as *Gilchristella*).

The mean and standard deviations of heavy metal concentrations for the muscle, liver, and fat quantified in Spotted Grunter, Garrick and Dusky Kob are indicated in Table 3.7. The metal concentrations in the liver of all three fish species were much higher than both their respective muscle and the fat concentrations. Grunter, which is a top predator in the SRE, showed the highest metal concentrations, followed by Kob and then Garrick.

Table 3.8 shows the length of each of the six individuals of the three species.

*Table 3.8. The forked length (cm) of each individual according to their species. The total length (cm) of Dusky Kob is given.*

	1	2	3	4	5	6	Mean
<b><i>Spotted Grunter</i></b>	49.7	50	49.6	59.5	39.6	49.4	49.6
<b><i>Garrick</i></b>	36.7	33.5	37.9	35.8	39	36	36.5
<b><i>Dusky Kob</i></b>	49.6	40	40.8	39.7	46.5	57.7	45.7

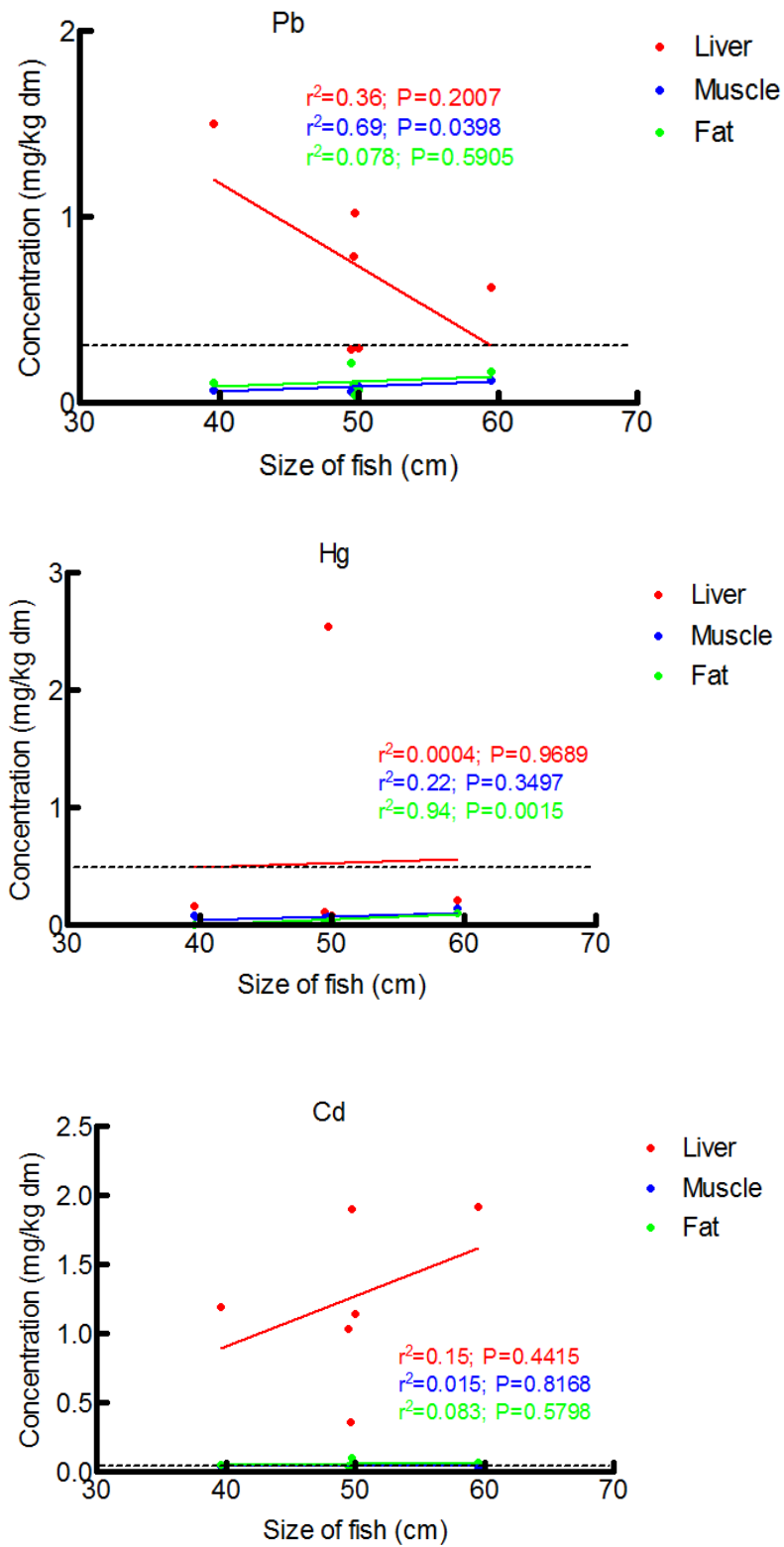


Figure 3.6. Heavy metal concentrations (Pb, Hg & Cd (mg/kg dm) in the liver, muscle, and fat of Spotted Grunter (*P. commersonnii*). Dotted line indicates the maximum concentration for contaminants in foodstuffs (EC, 2006).

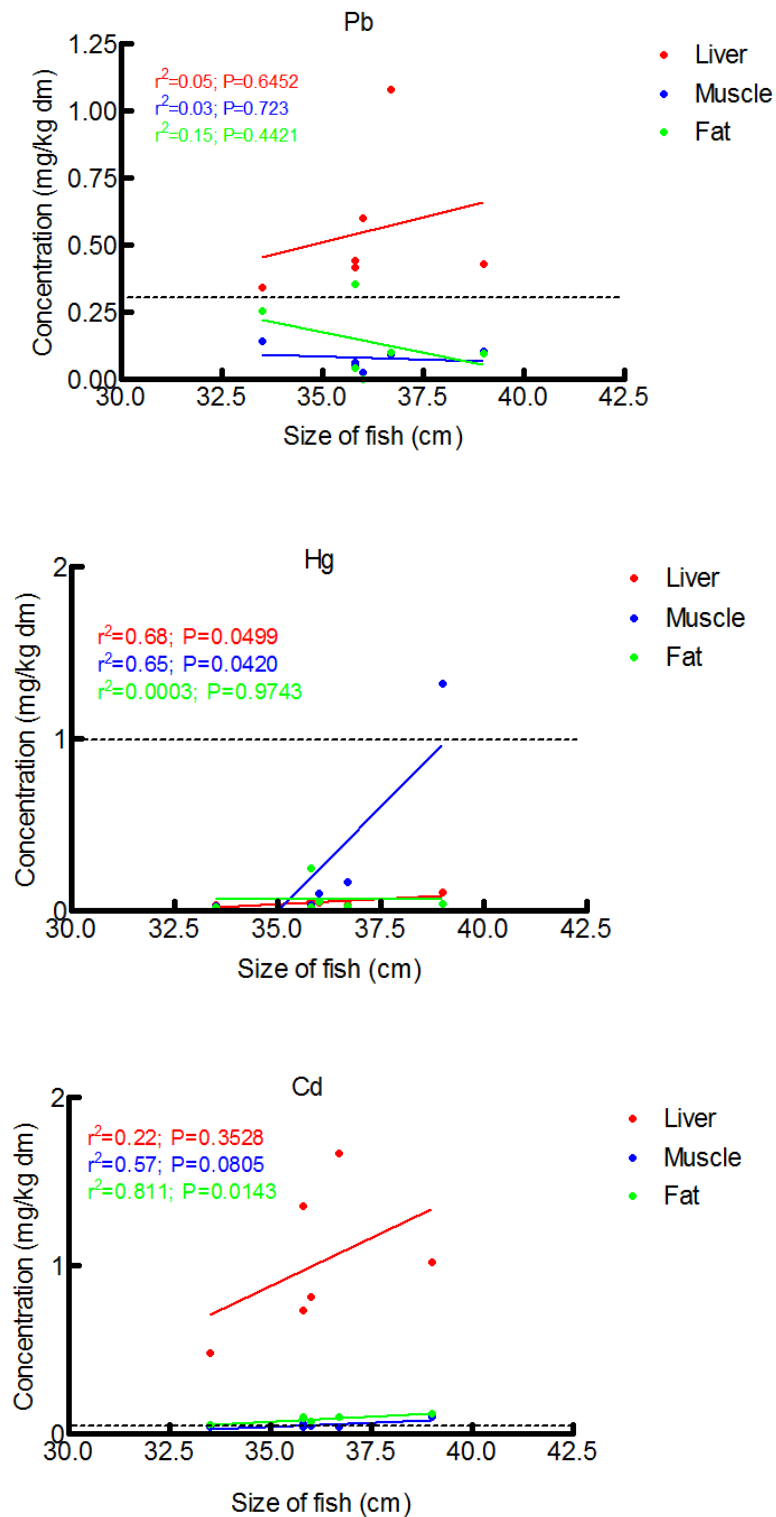


Figure 3.7. Heavy metal concentrations (Pb, Hg & Cd (mg/kg dm) in the liver, muscle, and fat of Garrick (*L. amia*). Dotted line indicates the maximum concentration for contaminants in foodstuffs (EC, 2006).

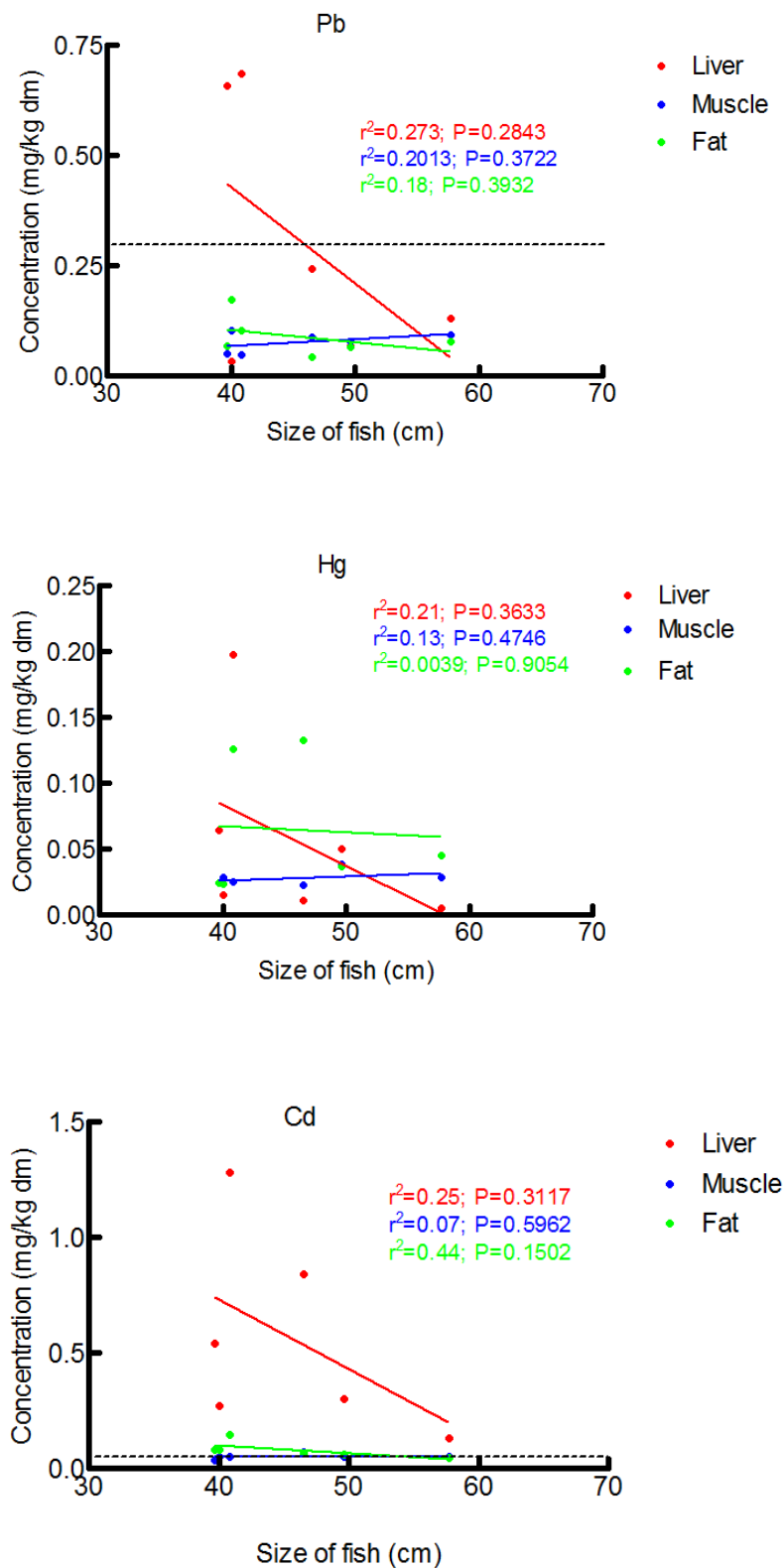


Figure 3.8. Heavy metals (Pb, Hg & Cd (mg/kg dm) in the liver, muscle, and fat of Dusky Kob (*A. japonicus*). Dotted line indicates the maximum concentration for contaminants in foodstuffs (EC, 2006). The maximum concentrations for Hg is 0.5 mg/kg, therefore the line is not visible).

In Figures 3.6 – 3.8, the linear regression for each of the different tissues (liver, muscle, and fat) is shown to assess whether the amount of pollutants accumulated can be attributed to the size of the fish. For Spotted Grunter (Fig. 3.6), only the regressions for Pb in the muscle ( $r^2=0.69$ ) and the Hg concentrations in the fat ( $r^2=0.94$ ) showed significant deviations from zero. Metal concentrations in the muscle and fat were generally below the maximum concentration for contaminants in foodstuffs, although the same cannot be said for the liver. Cd concentrations exceeded the maximum concentration for foodstuffs in the liver, muscle and the fat. Only one negative deviation was seen and this was for Pb in the liver.

The heavy metal regressions with size for Garrick (Fig 3.7) showed slight deviation and it was only the Hg concentrations in the liver ( $r^2=0.68$ ) and the muscle ( $r^2=0.65$ ) as well as Cd in the fat ( $r^2=0.8$ ) where significant deviations was determined. The majority of the metals showed a positive deviation, with only Pb in the fat that showed a negative deviation.

The heavy metal regressions for Kob with size (Fig 3.8) showed no significant deviation for any of the metals, which suggest that the amount of pollutants present in the species is not necessarily dependant on the size of the fish. Only Hg in the fat and Cd in the muscle showed positive deviations. Metal concentrations were generally below the maximum concentrations for foodstuffs, and only the Pb and Cd in the liver, and Cd in the fat of larger species were above the given guideline.

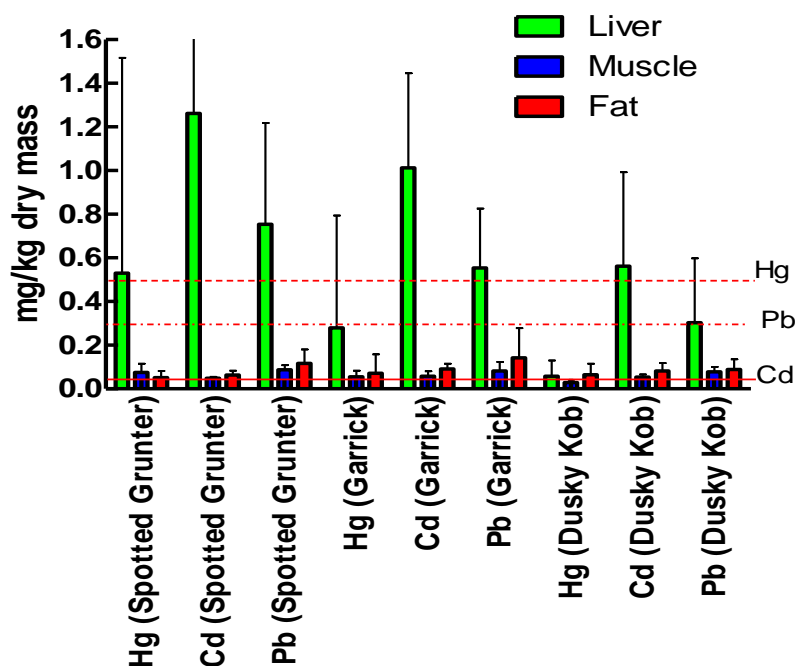


Figure 3.9. Concentrations for the metals of importance (Pb, Hg & Cd) in the liver, muscle and fat of the three large predatory fish (Spotted Grunter, Garrick & Dusky Kob). Means and standard deviations are indicated. The maximum concentration for foodstuffs (MCF) for each metal is also given as horizontal lines (EC, 2006).

The heavy metal concentrations found in the liver, muscle and fat for each of the three fish species is shown in Fig 3.9. Higher concentrations were found in the liver for all species, and descended in the following order: Cd>Pb>Hg, with Cadmium being the highest. The concentrations found in the muscle and fat were much lower compared to the liver, and similar profiles were seen for both the muscle and fat. Concentrations found in the liver for all fish species exceeded all given MCF values, except Hg in both Garrick and Dusky Kob and Pb in Dusky Kob. Cd was the only element that exceeded all of the given guidelines in the tissue sampled.

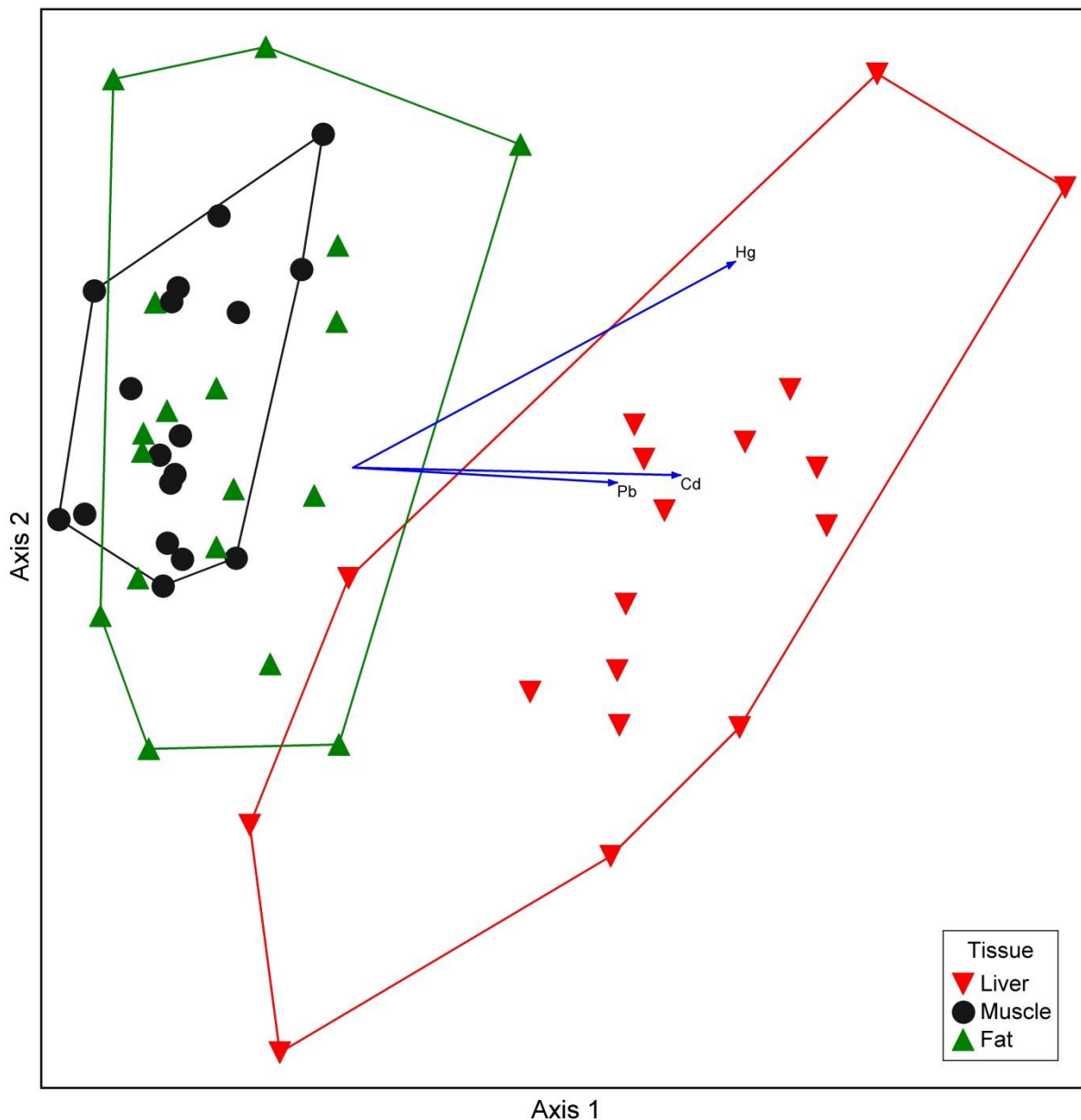


Figure 3.10. NMS (Nonmetric Multidimensional Scaling) bi-plot ordinations for the heavy metals found in the tissue. Data were relativised. Convex hulls group the three tissues. Axis 1 explains 60.5%, and Axis 2 explains 2.1% of the ordination.

Fig 3.10 shows a NMS for the heavy metals in the tissue of the fish in a bi-plot with the compounds indicated by vectors. The muscle and tissue showed similar pollutant profiles for Pb and Cd, whereas a different pollutant profile was seen in the liver. The liver indicates the highest concentrations, thus more suitable to use as an environmental indicator.



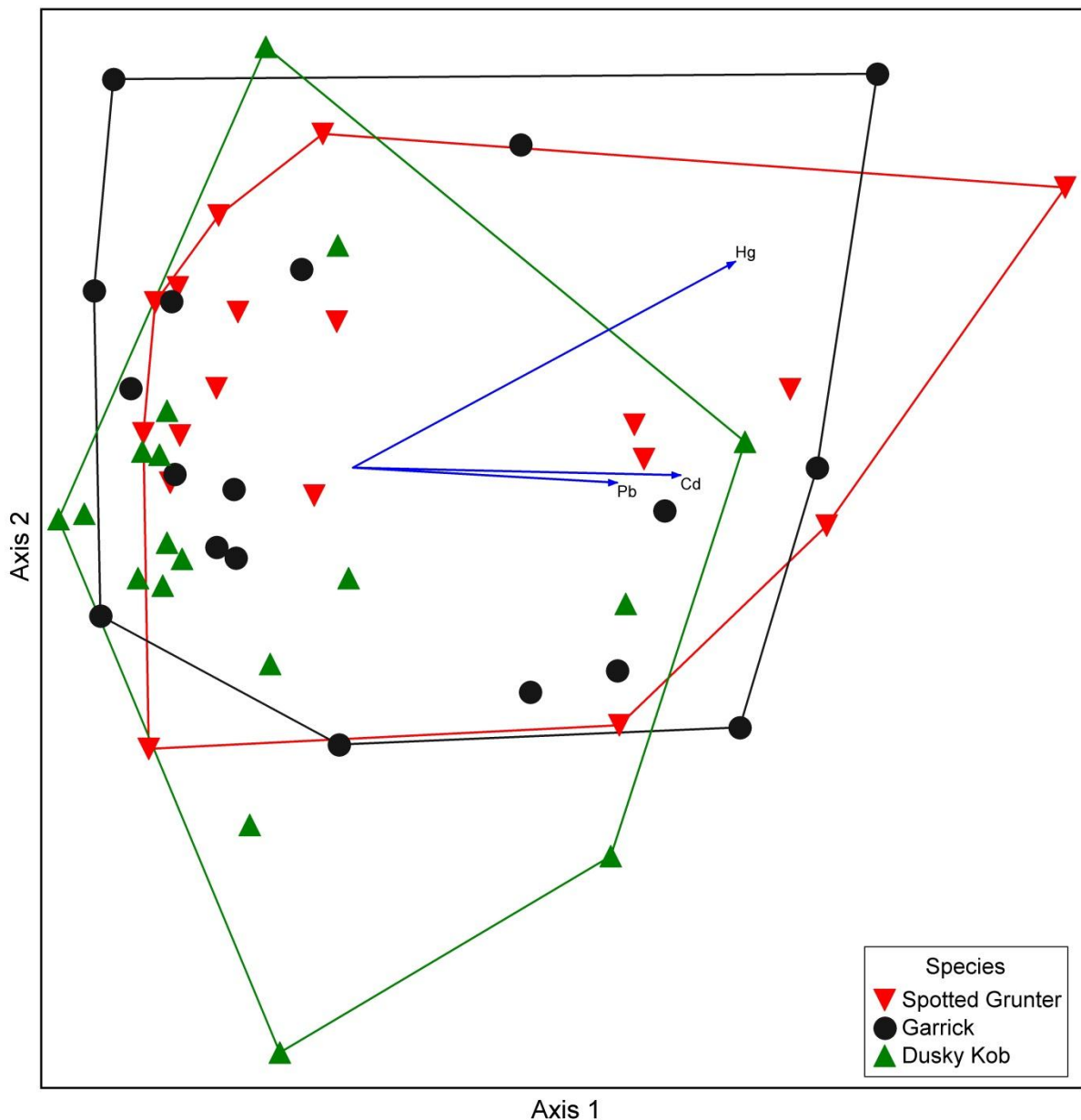


Figure 3.11. NMS bi-plot ordinations for the heavy metals found in the three fish species. Data were relativised. Convex hulls group the three species. Axis 1 explains 60.5%, and Axis 2 explains 2.1% of the ordination

Figure 3.11 shows the same NMS plot as in Fig 3.10 (but with the species grouped in convex hulls) for the heavy metals found in the three fish species in a bi-plot with the compound indicated by vectors. Similar pollutant profiles were observed for all the fish species which indicate that any of the species can be used as indicators, and that the fish serve as SRE system indicators, rather than indicators of the sites where they were caught.

References indicate that mud prawn is the preferred prey species for Spotted Grunter, Knysna Sand Goby is the main prey for Garrick and Estuarine Round Herring (indicated as *Gilchristella*) is the main prey of Dusky Kob (Smit, 2008; Griffiths, 1997, Hanekom & Baird, 1992). In Figs 3.12 a-h the trophic level relationships are plotted from left to right, with sediment at the left, followed by increasing trophic levels to the right. Flathead Mulletts were not included here as they were collected far away from the others and not considered part of the ecosystem where all the others occurred.

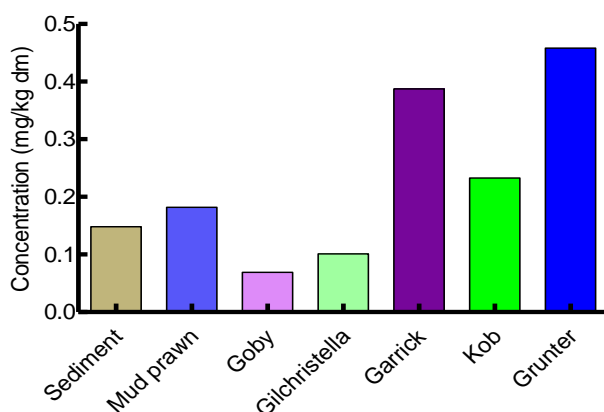


Figure 3.12 a. Cadmium (Cd) concentrations in prey and predators (dry mass based).

Cadmium (Fig 3.12 a) shows a factor of seven difference in concentrations between the sediment, prey and predators. Concentrations of Cd the sediment and mud prawn were much the same.

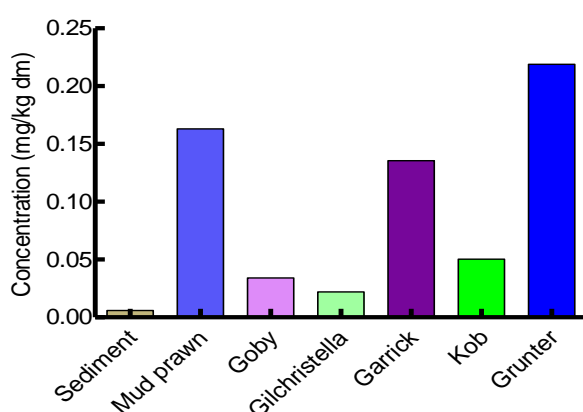


Figure 3.12 b. Mercury (Hg) concentrations in prey and predators (dry mass based).

Mercury (Fig 3.12 b) shows three orders of magnitude difference between the heavy metal concentrations in sediment, prey, and predator.

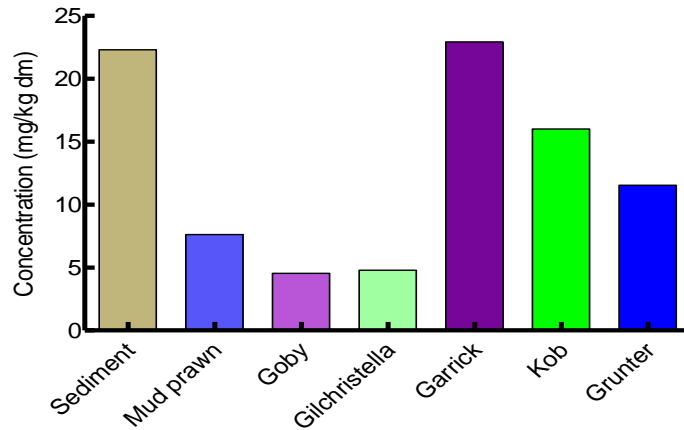


Figure 3.12 c. Chromium (Cr) concentrations in prey and predators (dry mass based).

Chromium (Fig 3.12 c) shows a factor of five difference between the heavy metal concentrations in sediment, prey and predator.

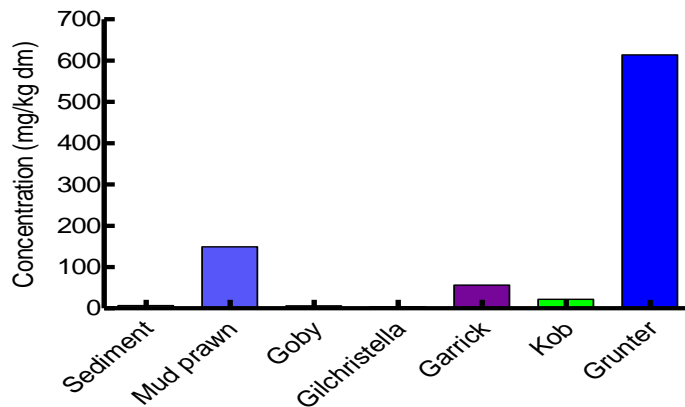


Figure 3.12 d. Copper (Cu) concentrations in prey and predators (dry mass based).

Copper (Fig 3.12 d) shows one orders of magnitude difference between the heavy metal concentrations in sediment and Grunter.

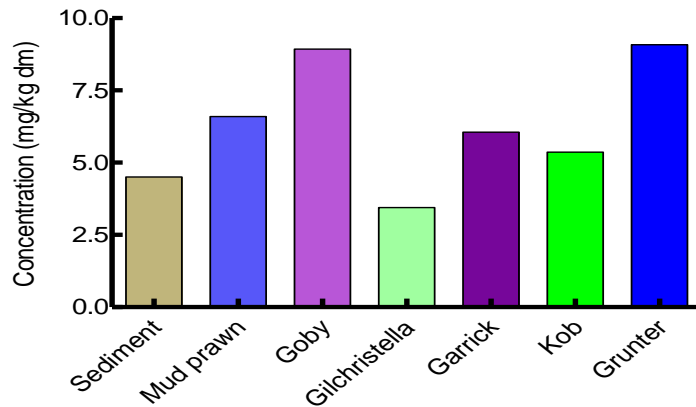


Figure 3.12 e. Arsenic (As) concentrations in prey and predators (dry mass based).

Arsenic (Fig 3.12 e) shows a factor of two difference between the heavy metal concentrations in sediment, prey and predator. The concentrations in Grunter and Kob were higher than their prey, but Garrick had lower concentrations compared to the Goby. The metal concentrations in mud prawn were higher than the sediment, and Goby showed a similar trend.

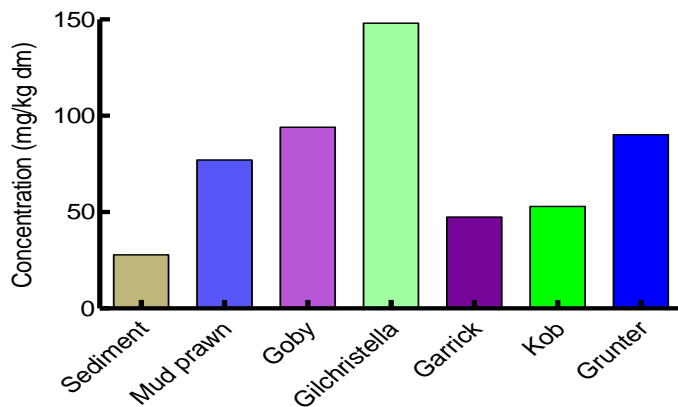


Figure 3.12 f. Zinc (Zn) concentrations in prey and predators (dry mass based).

Zinc (Fig 3.12 f) shows a factor of five difference between the heavy metal concentrations in sediment, prey and predator.

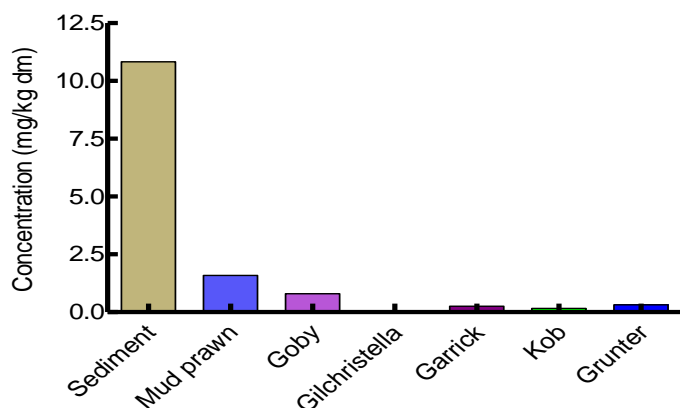


Figure 3.12 g. Lead (Pb) concentrations in prey and predators (dry mass based).

Lead (Fig 3.12 g) shows three orders of magnitude difference between the heavy metal concentrations in sediment, prey and predator. Small differences were seen between *Gilchristella* and Kob. Sediment had much higher concentrations than the biota.

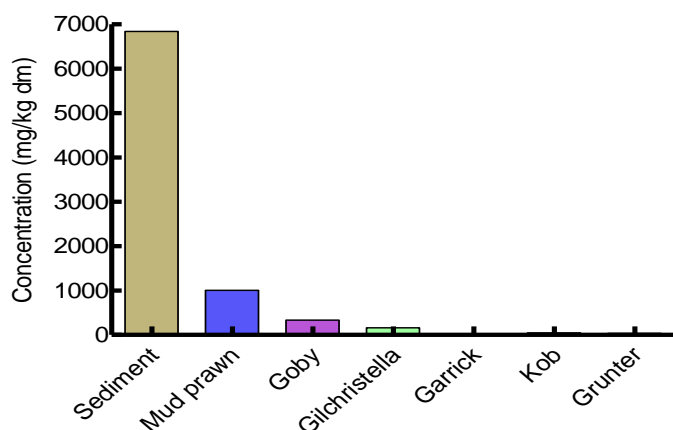
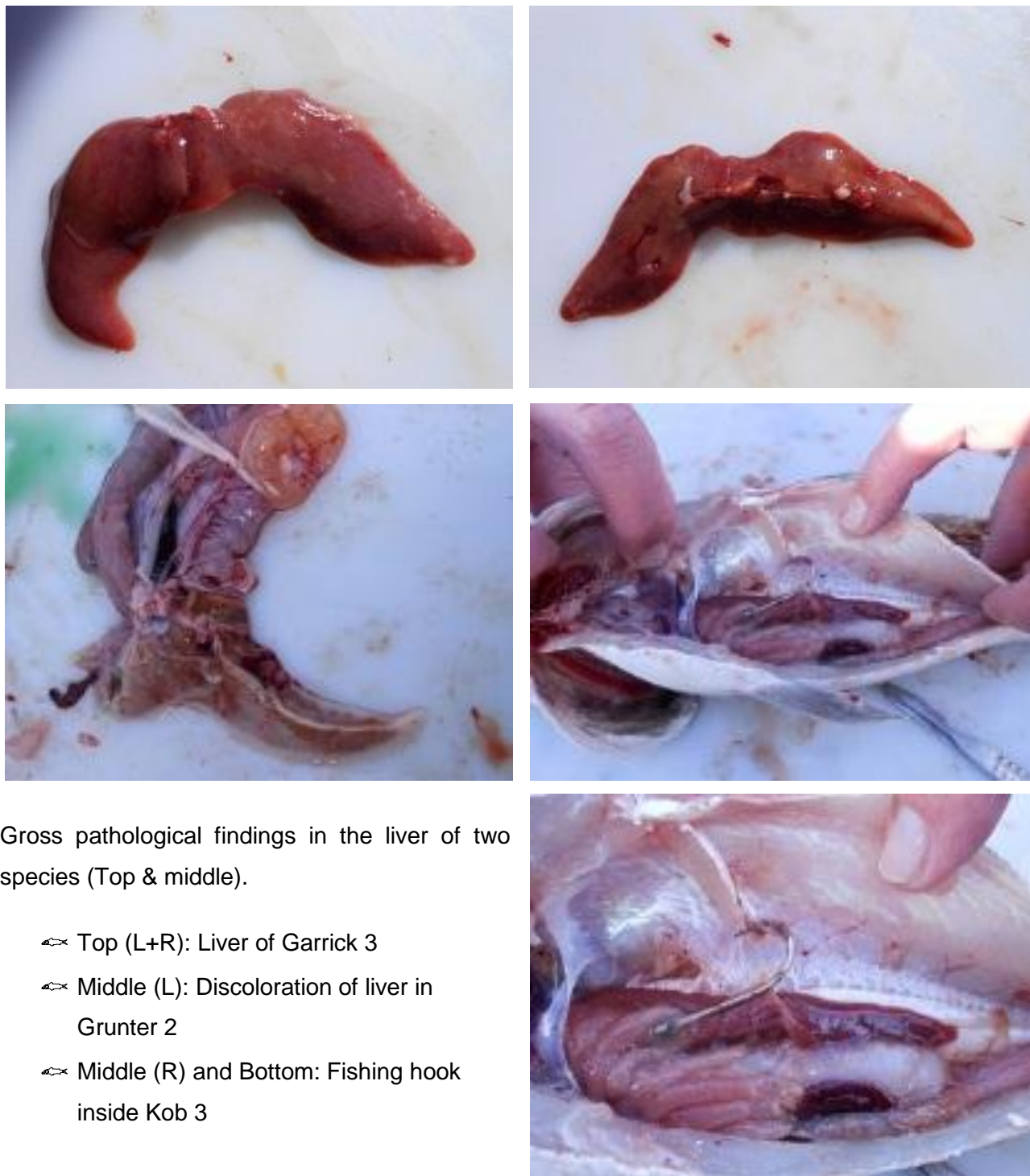


Figure 3.12 h. Aluminium (Al) concentrations in prey and predators (dry mass based).

Aluminium (Fig 3.12 h) shows three orders of magnitude difference between the heavy metal concentrations in sediment, prey and predator.

Cd (Fig 3.12 a), Hg (Fig 3.12 b), Cr (Fig 3.12 c) and Cu (Fig 3.12 d) showed a trend of increasing concentrations from the sediment and prey towards the predators. As (Fig 3.12 e) and Zn (Fig 3.12 f) showed similar trends but metal concentrations in the predators was not necessarily higher than the prey. Pb (Fig 3.12 g) and Al (Fig 3.12 h) suggested no evidence of bioaccumulation in higher predators, but higher concentrations were apparent in the sediment for both these elements.



Gross pathological findings in the liver of two species (Top & middle).

- 🐟 Top (L+R): Liver of Garrick 3
- 🐟 Middle (L): Discoloration of liver in Grunter 2
- 🐟 Middle (R) and Bottom: Fishing hook inside Kob 3

*Figure 3.13. Gross pathological images from the fish in the Swartkops River Estuary.*

### *3.2. Organic pollutants*

The majority of the organic pollutants in samples were below concentrations of quantification (LOQ). These included sediment, bird eggs, fish muscle, and invertebrates. The LOQs was as follows:

- PAHs < 100 µg/kg,
  - naphthalene < 500 µg/kg,
- OCLs < 2 µg/kg and
- PCBs < 5 µg/kg

For both PAHs and OCLs, samples were below LOQ and could therefore not be used.

PCBs showed quantifiable concentrations in all bird egg samples, but only some liver, muscle and fat samples for the three fish species had quantifiable concentrations. All further data was below quantification limits.

The PCBs that were quantified were CB-101, CB-118, CB-138, CB-153, CB-180, CB-28 and CB-52.

### 3.2.1. Bird eggs

Due to logistical reasons, the contents of egg 1A could not be analysed. The concentrations of PCBs found in the Kelp Gull eggs are indicated in Table 3.9.

Table 3.9. The concentrations of PCBs found in Kelp Gull eggs ( $n=15$ ). Concentrations are given in  $\mu\text{g/kg wm}$ .

	Mean	SD	Median	Min	Max
<b>CB-101</b>			< LOQ		
<b>CB-180</b>	30	13	27	15	51
<b>CB-28</b>	11	5	13	5	17
<b>CB-52</b>	6	6	6	<LOQ	6
<b><math>\Sigma\text{PCB}</math></b>	180	87	160	83	360

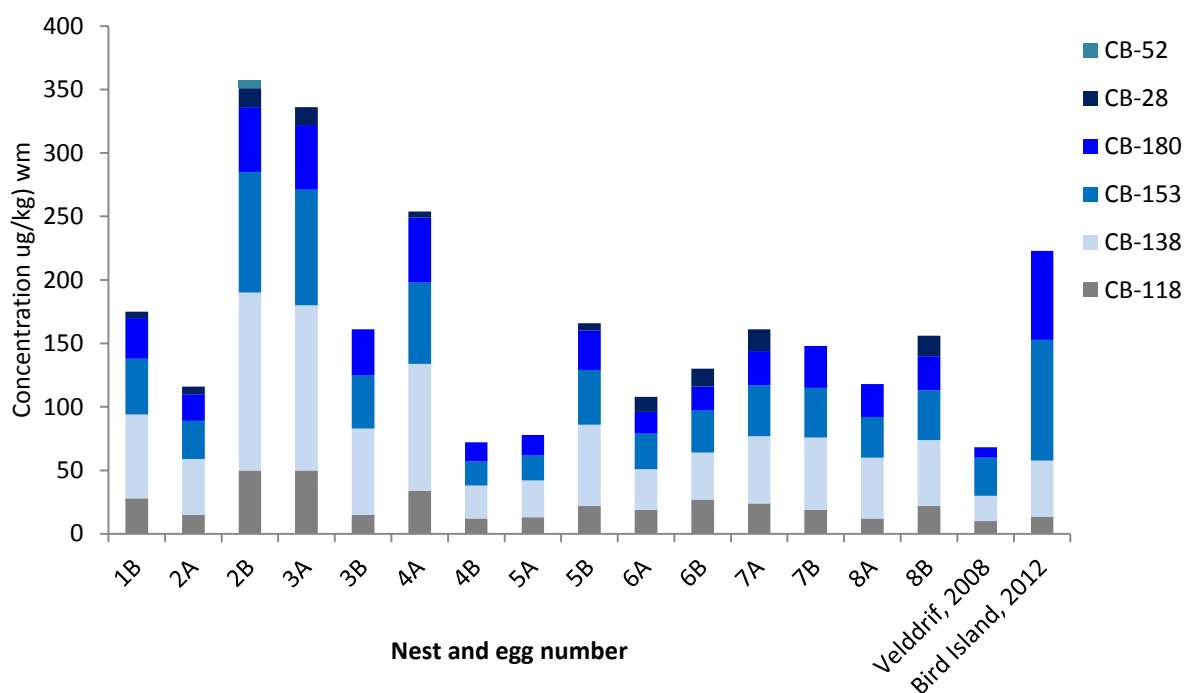


Figure 3.14. Concentrations of four congeners of PCBs found in the eggs of Kelp Gull. Concentrations are given in  $\mu\text{g/kg wm}$

Seven PCB congeners were quantified in the eggs of Kelp Gull (*Larus dominicanus*) (Fig 3.14). Four congeners (CB-118, CB-138, CB-153, and CB-180) were quantifiable in all of the samples. The concentrations for CB-101 were below the quantification concentrations in all samples. CB-28 was quantifiable in 15/17 eggs, while CB-52 was quantifiable in only one egg (2B). Decreasing concentrations in eggs were  $\text{CB-138} > \text{CB-153} > \text{CB-180} > \text{CB-118} > \text{CB-28} > \text{CB-52}$ . Additional data from previous studies have been added, namely single Kelp Gull eggs from Velddrif, 2008 (Bouwman *et al.*, 2008) and Bird Island, 2012



(unpublished results). The single egg from Bird Island (approximately 61 km east from the SRE mouth) showed similar concentrations to the SRE Kelp Gulls for CB-138 and CB-118, but concentrations were much higher for CB-153 and CB-180.

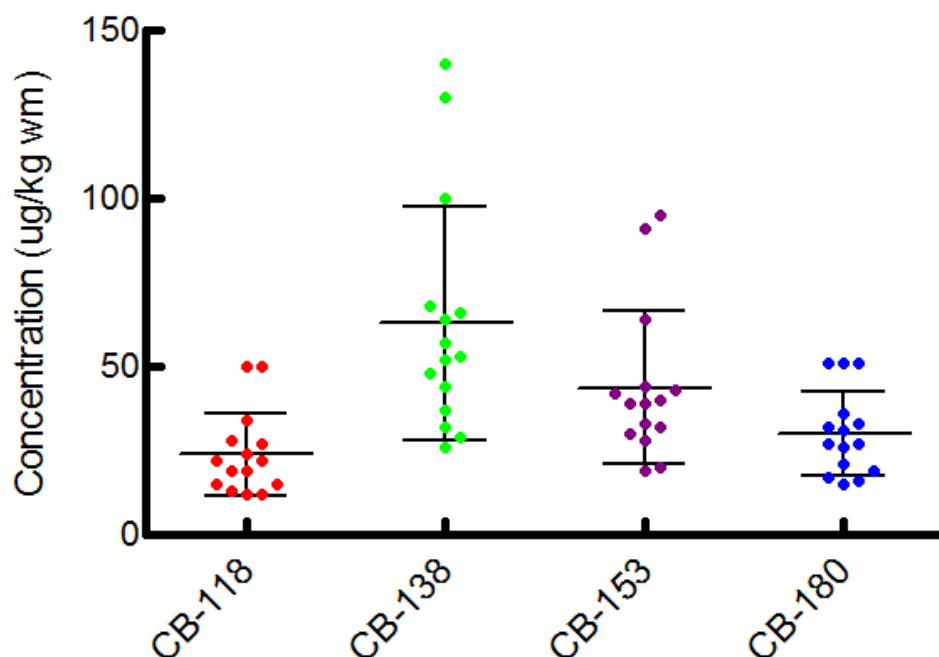


Figure 3.15. Scatter plot for the four PCB congeners quantifiable in all eggs of *L. dominicanus* collected from the SRE (Concentrations are given in ug/kg wm). Means and standard deviations are indicated.

The concentrations of PCBs measured in all of the eggs are indicated in Fig 3.13. PCB concentrations were relatively similar in the eggs except for CB-138, where concentrations were more dissimilar. The two highest concentrations of CB-118, CB-138 and CB-153 were from the same two eggs; 2B and 3A.

The eggshell thickness was regressed against the total pollutant concentrations in the Kelp Gull eggs (illustrated in Fig 3.16 a-c).

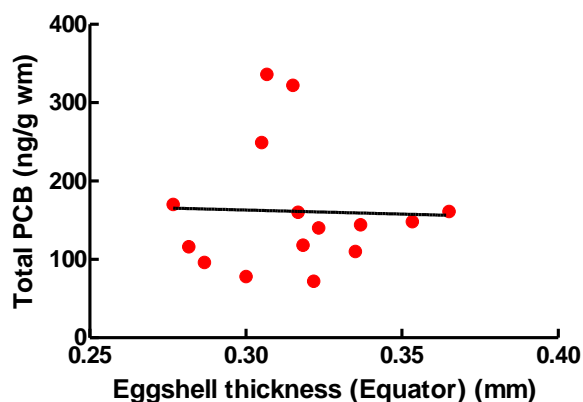


Figure 3.16 a. Linear regression of eggshell thickness at the equator of each egg.

The linear regression of the eggshell thickness at the equator against  $\Sigma$ PCB is indicated in Fig 3.16 a. There was no significant association between the  $\Sigma$ PCBs and the eggshell thickness ( $r^2 = 0.001$ ; P value = 0.0949).

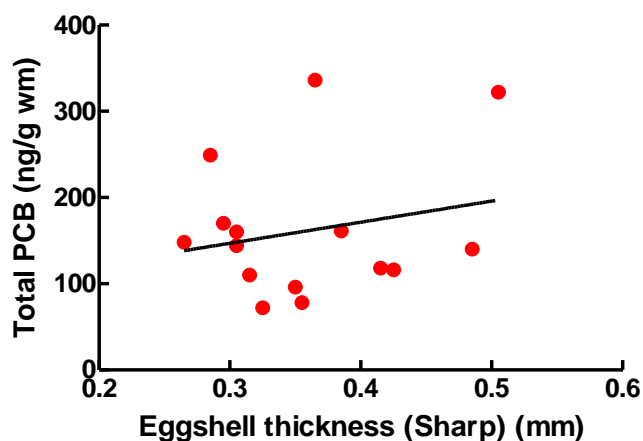


Figure 3.16 b. Linear regression of eggshell thickness at the sharp end of each egg.

The linear regression of the eggshell thickness at the sharp end against  $\Sigma$ PCB is indicated in Fig 3.16 b. There was no significant association between the  $\Sigma$ PCBs and the eggshell thickness ( $r^2 = 0.05$ ; P value = 0.0629).

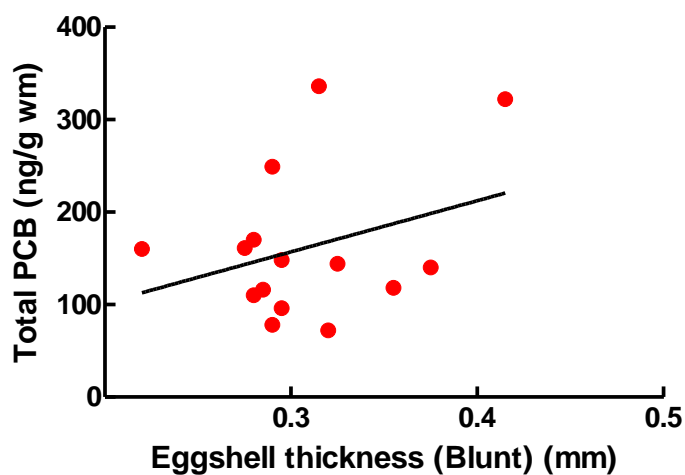


Figure 3.16 c. Linear regression of eggshell thickness at the blunt edge of each egg.

The linear regression of the eggshell thickness at the blunt end against  $\Sigma$ PCBs is indicated in Fig 3.16 c. There was no significant association between the  $\Sigma$ PCBs and the eggshell thickness ( $r^2 = 0.1$ ; P value = 0.3427).

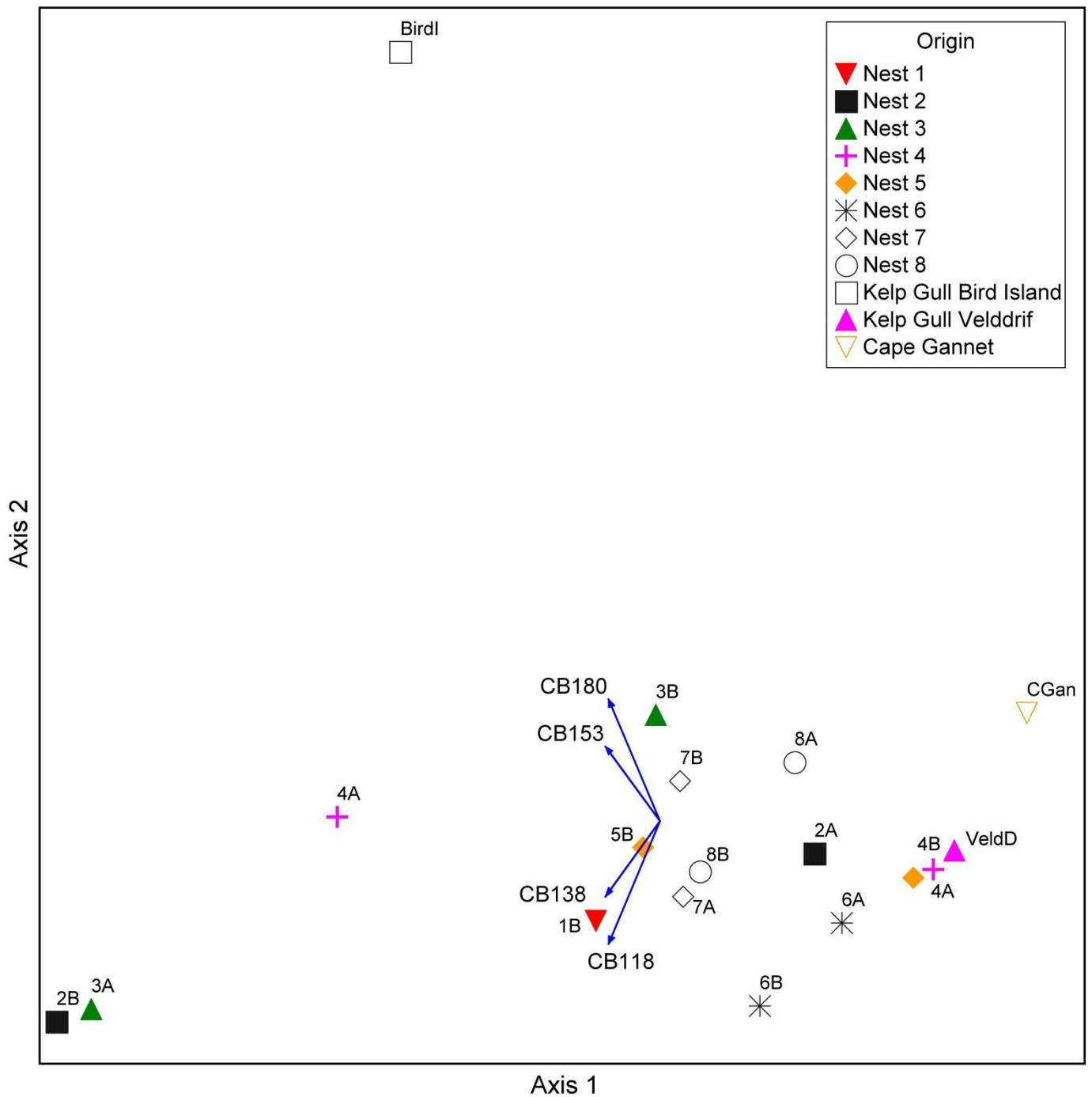


Figure 3.17. PCA plot of PCBs in eggs. 1-8 identifies the Kelp Gull egg clutches sampled at the Swartkops River Estuary. Additional information from other studies have been added, namely single eggs of a Kelp Gull (BirdI) and a Cape Gannet (CGan), both from Bird Island (unpublished results), and a Kelp Gull egg from Velddrif (VeldD) (Bouwman et al., 2008). Axis 1 explains 76%, and axis 2 explains 21% of the ordination.

Fig 3.17 shows a principal component analysis (PCA) of PCB congeners with their respective eggs. There was a clear clustering (sharing the same pollutant profile) for the

majority of the Kelp Gull eggs. However, the Kelp Gull eggs from Bird Island showed a much different pollutant profile from those collected at SRE as well as the one from Velddrif. Shared symbols and colours indicate eggs from the same nest, and presumably, the same maternal parent. The eggs from nests 2 and 3 for instance show large differences in pollutant profiles for eggs from the same nest. For these two nests, the difference seems to be associated with CB-188 and CB-138.

Table 3.10. Mean  $\Sigma$ PCB concentrations ( $\mu\text{g/kg}$  ww) in the eggs of previous studies.

Species	n	Locality	Year	n- PCB*	$\Sigma$ PCB	Reference
African Darter	13	Gauteng RSA	2010	7	210	Quinn, 2010
African Darter	14	Parys RSA	2008	7	206	Bouwman <i>et al.</i> , 2008
African Sacred Ibis	16	Gauteng RSA	2010	7	30	Quinn, 2010
African Sacred Ibis	2	Parys RSA	2008	7	30	Bouwman <i>et al.</i> , 2008
Common Tern	13	Axios Greece	2005	6	94	Goutner <i>et al.</i> , 2005
Kelp Gull	1	Velddrif RSA	2008	5	68	Bouwman <i>et al.</i> , 2008
Kelp Gull	1	Bird Island RSA	2011	4	223	Unpublished results
Kelp Gull	15	<b>SRE RSA</b>	<b>2012</b>	6	<b>180</b>	<b>Present study</b>
Kelp Gull	1	<b>Nest 2B, SRE, RSA</b>	<b>2012</b>	6	<b>351</b>	<b>Present Study</b>
Kelp Gull	1	<b>Nest 3A, SRE, RSA</b>	<b>2012</b>	6	<b>336</b>	<b>Present Study</b>
African Penguin	10	Bird Island RSA	2009	17	64	Unpublished results
African Penguin	10	Robben Island RSA	2010	17	42	Unpublished results
Glaucous Gull	3	Chuckchi Sea, Alaska	2008	36	1720	Vander Pol <i>et al.</i> , 2009
Glaucous Gull	3	Bering Sea, Alaska	2008	36	1690	Vander Pol <i>et al.</i> , 2009
Glaucous –Winged Gull	3	Bering Sea, Alaska	2008	36	1400	Vander Pol <i>et al.</i> , 2009
Glaucous –Winged Gull	3	Gulf of Alaska, Alaska	2008	36	1710	Vander Pol <i>et al.</i> , 2009
Mallard	6	Italy	2007	5	489	Antoniadou <i>et al.</i> , 2007
Mediterranean Gulls	15	Evros Delta Greece	2005	7	55	Goutner <i>et al.</i> , 2005
Mediterranean Gulls	15	Axios Delta Greece	2005	7	35	Goutner <i>et al.</i> , 2005
Mediterranean Gulls	13	Porto Lagos Greece	2005	7	43	Goutner <i>et al.</i> , 2005
Reed Cormorant	2	Parys RSA	2008	7	77	Bouwman <i>et al.</i> , 2008
Yellow-legged Gull	11	Lipsos, Greece	2003	8	42	Albanis <i>et al.</i> , 2003
Yellow-legged Gull	15	Arki, Greece	2003	8	43	Albanis <i>et al.</i> , 2003
Yellow-legged Gull	12	Kinaros, Greece	2003	8	87	Albanis <i>et al.</i> , 2003
Yellow-legged Gull	7	Envros Delta, Greece	2003	8	41	Albanis <i>et al.</i> , 2003
White Breasted Cormorant	4	Gauteng RSA	2010	7	198	Quinn, 2010

n= number of eggs

\* number of PCB congeners

The mean  $\Sigma$ PCBs concentrations in bird eggs from previous studies are shown in Table 3.10. Data from the present study are highlighted in bold. Gull eggs from Alaska (Glaucous Gull & Glaucous-winged Gull) had the highest concentrations overall keeping in mind that there were only three eggs, and the number of PCB congeners analysed was much higher. The single eggs from Nest 2A and 3A was reported separately, since they had the highest

PCB concentrations from the SRE. The  $\Sigma$ PCB concentrations in these eggs were higher than the Kelp Gull eggs from Velddrif and Bird Island although the mean of 15 eggs only exceeded the Velddrif study. The concentrations in gull eggs of the current study exceeded other species such as the African Penguin (including 10 eggs from Bird Island near SRE), African Sacred Ibis, Reed Cormorant and Common Tern.

### 3.2.2. Fish

The PCB concentrations found in the liver, muscle and fat for each of the three fish species are showed in Table 3.11. In most cases, concentrations of  $\Sigma$ PCBs in fish were below the LOQ, which was 5  $\mu\text{g/kg dm}$ . Table 3.11 shows the PCB concentrations found in the liver, muscle, and fat for the three fish species. For Spotted Grunter, the descending  $\Sigma$ PCB trend was CB-118 > CB-153 > CB-138 > CB-180, with means of 46, 24, 18 and 11  $\mu\text{g/kg dm}$ , respectively. For Garrick, CB-138 > CB-153 > CB-180, had respective means of 15, 13 and 8  $\mu\text{g/kg dm}$ . The trend for Kob was CB-153 > CB-180 > CB-138 > CB-52 with respective  $\Sigma$ PCB means of 35, 29, 22 and 6  $\mu\text{g/kg dm}$ .

PCB concentrations found in the liver for the three fish species was higher than the muscle or fat. The  $\Sigma$ PCB concentrations in Dusky Kob were significantly higher than Grunter or Garrick. The size of fish did not appear to influence the concentrations of  $\Sigma$ PCBs among individuals of the same species, but appeared to differ between the three fish species, with Garrick highest and Kob the lowest> Grunter >Kob.

Table 3.11. PCB concentrations found in the liver, muscle and fat for the three fish species. Highlighted values are those above the level of quantification (LOQ). Concentrations are given in µg/kg wm

<i>Spotted grunter (P. commersonnii)</i>																		
	Liver	Liver	Liver	Liver	Liver	Liver	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Fat	Fat	Fat	Fat	Fat	Fat
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
CB 101	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 118	<LOQ	<LOQ	35	32	72	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 138	<LOQ	<LOQ	33	17	50	5	<LOQ	<LOQ	6	5	7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 153	<LOQ	<LOQ	43	24	62	7	<LOQ	<LOQ	5	<LOQ	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 180	<LOQ	<LOQ	14	<LOQ	8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 52	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
<i>Garrick (L. amia)</i>																		
	Liver	Liver	Liver	Liver	Liver	Liver	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Fat	Fat	Fat	Fat	Fat	Fat
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
CB 101	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 118	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 138	7	40	11	12	<LOQ	10	8	18	<LOQ	<LOQ	<LOQ	10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 153	<LOQ	36	10	12	<LOQ	5	6	14	<LOQ	<LOQ	<LOQ	7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 180	<LOQ	15	<LOQ	5	<LOQ	5	<LOQ	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 52	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
<i>Dusky Kob (A. japonicus)</i>																		
	Liver	Liver	Liver	Liver	Liver	Liver	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Fat	Fat	Fat	Fat	Fat	Fat
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
CB 101	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 118	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 138	83	41	14	12	7	8	<LOQ	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7
CB 153	86	54	16	18	<LOQ	30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6
CB 180	29	<LOQ	<LOQ	9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
CB 52	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

### 3.2.3. Sediment

#### 3.2.3.1. PAHs (Polycyclic aromatic hydrocarbons)

Since all PAHs were below the levels of quantification of the contract laboratory, a single sample was reanalysed by National Metrology Institute of South Africa (NMISA) that had lower LOQs.

Table 3.12. PAHs concentrations in the sediment at Site 5 in the Swartkops Estuary, Port Elizabeth.

Compound	µg/kg dm	ISQG (µg/kg dm)	PEL (µg/kg dm)
Naphthalene	1	35	390
Acenaphthene	ND	7	90
Anthracene	0.4	47	250
Benzo[a]anthracene	0.4	75	690
Benzo[b]fluoranthene	0.7	-	-
Dibenz[a,h]anthracene	ND	6	140
Fluorene	1	21	140
Fluoranthene	1	113	1500
Chrysene	1	108	850
Benzo[a]pyrene	0.01	90	760
Indeno[1,2,3-cd]pyrene	2	-	-
Acenaphthylene	ND	6	200
Phenanthrene	0.1	90	540
Pyrene	2	150	1400
Benzo[k]fluoranthene	2	-	-
Benzo[ghi]perylene	0.4	-	-
ΣPAHs	10	-	-

The results in Table 3.12 show the PAHs concentrations for Site 5. Concentrations were generally low. The ISQG and PEL guidelines for PAHs in marine sediments as set out by the Canadian Sediment Quality Guidelines, 1999 is also shown. No concentrations exceeded any of the given guidelines. The ERL and ERM for the ΣPAHs is 4000 and 45000 µg/kg dm respectively. The ΣPAH concentration is three and four orders of magnitude less, respectively.



## Chapter 4

### Discussion

#### *4.1. Heavy metals*

##### *4.1.1. Sediment*

According to the hypotheses of this study, pollutant concentrations in the sediments should be higher at discharge points due to legacy and on-going contamination (Redfern, 2006). This tendency was seen by Watling & Watling (1982) and Binning & Baird (2001) that also reported higher metal concentrations close to discharge points. The effect of mineralogical background is not known and the scale may be too small to recognize geological origin. However, the Swartkops River is mainly entrenched in Table Mountain Sandstone and is joined by the Eland River that is entrenched in Bokkeveld Shales. For this study, consistently higher concentrations were found in the sediments of Site 6, followed by Site 2 and Site 4 (Table 3.1). Sites 4, 5 and 6 are at the main effluent discharge points into the estuary with pollutants entering the system from both industrial and residential areas. The largest of these discharging points is the Chatty River, a tributary that runs through dense residential areas directly into the estuary (Fig 3.1; Enviro-Fish Africa, 2009). The sites will be discussed from upstream to downstream with reference to Fig 3.1 and Table 3.1.

Site 7 was used as a reference site, because it was well above the Motherwell Canal, (~ 2.7 km) and not influenced by direct industrial discharges. This site was however bordering the small neighbourhood of Redhouse with activities that include two boat clubs, a park, and a number of private jetties. Although Site 7 is in close proximity to the Perseverance industrial area, no direct drainage or canals were present. Sediment concentrations in this site were the lowest for all metals, but Cu showed similar concentrations to Sites 2, 4 and 5 (Fig 3.1). Sources of these metals may include motorboat fuels, and atmospheric deposits from the Perseverance industrial area such as alloys, pigments, and steelworks (Table 1.1). The activities in the Perseverance industrial area include inter alia SAB (South African Breweries), a metal refinery, concrete manufacturing, Coca Cola, Clover, steelworks, and the manufacturing of wheels, and roofing tiles.

Site 6, located near the discharge points of all Motherwell's sewage and untreated waste, showed the highest concentrations of all the sampled sites. The highest concentrations was Al, Cu, As, and Hg. The Motherwell residential area is home to many low-income residents, as illustrated in Fig 4.1 bottom left. The level of pollution seen in this canal is illustrated in Fig 2.4. High rainfall in the Swartkops catchment area probably contributes to the transportation and leaching of pollutants by storm water eventually ending

up in the estuary (Binning & Baird, 2001; Redfern, 2006; Orr, 2007). The mercury concentration at Site 6 was also higher than at any of the other sites and this might be ascribed to the dumping of waste. Domestic wastes would include plastics, tyres, batteries, cosmetics, pesticides, and paints for example, which can all contain mercury (Table 1.1). Adjacent to this site and the next is a large brick manufacturing industry, which may contribute to the increasing metal concentrations.

The canal leading from the Markman Industrial to Site 5 was constructed differently from the Motherwell Canal, and this presumably one of the reasons why lower metal concentrations were found at this site (Fig 4.1 top). The Motherwell Canal (Fig 4.1 top middle) is a concrete canal in which the water flows unrestricted. The Markman Canal (Fig 4.1 top left) on the other hand, is an excavated (earth-lined) canal in which natural vegetation has established in some parts. In the lower parts of this canal, vegetation has taken over to such an extent that there is virtually no sign of the canal. The effect of vegetation on heavy metals will be discussed later. When heavy rain or major floods occurs, standing water with presumably accumulated pollutants (Fig 4.1 top right) will reach the estuary.

Site 4, located at the confluence of the Chatty and Swartkops rivers, showed higher concentrations for Al, As, and Cu (Fig 3.1). The Chatty River runs through a dense informal settlement where waste is dumped directly into the river (Fig 4.1 bottom left). Some sectors along the Chatty River are also used for agriculture, but the major land-use is for livestock such as cattle, sheep, or fowl (Enviro-Fish Africa, 2009). The Chatty River with its tributaries is often used for washing, bathing, and irrigation; according to public reports, animal carcasses have also been dumped here. Metals like Al, As, and Zn is generally introduced via domestic runoff, and the production of chemicals, motor vehicles, cosmetics, etc. (Table 1.1). Industrial activities adjacent to this site are the now discontinued Swartkops power station, railway yards, the Fishwater Flats wastewater treatment plant, and the Algorax Carbon black production plant that may contribute to the high concentrations found at this particular site.

Sites 3 and 4 showed similar pollutant profiles. Site 3, much like Site 4, showed higher concentrations of Al, Cu and As (Fig 3.1). The pollutant sources at Sites 3 and 4 are therefore likely to be similar. Recreational activities between these two sites include boat clubs and private jetties, probably contributing little pollution. The reduction in concentrations between Sites 4 and 3 is likely due to dilution. Site 3 was the lowest downstream site sampled in the estuary, and had concentrations similar to Site 7.

Site 2 had the second highest metal concentrations (Table 3.1 & Fig 3.1). Despite being located away from major discharge sources, it is also isolated from the main flow of

the river, presumably by pollutants being transversely transported and incompletely flushed by tidal action, resulting in increasing metal build-up in this area (Watling & Watling, 1982). Geographically, this site lies at the foot of a hill (Fig 2.1) bordering residential housing developments with a number of storm water canals draining directly into the estuary. Numerous private jetties are found at this site, thus more fuel emissions from boats and the jetty slope causes more storm water runoff, which presumably adds to the pollution. Located directly north of this site, are a smaller informal community and the Markman Industrial area. Increased concentrations of heavy metals from industrial activities such as brick manufacturing (close to Sites 5 and 6) and carbon black production (across the estuary) are probably from atmospheric deposits and storm water canals (Table 1.1). The titanium concentration at this site was higher than at any other site (Table 3.1). The increasing titanium concentrations were also seen in Fig 3.3. Titanium is widely used in the automotive and steel industry as an excellent anti-corrosion compound (RSC, 2013). Port Elizabeth is home to two major automotive industries including Volkswagen South Africa and General Motors. There are also a number of construction industries responsible for the manufacturing of steel products around the SRE. Since seawater is known for its corrosive effects, it can be assumed that these anti-corrosion products are frequently used in the construction industry, for vehicles, and the hulls of ships and vessels.



*Figure 4.1. Top left and middle: Storm water canals leading directly into the estuary. Left: Markman Canal (Site 5) - flow is often restricted due to vegetation. Middle: Motherwell Canal (Site 6) – unrestricted and regular flow. Top right: The extent of natural and invasive vegetation causes water in the Markman Canal (Site 5) to accumulate in pools. Bottom left: Low-income residential housing alongside the Chatty River (Site 4). Pollution seen in the foreground is similar seen to that in Motherwell. Bottom right: The Chatty River and the dense stands of *Phragmites australis*.*

#### 4.1.2 Comparisons of heavy metal concentrations with previous studies

Since heavy metal concentrations can be affected by sediment grain size (Binning & Baird, 2001), comparisons between these two studies are only used as a point of reference and for comparisons with international sediment quality guidelines that does not take sediment parameters into account.

Two previous studies, focussing on the heavy metal concentrations in the sediment have been conducted in the SRE. Both Watling & Watling (1982) and Binning & Baird (2001) did extensive studies on the heavy metals, surveying the area as far as the tidal reach and Uitenhage, respectively. The present study, however, focussed only on the central and lower reaches of the estuary and excluded the dynamic estuary mouth and freshwater reaches.

In the studies of Watling & Watling (1982) and Binning & Baird (2001) (Fig 3.2), sediment cores with a depth of 10-20 cm were collected. This is a different sampling protocol from the present study; six cores of approximately 6 cm were collected from each site and mixed together a bucket. This sampling protocol was discussed in Chapter 2. Higher concentrations found deeper in the sediment will be due to historical contamination whereas surface sediments are more likely to indicate contributions from more recent activities (Redfern, 2006). It was therefore hypothesized that in the absence of any pollution mitigating measures, metal concentrations in the sediment will increase with time and that the higher concentrations seen in previous studies will be reflected in the concentrations in surface sediments from recent contributions. The samples of the current study (Fig 3.3) are therefore surface sediment, presumably representing recent activity and current exposure to most benthic organisms.

The sediment concentrations from other studies are indicated in Table 3.3 and compared to international guidelines; ISQG, ERL, and ERM. The ERL (effects-range low) and ERM (effects-range median) guidelines are concentrations developed by Long *et al.*, (1995) for a range of heavy metals. This was developed to indicate possible effects on a variety of aquatic biota. ERL and ERM delineate three concentration ranges for a particular chemical. Concentrations below the ERL indicate that effects on biota would rarely be observed. A possible-effects range where occasional effects would be seen is when concentrations are above the ERL but below the ERM. Concentrations above the ERM represent the probable effects-range; at these concentrations, biota could be affected negatively. The Canadian Quality Guidelines are considered as one of the strictest set of environmental guidelines. Because equivalent quality guidelines for South Africa are limited (Roos, 2010), the aforementioned was used throughout this project. Based on Table 3.3, it can be deduced that the metal concentrations in the sediment of the SRE are still moderate when compared to international estuaries.

#### 4.1.3. Metal concentrations in aquatic plants

\*Assumed declining metal concentrations (Fig 3.4; - Mn, Zn, Pb and Cr) observed in the sediment of the Chatty River can be attributed to the increase in the cover of the common reed (*Phragmites australis*). The highest occurrence of common reed was found around industrial settlements where human activities and disturbance was highest (Ailstock *et al.*, 2001) (Fig 4.1 bottom right). The same effect was observed in the Markman Canal (Site 5), where these plants have overgrown the canal in some stretches. Although sediment concentrations in this area could not be directly compared to previous studies, it is assumed that the metal concentrations would also be lower, as it was already comparatively low. The newly constructed wetland (Fig 2.3) at the mouth of the Motherwell Canal may therefore have contributed towards less pollution. The bioaccumulation of pollutants via aquatic plants was discussed in the first chapter.

\*Since sampling protocols differed (and different sediment layers were analysed) it is not that the concentrations here have decreased.

#### 4.1.4. Heavy metals in biota

##### 4.1.4.1. Mud Prawn

Although limited studies have been performed on the growth of mud prawn, Hanekom & Baird (1992) noticed that larger adult prawn are more apparent in the lower reaches than in the middle reaches and creeks of the SRE. In addition, the size of mud prawn may also tend to increase with decreasing tidal heights (Hanekom & Baird, 1992; Cretchley, 1996). These differences are likely due to growth rates influenced by differences in the tidal transportation of suspended food materials (Hanekom & Baird, 1992; Spencer & MacLeod, 2002) at the various sites. Although the burrows of mud prawn reaches much deeper than the top layer of sediment, the animal feeds on both suspended particles in the water pumped through the burrow, or by collecting surface particles from the immediate surroundings of the burrow openings. Heavy metal concentrations in the lower strata of the sediment would presumably, therefore, have less of an effect on the concentrations in the animal than the concentrations in suspended particles pumped through the burrow (that contribute towards the surface layers of the sediment), or the concentrations in the top layer itself (Dworschak, 1987).

Metal concentrations found in the sediment in relation to mud prawn are illustrated in Fig 3.4. For Al, Cr, and Pb, the concentrations in sediments were much higher than in the mud prawn. For As and Cd the concentrations were similar, while exceeding mud prawn concentrations for Cu and Zn were seen at all the sites and only once for Hg. Metal uptake in sediment-dwelling organisms occurs via the ingestion of sediment particles,

bioconcentration, and biomagnification (Spencer & MacLeod, 2002). Not all metals have strong accumulation properties and other metals may not be as bioavailable to the same extent; therefore, their effects would be minimal in higher trophic levels. However, for this study it was clear that Zn, As, Cd, Cu and Cr showed strong biomagnification properties (Erasmus, 2004; Redfern, 2006; Mohamed, 2008) between the mud prawn and the sediment, and considering that three of these metals falls under the “top ten” of the ATSDR priority list, they will be more closely considered here.

#### *4.1.4.2. Smaller organisms*

The Knysna Sand Goby, Estuarine Round Herring, and mud prawn are less than 10 cm long, filter feed, and serve as primary prey for birds and larger fish (Enviro-Fish Africa, 2009). The highest metal concentrations were found in mud prawn and Estuarine Round Herring (Fig 3.5), the two species with the closest contact with sediment. Higher concentrations of heavy metals in mud prawn can also be ascribed to the fact that they were collected below the major discharge-points of the estuary (Motherwell Canal, Markman Canal & Chatty River), whereas Estuarine Round Herring and Knysna Sand Goby were collected randomly. According to the Commission Regulation (EC, 2006), the maximum concentrations for Hg, Pb and Cd in crustaceans are 0.5 mg/kg. Only lead (Pb) in both mud prawn and Knysna Sand Goby exceeded this value.

#### *4.1.4.3. Larger vertebrates (Fish)*

##### *4.1.4.3.1 Feeding*

Feeding is mostly associated with the size of the predator and the availability of prey (Smit, 2008). The highest metal concentrations were frequently seen in Spotted Grunter and Garrick. Heavy metals concentrations were the highest in the liver of all fish species, followed by muscle and fat (Table 3.7). Metal concentrations tend to be higher in organs such as the liver, kidney and even gills in relation to muscle or fat (Al-Weher, 2008), and this was seen in the present study. The concentrations of lead, cadmium, and mercury in each species were compared to the maximum concentrations for certain foodstuffs (EC, 2006) are as follows: Pb = 0.3 mg/kg dm, Cd= 0.05 mg/kg dm, and Hg= 0.5 mg/kg dm. The Food and Agricultural Organization (FAO), 1983 set a maximum concentration of 0.5 mg/kg for fishery products.

Accumulation of heavy metals mostly depends on the organism's ability to digest and eliminate the metal, as well as the concentration of the metals to which they are exposed (Eneji *et al.*, 2011). The different concentrations seen in the organs and muscle of the same species and individuals can mainly be attributed to the different physiological roles and properties of the organs. The high concentrations of copper seen in the liver of all three fish

species are a concern, since concentration as low as 10 mg/kg is considered detrimental (Erasmus, 2004). Higher concentrations of Cu are often seen in the liver because of its high retention capacity towards this metal. The liver can be considered as the selective organ for the storage of Cu (Mohamed, 2008). The same principle that was seen for Cu can be suggested for Zn and Fe, where very high concentrations were found in the liver compared to the muscle or fat (Table 3.7). The high concentrations of Cu may come from a number of widespread anthropogenic sources (e.g. industrial activities, plumbing, wiring and electronics) which is then released to the atmosphere and storm water canals (Erasmus, 2004; Redfern, 2006; Newman, 2010), eventually reaching the estuary. Fish plays a major role in the SRE and overfishing and consumption of potentially polluted fish may have detrimental consequences to ecology and society (Enviro-Fish Africa, 2009).

#### 4.1.4.3.2. Fish size

The following are recorded lengths of the fish species.

- Adult Spotted Grunter can reach up to 85 cm and weigh up to 9.5 kg (Childs, 2005)
- Garrick are dependent on estuaries, they enter these systems as juveniles (40-120 mm), and can reach up to 50cm before they leave the estuary (Smit, 2008).
- Dusky Kob enters estuaries as juveniles (30-150 mm) and reaches up to 15-107 cm before they leave into the open sea where they can reach up to 1.8 meters (Griffiths, 1997).
- Flathead Mullet commonly reach up to 20 cm, although they may reach up to 80-120 cm (Saleh, 2008).

According to literature, higher metal concentrations tend to be more apparent in larger or older fish (Eneji *et al.*, 2011; Karimi *et al.*, 2013; Oyakhilome *et al.*, 2013). According to this study, the size of individual fish did not appear to have a strong correlation with metal concentrations (Figs 3.6-3.8), meaning; higher concentrations were not necessarily associated with larger or heavier fish. However, to confirm this, smaller fish for each species should be sampled and analysed. Chemical (e.g. pH, dissolved organic carbon) and biological factors (e.g. water, diet, food chain length, body length, consumption rate, etc.), ecological needs and metabolic activities influence the bioavailability and accumulation of metals in fish (Ebrahimpour *et al.*, 2011; Murtala *et al.*, 2012; Karimi *et al.*, 2013). Numerous interactions between different elements may also influence the toxicity of the metals (Ebrahimpour *et al.*, 2011). The concentration of metals and the process by which they accumulate in tissues and organs are species-dependent and related to detoxification mechanisms and metabolism. Thus, one may find various species in the same environment with different metal concentrations (Eneji *et al.*, 2011; Jamiska *et al.*, 2011). Results from



many previous studies have also shown that target organs (liver, kidney & gills) tend to accumulate heavy metals in high concentrations (also seen in this study (Fig 3.9). Fish liver is therefore often recommended as an environmental indicator for water pollution (Ebrahimpour *et al.*, 2011; Eneji *et al.*, 2011). Flathead Mullet showed the lowest metal concentrations overall probably because they were collected higher up in the estuary (~3 km from Redhouse), where concentrations were much lower compared to those collected in the middle and lower reaches (based on the sediment concentrations seen in Table 3.1).

NMS (Nonmetric Multidimensional Scaling) bi-plots shown in Figures 3.10 and 3.11 indicate that the liver of any of the three fish species would be suitable as an environmental indicator (Fig 3.10). This mean that future studies would require only the liver and since the liver of fish is not consumed by most users, these can be collected from fishers, and less fish would need to be sacrificed. It was also shown that any of the three fish species would be suitable as environmental indicators in the SRE (Fig 3.11). Since the Spotted Grunter is the most preferred and abundant species in the SRE, less time needs to be focused on endangered species likely to be found in the estuary.

#### 4.1.4.3.3. Bioaccumulation

Revisiting the definition of bioaccumulation (the increase of a contaminant's concentration from one trophic level to the next), it is apparent that this progression is also present in the SRE. The relationship between these trophic levels is important to consider since top predators are the ones more likely to be affected by accumulated pollutants than those at lower trophic levels. The discharge of industrial and domestic wastewaters, which contain high concentrations of toxic metals, may not only affect smaller marine organisms, but also larger predatory fish, and eventually humans eating these fish (Eneji *et al.*, 2011).

In Fig 3.12 a-h, the species are arranged according to approximate trophic level, with the lowest trophic level to the left. Assuming this trophic level arrangement, trends of increasing concentrations towards higher trophic levels were seen for Cd, Hg and Cu (Fig 3.12 a-c) where concentrations for each of the predators in succession were generally higher than its related prey. A similar trend was also seen between the sediment and mud prawn. Mud prawn is a typical sediment dweller, spending the majority of their lifecycle close contact with sediment. Metal uptake in crustaceans occurs directly from the surrounding water across the permeable body surface, via water, and from their food (Kumar & Achyuthan, 2007). The sediment can therefore be considered as a contributing factor to higher metal concentrations seen in mud prawn. Higher concentrations were not seen in top predators for Cr (Fig 3.121 c) and only Garrick had a mean concentration slightly higher than the sediment.

As and Zn showed indications of accumulation from sediment to the lower trophic levels (Figs 3.12 e-f), but at higher trophic levels, this trend was unclear. Although strong accumulation of As and Zn has been described (Erasmus, 2004; Redfern, 2006; Cooksey, 2012), there was no particularly strong trend in the SRE. Sediments are considered major sinks for Zn and As (Redfern, 2006), and may be a reason for higher concentrations seen in mud prawn.

Sediment concentrations for Pb and Al were much higher than any of the marine organisms sampled (Fig 3.12 g-h). These elements are both abundant metals in the earth's crust with Pb being the 36<sup>th</sup> most abundant and Al as the 3<sup>rd</sup> most abundant (Erasmus, 2004). Although literature suggest that Pb is somewhat accumulative in marine organisms (Erasmus, 2004), this was not apparent here. The investigation of detailed in situ bioaccumulation properties of individual metals did not form part of this study.

Continuous bioaccumulation may lead to high mortalities and/or cause biochemical and histological modifications in exposed fish (Mohammed, 2008). Gross pathological changes such as liver discoloration were seen in some individuals (Fig 3.13), suggesting that toxic injury may be involved. However, further investigations are needed to confirm the causes of the pathology seen.

## 4.2. Organic Pollutants

### 4.2.1 Bird eggs

The concentrations of organochlorines found in the eggs of birds, such as the Kelp Gull reflect the diet as well as the concentration of accumulated pollutants in female gulls at the time the eggs was laid (Goutner *et al.*, 2005). The Kelp Gull's omnivorous nature therefore makes them a good indicator of the chemical pollution found in and around the SRE (Muñoz & Becker, 1999). The Kelp Gull is one of the most abundant bird species found at SRE and because of its scavenging nature, pollutant concentrations were expected to be higher.

Seabird eggs are frequently used to monitor POP trends in marine ecosystems (Leat *et al.*, 2011). Differences in congener-specific polychlorinated biphenyls have been associated with differences in diets and their metabolic capacities. PCBs differ in their biodegradation and bioaccumulation patterns and pose different toxicities to wildlife (Mora, 1996). Seven PCB congeners were quantified in the eggs of Kelp Gull (Fig 3.14). It is often reported that CB-138, CB-153 and CB-180 are most common in bird tissues (Mora, 1996), and this was also found during this study. There were differences between the eggs in each nest and between clutches (Fig 3.14), but despite the concentration differences, the

congener distribution patterns seemed mostly the same. Distribution profiles were further investigated in Fig 3.15.

Eggshell thickness was regressed against  $\Sigma$ PCBs for the Kelp Gull (Fig 3.16 a-c). No significant ( $p < 0.05$ ) association was evidenced for the sharp, equator and blunt end shell thicknesses, thus for this study, thinner eggshells were not associated with increasing PCBs; if anything, a slight increase in thickness can be seen at the sharp end and the equator, but this needs further investigation.

The PCA plot of PCBs in the bird eggs (Fig 3.17) showed a clear clustering for the majority of the eggs. The egg from Velddrif seems to be associated with stronger contributions from CB-180 and CB-153. It is clear however, that the pollutant profiles differ as much between eggs as the pollutant profiles differ between clutches. The large pollutant differences between the eggs of nests 2 and 3 may reflect on the laying order of the eggs as it was assumed that pollutant loadings in the last laid egg would have higher pollutant loadings than the first laid egg (Pickard, 2010). Different pollutant profiles also reflect on the pollutant uptake through their diet prior to egg laying (Pickard, 2010; Quinn, 2010).

PCB concentrations in gull eggs from the Alaskan study showed the highest of any of the other studies listed in Table 3.10. Mediterranean Gull and Yellow-legged Gull eggs were both sampled in Greece (Albanis *et al.*, 2003; Goutner *et al.*, 2005). The Yellow-legged Gulls from 2003 showed lower concentrations than the Mediterranean Gulls from 2005 for approximately the same number of PCB congeners. The single eggs from nest 2 and 3 showed the highest concentrations compared to other Kelp Gull eggs. Two of the bird species reported by Bouwman *et al.* (2008) and Quinn (2010) had similar concentrations (African Darter and African Sacred Ibis) with African Darter being the highest and exceeding the concentrations of the present study. Different concentrations found in each study are due to the environment, the proximity of activities releasing PCBs, and the food the birds consume (Goutner *et al.*, 2005). Physiological and environmental factors may also affect the concentration of pollutants found in the eggs. These include differences in environmental input, trophic position, the clutch size and the order in which eggs were laid (Quinn, 2010). High concentrations found in SRE are likely due to the high industrial and recreational activities surrounding the estuary.

The Food and Drug Administration (FDA) requires the concentrations of PCBs to be no more than 300  $\mu\text{g/kg}$  ww for eggs. In the present study, the mean concentration of seven congeners in 15 eggs was 178  $\mu\text{g/kg}$  ww - well below the concentration set by the FDA. If humans were to consume these eggs, the PCB-associated risk will be negligible. However, the concentrations may be a problem for egg-eating predators such as mongoose and snakes occurring on these islands. Higher chlorinated PCBs such as CB-138 and CB-153

were apparent in all of the Kelp Gull eggs, reflecting the potential of enhanced bioaccumulation compared to lower chlorinated congeners (Fliedner *et al.*, 2012).

It should be kept in mind, however, that only seven congeners were determined in this study and that the actual PCB concentrations might exceed the FDA limit. The concentration in the egg from Bird Island ( $\Sigma$ PCB = 310  $\mu\text{g/g}$  wm for 16 congeners) could be indicative of such an effect. Therefore, additional eggs and also eggs from other species using the SRE should be investigated for a more complete suite of PCB congeners.

#### 4.2.2. Fish

Both the Food and Agricultural Organization (FAO, 1983) and ATSDR concentration limits for  $\Sigma$ PCBs in the edible parts of the fish are 2000  $\mu\text{g/kg}$  wm. The highest concentrations were found in the liver of the fish, and almost all other muscle (muscle and fat) was below the LOQ of 5  $\mu\text{g/kg}$  wm. It is therefore presumed that the PCB concentrations in the fish of SRE have no human health consequence even considering that not all congeners were measured.

In a similar study,  $\Sigma$ PCBs in the tissue burdens of Largemouth Bass from Escambia River Delta, Florida exceeded the USEPA screening value (20  $\mu\text{g/kg}$  wm) ranging between 23.4- 61.0  $\mu\text{g/kg}$  for the sum of four congeners (Snyder & Karouna-Renier, 2009). In San Joaquin River watershed and delta, California, the  $\Sigma$ PCBs in fish showed that 83% of 92 congeners analysed were less than the reporting levels, and concentrations were neither extensive nor extreme (De Vlaming, 2008). This would also apply to the present study, where only 29% of the concentrations exceeded this value. Both the studies of Snyder & Karouna (2009), and De Vlaming (2008) showed higher concentrations than the present study.

Therefore, because the concentrations measured in fish were mostly below safety limits and those reported in other studies, there is little indication of concern to human and environmental health.

#### 4.2.3. Sediment

The concentration of PAHs for the single sediment sample of Site 5 is shown in Table 3.12. Polycyclic (or polynuclear) aromatic hydrocarbons (PAHs) consist of two or more fused benzene rings, generally considered to be the most widespread of the organic contaminants, and are mostly produced during the incomplete burning of wood, coal, or petroleum (Newman, 2010; Wanjeri *et al.*, 2013). Studies from estuaries elsewhere showed that in the Pearl River Delta (China),  $\Sigma$ PAH concentrations ranged from 323-14812  $\mu\text{g/kg}$  dm (Mai *et al.*, 2002). In three estuaries from Malaysia, South East Asia,  $\Sigma$ PAH concentrations ranged from 6-924  $\mu\text{g/kg}$  dm (Zakaria *et al.*, 2002). In the Tana Estuary in Kenya, the  $\Sigma$ PAH

concentration was 13 µg/kg dm (Wanjeri *et al.*, 2013). No single congener concentration exceeded the ISQG, indicating that the system is relatively clean and explains the non-detection of PAHs in biological samples.

## Chapter 5

### Conclusions

The aim of this project was to determine and interpret the presence, levels, distribution, and potential threats of pollutants in the estuarine food web using candidates from the Swartkops River Estuary, South Africa. This study has shown that environmental contaminants continue to threaten the estuary. The presence of contaminants was evident throughout the sediment and food web. From previous studies, it seems that the concentrations are increasing, attributable to increased industrial and recreational activities surrounding the estuary. Even if industries and other sources of pollutants become cleaner, the increase in these will add to the contaminants already present.

Three hypotheses were formulated in Chapter 1. These hypotheses were tested by measuring the concentrations of pollutants, comparisons with historic data and comparisons between matrices.

*Hypotheses 1: Bottom sediment concentrations will be higher at discharge points.* The corresponding null-hypothesis would be that bottom sediments would not be higher at the discharge points. Varying pollutant concentrations are likely to be found throughout the system, some being much higher than others, and can reach critical levels. Three major water pollution sources to the SRE were identified - the Markman- and Motherwell canals, and the Chatty River. These discharges act as point sources and were assumed to carry higher concentrations of pollutants from neighbouring residential and industrial areas, which eventually end up in the estuary.

The pollutant concentrations found in the sediment were compared to previous work in the SRE, which also found high concentrations associated with the discharge points compared to any of the other sites analysed. Site 7 was chosen as an upstream reference site since it is not influenced by direct discharges. The concentrations here were much lower. The major discharge points of this study were Sites 4, 5, and 6. Similar pollutant profiles were observed for Al, Cu and As throughout the estuary with Site 6 being the highest. Site 6 showed the highest pollutant loads compared to any of the other sites. The lower concentrations at Sites 4 and 5 may be attributed to dense vegetation found in the waterway that may retain the pollutants. Thus, this null hypothesis may not be valid and more sampling and analyses are needed. In general, the heavy metal concentrations in the sediment of the SRE were low compared to estuaries elsewhere in the world. The concentrations were also well below the international sediment quality guidelines (ISQG, ERL, & ERM). The SRE can therefore be classified as relatively clean since all the heavy

metal concentrations in the sediment were below the ERL, negative effects on biota would rarely be observed.

*Recommendations:*

- More comprehensive and consistent studies should be conducted in order to determine the full extent of pollutants in the Swartkops River Estuary. These follow-up studies should use the same sampling protocols, thus ensuring comparable data. Various matrices (i.e. sediment, water, fauna and flora) should also be included in these studies.
- Sampling should also be done within the canals and river to determine their relative contributions. From the fish data, there seems to be more than one major source. With more data, the relevant sources might be identified. A better understanding of the differences may provide targets for intervention for pollution reduction.

*Hypothesis 2: Pollutant concentrations will be higher within organisms in the lower and middle reaches of the estuary since they are exposed to direct discharges.* The corresponding null-hypothesis would be that pollutant concentrations would not be higher within those organisms situated in the middle and lower reaches of the estuary. Based on the first hypothesis, it was clear that higher concentrations were more likely found at the discharge points, and reflected higher concentrations in the mud prawn. Therefore, the null-hypothesis is not rejected, and more samples from these regions may have shown a much clearer trend. Not all contaminants showed strong accumulative tendencies in the SRE as the uptake and elimination of these contaminants differ for each species and/or individual. Although low concentrations may be present in these organisms, their concentrations may have detrimental effects on higher trophic levels. Estuaries provide well-maintained ecosystems whilst providing feeding areas for many species of bird and fish - the SRE is one such system, although, as was concluded from Hypothesis 1, that the concentrations, although reflecting discharge points, were still below general levels of concern and quality. One should keep in mind that these levels and guidelines were developed for regions, biota, and ecosystems functioning different from the SRE specifically, and estuaries in South Africa in general.

Therefore, the continuous pollution of the estuary may well contribute towards unpredictable adverse effects throughout the food web, including humans, motivating expanded studies and surveys.

*Recommendations:*

- Additional invertebrate species should be included in the study, such as crab and mussel.
- More sites should be analysed, including those sites not directly affected by inflow
- Smaller organisms such as the Estuarine Round Herring and Sand Gobies should be analysed at more sites in the SRE.
- Changes in ecosystem functioning, in the face of increased pollution and other pressures on the estuary should be closely investigated and monitored. This study has identified some of the end-points that can be included.

*Hypothesis 3: Toxic pollutants will bio-accumulate from lower to higher trophic levels.* The corresponding null-hypothesis would be that toxic pollutants would not bio-accumulate from the lower to higher trophic levels. During this study, organisms from different trophic levels within the estuary were tested for pollutants. These toxic chemicals accumulate in fish and shellfish, water birds, and other freshwater and marine animals. Higher concentrations were generally observed in the higher trophic levels and top predators. Therefore, the null-hypothesis is not rejected and more data is needed to provide meaningful statistics. Since the data analysed in the Kelp Gull could not be compared to other organisms of the estuary, such as mud prawn, Estuarine Round Herring and Sand Goby, the results are difficult to interpret. PCBs do accumulate, but they were not detected in matrixes other than bird eggs. Therefore, PCBs should be present in the other matrixes such as sediment and Mud Prawn, but at concentrations below detection limits.

Although the Kelp Gull is also a scavenger feeder (meaning that it would be exposed to PCBs in rubbish dumps and landfills), it also feed on mud prawn. The PCB sources are therefore from both scavenging and predation. Better detection limits would have enabled fingerprint analyses to identify the apportionment of these two sources. It is unlikely that there would be an even contribution from both. If the main contribution came from mud prawn, the functioning of the SRE and human health may be compromised. Pathological changes were seen in the livers of some fish, and although this was not further studied, it suggests that the fish are already subjected to injury from some form of pollutant. The highest trophic level in the SRE is the human population and since this estuary forms an important recreational asset in Port Elizabeth especially for fishing, human health may be of concern. Everyday nearly 20 boats and numerous subsistence fishers can be found around the estuary, and doubles over weekends and holidays. Heavy metal concentrations found in the edible part of the fish (i.e. muscle & fat) were not very high, and were mostly below nearly all of the maximum food concentration guidelines. Only Cd exceeded concern



concentrations in both muscle and fat. The continuous exposure to Cd may result in kidney failure, chronic lung diseases, and respiratory irritation. The pathology observed cannot be ascribed to any single pollutants, but combinations of pollutants may be involved, including PAHs and other not measured.

*Recommendations:*

- Future studies should employ analytical capacity with much better detection limits.
- More fish sizes should be included. It is recommended that they should range from juvenile up to the point where they leave the estuary.
- The liver of one species of fish should be more than adequate for monitoring changes in heavy metal concentrations in the SRE.
- For this study, only one bird species was selected. More species should be included to determine risks to the breeding birds associated with the estuary. Different breeding sites should also be included.
- Human health studies should be encouraged to determine how SRE's contaminated resources may be affecting the users of the estuary.
- Observed pathology should receive more attention.
- The sources of PCBs should be investigated.

Finally, the results of this study should be communicated to the relevant authorities and managers to increase awareness, inform management, and potentially lead to pollution reduction interventions.

## Chapter 6

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