Investigating morphological changes in fish tissue, due to the presence of persistent organic pollutants and metals

W. PHEIFFER

20545959

Dissertation submitted in fulfilment of the requirements for the degree Master of Science in Environmental Sciences at the Potchefstroom Campus of the North-West University

Supervisor: Doctor Rialet Pieters

Co-Supervisor: Professor Nico Smit

November 2012

Here, ons Here, hoe wonderbaar is u Naam oor die hele aarde, hoe glansryk alles wat U in die hemelruim geplaas het!

Psalm 8:2

Table of Contents

List of Tables and Figures

List of Abbreviations

1,2,3,4,6,7,8-HpCDD	1,2,3,4,6,7,8-Heptachlorinated dibenzo-*p*-dioxin
1,2,3,4,6,7,8-HpCDF	1,2,3,4,6,7,8-Heptachlorinated dibenzofuran
1,2,3,4,7,8,9-HpCDF	1,2,3,4,7,8,9-Heptachlorinated dibenzofuran
1,2,3,4,7,8-HxCDD	1,2,3,4,7,8-Hexachlorinated dibenzo-*p*-dioxin
1,2,3,4,7,8-HxCDF	1,2,3,4,7,8-Hexachlorinated dibenzofuran
1,2,3,6,7,8-HxCDD	1,2,3,6,7,8-Hexachlorinated dibenzo-*p*-dioxin
1,2,3,6,7,8-HxCDF	1,2,3,6,7,8-Hexachlorinated dibenzofuran
1,2,3,7,8,9,-HxCDF	1,2,3,7,8,9,-Hexachlorinated dibenzofuran
1,2,3,7,8,9-HxCDD	1,2,3,7,8,9-Hexachlorinated dibenzo-*p*-dioxin
1,2,3,7,8-PeCDD	1,2,3,7,8-Pentachlorinated dibenzo-*p*-dioxin
1,2,3,7,8-PeCDF	1,2,3,7,8-Pentachlorinated dibenzofuran
2,3,4,6,7,8-HxCDF	2,3,4,6,7,8-Hexachlorinated dibenzofuran
2,3,4,7,8-PeCDF	2,3,4,7,8-Pentachlorinated dibenzofuran
2,3,7,8-TCDD	2,3,7,8-Tetrachlorinated dibenzo-*p*-dioxin
2,3,7,8-TCDF	2,3,7,8-Tetrachlorinated dibenzofuran
ADD	Average Daily Dosage
Ag	Silver
ANOVA	Analyses of variance
ANZECC	Australian & New Zealand Environment And Conservation Council
As	Arsenic
Au	Gold
BM	Body mass
BF	Bio-accumulation factor
Cd	Cadmium
Cf	Contamination Factor
C_{Fe}	Concentration of Fe

C_{HM}	Heavy metal concentration
C_m	Average concentration of pollutant in food substance
Co	Cobalt
CR	Cancer Risk
Cr	Chromium
Cu	Copper
DAFF	Department Of Agriculture, Forestry And Fisheries, South Africa
DDD	Dichloro diphenyl dichloro ethane
DDE	Dichloro diphenyl dichloro ethylene
DDT	Dichloro diphenyl trichloro ethane
dl	Dioxin like
DNA	Deoxyribonucleic acid
DWA	Department of Water Affairs, South Africa
ED	Exposure duration
Ef	Enrichment factor
Fe	Iron
FOA	Food And Agriculture Organisation
HCB	Hexachlorobenzene
HDPE	High density polyethylene
Hg	Mercury
HI	Hazard Index
HPA	Health Protection Agency, United States of America
HRGC-HRMS	High resolution gas chromatography- high resolution mass spectrometer
ICP-MS	Inductively coupled plasma mass spectrometry
I_{Fish}	Mean fish Index
I_G	Mean gill Index
I_{geo}	Geo-accumulation index

I_K	Mean kidney Index
I_L	Mean liver Index
IQ	Intelligence quotient
IRIS	Integrated Risk Information System
IR_m	Average intake rate
ITIS	Integrated Taxonomy Identification System
km	Kilometre
LADD	Lifetime Average Daily Dose
LRAT	Long range atmospheric transport
LT	Expected lifetime
mdnc	Mean degree of non-compliance
Mn	Manganese
MPI	Metal Pollution Index
NCB	Northern Cape Business
NCSU	North Carolina State University
Ni	Nickel
OC	Organo-chloride (pesticides)
OCDD	Octachlorinated dibenzo-*p*-dioxin
OCDF	Octachlorinated dibenzofuran
Orasecom	Orange-Senqu Commission
OSHA	Occupational Safety And Health Administration
PAH	Poly-aromatic hydrocarbons
Pb	Lead
PBB(s)	Polybrominated biphenyls
PBDE(s)	Polybrominated diphenyl ethers
PCB(s)	Polychlorinated biphenyl(s)
PCDD(s)	Polychlorinated dibenzo-*p*-dioxin(s)
PCDF(s)	Polychlorinated dibenzofuran(s)

PFCs	Perfluorinated compounds
PFOS	Perflourooctane sulfonate
PLI	Pollution Load Index
POPs	Persistent organic pollutants
Pt	Platinum
RfD	Reference Dose
Se	Selenium
Sf	Slope factor
SL	Standard length
SQG-I	Sediment quality guideline index
SQI	Sediment Quality Index
TEF	Toxic equivalent factor
TEQ	Toxic equivalency quotient
Tl	Thallium
U	Uranium
UNEP	United Nations Environmental Programme
US DOI	United States Department Of The Interior
US EPA	United States Environmental Protection Agency
WRC	Water Research Commission, South Africa
ww	Wet weight
Zn	Zinc
α-HCH	α-hexachlorocyclohexane
β-HCH	β-hexachlorocyclohexane

Acknowledgements

I would like to express my gratitude and thanks to:

- the Lord, for His love, support and for giving me the privilege and abilities to study;
- Dr R Pieters, for her guidance, patience and support throughout this project;
- Prof NJ Smit, for his advice and contribution in the completion of this dissertation;
- ORASECOM (UNDP-GEF) for financial support;
- Dr JC van Dyk, for his selfless contribution with the histological assessment
- the School of Biological Sciences and its staff , especially Prof L du Preez and Dr C Weldon, for the use of the facilities and infrastructure;
- my fellow students, for assistance and support;
- my family and loved ones for their encouragement, support, and love throughout this study.

Investigating morphological changes in fish tissue, due to the presence of persistent organic pollutants and metals.

Levels of selected metals and persistent organic pollutants (POPs) were investigated in sediment and fillet tissue of the sharptooth catfish (*Clarias gariepinus*), from sites along the Orange-Vaal River system, South Africa.

A histological assessment was done on the livers, kidneys, and gills of the fish sampled to determine morphological changes. The sediment and fish sampling sites were selected up- and downstream of major pollution sources such as mining, industrial and agriculture. The concentrations of the metals in the fish and sediment was determined with an inductively coupled plasma mass spectrometry and the POPs with a high resolution gas chromatography-high resolution mass spectrometer. Indices were calculated to describe the quality of the sediment The enrichment factor (Ef) of individual heavy metals evaluated elevation in levels above natural geology. The geo-accumulation index (Igeo) determined the degree of pollution by the enrichment levels of the individual metals. The pollution effect of the total mixture of the heavy metals was investigated by the metal pollution index (MPI) and pollution load index (PLI). Ecological risk was determined by calculating the sediment quality guideline index (SQG-I) and a sediment quality index (SQI) to determine the quality of the sediment. For the SQG-I and SQI, international sediment quality guidelines were used, since South Africa does not have them. The bio-accumulation factor (BF) was calculated between sediment and fish. A limited human health risk assessment was done for the consumption of *Clarias gariepinus*. A semi-quantitative histopathological assessment was preformed and alterations found were numerically described with the aid of mean organ indices.

The POPs values were very low. The Igeo and Ef of Se, Hg, Ag and Au were the highest at all the sites. Parys had the most metals that were enriched to different degrees, but Rooipoort had the highest MPI and PLI. The SQG-I indicated that the sites had a moderate chance of posing an ecological risk to its biota, except for Rooipoort that had a high toxic probability. The SQI classified Rooipoort as “fair” in terms of sediment quality and the rest of the sites as “good”. The semi-quantitative histology based assessment results showed that the mean organ and fish indices fell within class 1 (normal tissue structure with slight histological alterations) or class 2 (normal tissue structure with moderate histological alterations). The mean gill, -liver and kidney indices for all the sites fell in class 1. The mean fish index for all the sites however, fell in class 2. The human health risk assesment showed high risk for non-carcinogenic effects from Ag, Hg, As and Cr if fish from the sample sites were to be consumed.

The results from this study identified that the river system is polluted by anthropogenic activities. Results showed that the pollutants of concern in the system were ultimately Ag, Hg and PFOS. Although the morphology of *Clarias gariepinus* was not altered, the results indicated that the Orange-Vaal River system is polluted and that these fish is unsafe for human consumption.

Keywords: Fresh water pollution, ecotoxicology, heavy metals, POPs, fish histology, sediment indices, human health risk

’n Ondersoek na morfologiese veranderinge in visweefsel in die teenwoordigheid van persisterende organiese besoedelstowwe en metale

Die vlakke van geselekteerde metale en persisterende organiese besoedelstowwe (POB) is vir sediment en vis (*Clarias gariepinus*) in die Oranje-Vaalrivierstelsel, Suid-Afrika ondersoek.

’n Histologiese assessering is op die niere, lewers en kieue van die vis gedoen om morfologiese veranderinge te bepaal. Die versamelingspunte was stroom-op en stroom-af van besoedelingsbronne soos landbou, mynbou en industrieë. Die konsentrasie van die metale is deur induktiewe gekoppelde plasmamassaspektrometriese analise bepaal en die POBe d.m.v. hoë resolusie gaschromatografie-hoë resolusie massaspektrometrie. Indekse is bereken om die kwaliteit van die sediment te beskryf: Die verrykingsfaktor (Vf) is vir individuele metale bereken om vas te stel tot watter mate die vlakke hoër is as die natuurlike geologie. Die geo-akkumuleringsindeks (Igeo) bepaal die graad van besoedeling wat veroorsaak is deur die verrykingsvlakke van die individuele metale by elke monsternemingspunt. Die besoedelingseffek wat veroorsaak word deur die totale elementmengsel is bepaal deur die elementkontaminasie-indeks (EKI) en die besoedelingladingsindeks (BLI) te bereken. Ekologiese risiko is bepaal deur die sedimentkwaliteitriglynindeks (SKR-I) en die sedimentkwaliteitsindeks (SKI) om die sedimentkwaliteit te bepaal. Die bio-akkumulering vir besoedelstowwe vanaf die sediment na die vis is ook bereken en word uitgedruk as die bio-akkumuleringsfaktor (Bf). ‘n Beperkte menslikegesondhiedsrisko-assesering was op die verbruik van *Clarias gariepinus* gedoen. ‘n Semi-kwantitatiewe histologiese assessering was gedoen en die afwykings was beskryf in orgaanindekse.

Die POBe waardes was baie laag. Die Igeo en Kf van Se, Ag en Au was die hoogste by al die versamelingspunte. Die metale by Parys was die meeste verryk maar Rooipoort het die hoogste EBI en BLI gehad. Die SKR-I het getoon dat drie van die vier studie-areas se metale ’n matige risiko het om toksies vir die biota te wees. Rooipoort het dus ’n groter waarskynlikheid om toksies vir biota te wees. Die SKI het Rooipoort geklassifiseer as “matig” i.t.v. sedimentkwaliteit en die res van die areas het as “goed” gekwalifiseer. Die semi-kwantitatiewe histopatologiese assessering resultate van die orgaan- en visindekse het geval in klas 1(normaal weefselstruktuur met effense histologiese veranderinge) of klas 2 (normaal weefselstruktuur met matige histologiese veranderinge). Die gemiddelde kieu-, lewer-, en nierindekse was in klas 1 en die gemiddelde visindeks was in klas 2. Die menslikegesondhiedrisko-assesering het hoë risiko vir nie-karsinogeniese effekte getoon vir Ag, Hg, As en Cr, indien die vis wat versamel is, geëet word.

Die resultate van die studie het aangedui dat die rivierstelsel besoedel is a.g.v. antropologiese aktiwiteite. Besoedelstowwe wat kommer skep in die stelsel was uiteindelik Ag, Hg en perfluoro-oktaan sulfonaat (PFOS). Alhoewel daar nie veranderinge in die morfologie van *Clarias gariepinus* was nie, het die resultate aangedui dat die Oranje-Vaalrivier stelsel besoedel is en dat dié visse nie veilig is as voedsel vir mens nie.

Sleutelterme: varswaterbesoedeling, ekotoksiekologie, swaarmetale, POBe, vishistologie, sediment indekse, menslikegesondhiedrisko

1. Introduction

1.1 General introduction.

The Orange-Senqu catchment covers an area of 1 000 000 km^2 of which 600 000 km^2 falls within South Africa. This makes it the largest catchment in the country, providing six of the nine provinces with water and other resources. The rest of the greater catchment is shared with neighbouring countries: Lesotho, Botswana and Namibia. The catchment receives a total of 12 000 million m^3 of run-off per year (DWA, 2011a). The Orange River has a total water demand of 65 000 million m^3 per year of which 64 % is destined for agricultural use and 30 % for urban and mining use (Earle *et al*., 2005).

The Vaal River is the biggest and most important tributary of the Orange River. The Vaal River provides water to the industrial centre of South Africa, supporting industries that produce 50 % of South Africa's gross domestic product and 80 % of the country's electrical production (DWA, 2011a). This is why it is called the hardest working river in SA (Braune & Rogers, 1987; Van Wyk, 2001; Brand *et al*., 2009).

According to Brand *et al* (2009), unfortunately South Africa has over-utilized its water resource, with the attitude that the resources are inexhaustible. This caused the deterioration of ecosystems. Anthropological influences like pollution, misuse and poor management of water resources created environmental problems like poor water quality and the diminishing of ecosystem health.

The Orange and Vaal Rivers are subject to pollution from agriculture, industry, mining wastewater treatment facilities, urban areas and informal settlements (Braune & Rogers, 1987; Van Wyk, 2001; Dikio, 2010; Wepener *et al*., 2011).

Persistent organic pollutants (POPs) are chemicals (industrial and pesticides) that persist in the environment, bio-accumulate in the food web and pose a risk of causing adverse effects to the environment and human health (Stockholm Convention, 2011). These chemicals can travel long distances to areas where they were never produced or used (UNEP, 2011). The Stockholm Convention on Persistent Organic Pollutants is a global treaty, that aims to ban and reduce the use of these chemicals in order to protect environmental- and human health (Stockholm Convention, 2011).

South Africa is one of the countries that has signed the treaty and has to adhere by the objectives of the convention (Bouwman, 2004).

Heavy metals are metallic elements that have high densities and atomic mass (Duffus, 2002). These metals are toxic at low levels. They are dangerous because they can bio-accumulate and are difficult to metabolise (Dallas & Day, 2004). They are released into the aquatic environment through anthropological sources, like mining and various types of industries (Dallas & Day, 2004).

This study will investigate the levels of POPs and heavy metals in the Orange-Vaal River system and whether these levels have histological effects on an indicator species, *Clarias gariepinus*, and possibly pose a threat to human health.

1.2 Aim and Objectives

1.2.1 Hypothesis:

There is evidence of effects of metals and persistent organic pollutants on fish *Clarias gariepinus* at various levels of biological organisation, likely due to anthropogenic activities in the Orange-Vaal catchment.

1.2.2 The aims for the study:

The aim of this study will be to determine the levels of heavy metals and POPs in the fish muscle and sediment of the Orange/Senqu catchment. Endpoints of pollution in the fish investigated include bio-accumulation and histological effects. Assessing possible risk to human health will be done by extrapolating measured pollution from the investigated aquatic environment.

1.2.3 The objectives for this project are to:

- assess the presence of heavy metals[1] and POPs in the sediment and in the target fish species, *Clarias gariepinus* from selected sites in the Vaal- and Orange Rivers,
- compare pollution levels between the rivers in terms of sediment and fish,
- determine bio-accumulation in the fish from the sediment
- determine if histological changes in target organs can be ascribed to observed pollution levels and
- perform limited human health risk assessment on the levels found in the sediment and fish muscle.

[1] Current scientific thinking does not approve of the use of the term 'heavy metal' (Hodson, 2004; Chapman, 2007; Chapman, 2012). This term however is used in this dissertation and is defined in Literature Review chapter (2.2.2)

2. Literature review

South Africa's water resources are limited and the increase in population has put the freshwater systems under immense pressure (Dallas & Day, 2004). A more pressing matter is the quality of the available water. Water quality can be described as the physical, chemical, biological and aesthetic properties of water, which determine its fitness for use and consumption and its ability to maintain the integrity and health of an ecosystem (DWA, 2010b). Water quality is lowered by anthropogenic influences such as pollution. In South Africa, freshwater pollution can be linked to diffuse surface run-off (agricultural and mining) and point source discharge (waste water and industrial effluents) (Du Preez *et al.*, 2002).

2.1 Orange and Vaal Rivers

The Orange-Vaal basin is South Africa's largest river basin covering approximately 45% of the country (600 000 km^2). The Orange River originates in the Lesotho Highlands (where it is called the Senqu River) (Figure 1) and stretches 2 300 km to the west where it flows into the Atlantic Ocean at Alexander Bay (DWA, 2011a). The Orange River is divided into three sections. The Upper Orange refers to the stretch of river from the Lesotho Highlands to the Gariep Dam (DWA, 2012a). The Middle Orange is the part of river from the Gariep Dam to the confluence with the Vaal River (DWA, 2012b). The Lower Orange is from the Orange-Vaal confluence to where the river mouths into the ocean (DWA, 2012c). The Orange River banks are heavily developed for irrigation, while the Gariep and Vanderkloof Dams (located in the Upper Orange water catchment) (DWA, 2004a) produce hyrdo-electricity during peak demand periods. Irrigation-, mining- and hydro-electricity activities forms the majority water usage of the river. The water of the Orange River is seldom used for domestic or industrial water with the exception of the water that is used in the Vaal River system (DWA, 2011a).

According to Bucas (2006), pollution on the Orange River is from mining, urban development and agriculture. The Upper Orange River, which is an area consisting predominantly of rural communities, receive minimal pollution from few industries and mining activities in the area (DWA, 2004b). Mining takes place along the Lower Orange and coastline at Alexander Bay for diamonds (DWA, 2004a). Within the catchment, in the central and lower parts of the Orange River, copper is mined in the Okiep Copper district (DWA, 2004a). Iron ore is mined at Sishen mine, north of the Orange River (DWA, 2011b). The mine has an average annual production of 41.3 million tonnes (Anglo-American, 2010). Mining along the river results in an increase of

metals and erosion, that causes and increase in dissolved salts and an increase in turbidity (Dallas & Day, 2004). Large cities within the Orange-Senqu catchment, like Bloemfontein, Kimberley, Botshabelo and Maseru, drain into the Orange River, which can increase the bacterial contamination, nutrient levels and lead to a high level of toxins, metals and hydrocarbons entering the system (Bucas, 2006). Agriculture also increases the salt load as fertiliser nutrients run-off into the river (Dallas & Day, 2004; Bucas, 2006). Included in the run-off from crops and pastures are pesticides and organic carbon loads (Dallas & Day, 2004). Agriculture in Lesotho is mainly subsistence farming with maize, wheat and sorghum as main crops. Livestock include mainly cattle and goats (FOA, 2005). South Africa, Namibia and Botswana have mainly a commercial farming sector, although the area of Botswana within the catchment consists of rural areas practicing subsistence farming (Orasecom, 2011). Along the Orange River in South Africa both grain and livestock agriculture is successful. Maize, wheat, oilseeds, cotton, olives, tea, citrus fruit and grapes are cultivated in the area as well the farming of sheep, goats and cattle. (DAFF, 2011; NCB, 2012)

The Vaal River originates in the Mpumalanga Province (Figure 1), near Ermelo, on the western slopes of the Drakensberg escarpment (Braune & Rogers, 1987). It is about 1 120 km long, is the largest tributary of the Orange River (Dikio, 2010) and covers 30% of the Orange-Vaal basin (Braune & Rogers, 1987). The river is subjected to point source pollution from waste water treatment facilities, industries and mines as well as non-point pollution from mining, urban development and agriculture (Braune & Rogers, 1987; Van Wyk, 2001; WRC, 2009). The Vaal River flows southwest towards the Orange River through the Vaal Triangle, an area considered to be one of the major industrial areas in South Africa. The Vaal Triangle is an area that is formed by three cities, Vereeniging, Vanderbijlpark and Sasolburg (Dikio, 2010). Within this area, industries include ferrous and non-ferrous metal production, petrochemical-, polymer plastic- and fertilizer manufacturing plants as well as coal generated power stations and coal and iron mines (Niewoudt *et al*., 2009; Engineernews, 2009; Mbedi.com, 2012). Within the Vaal River's catchment there are also gold, uranium, ferroalloys, platinum and silver mines and metal refineries (US DOI, 2007).

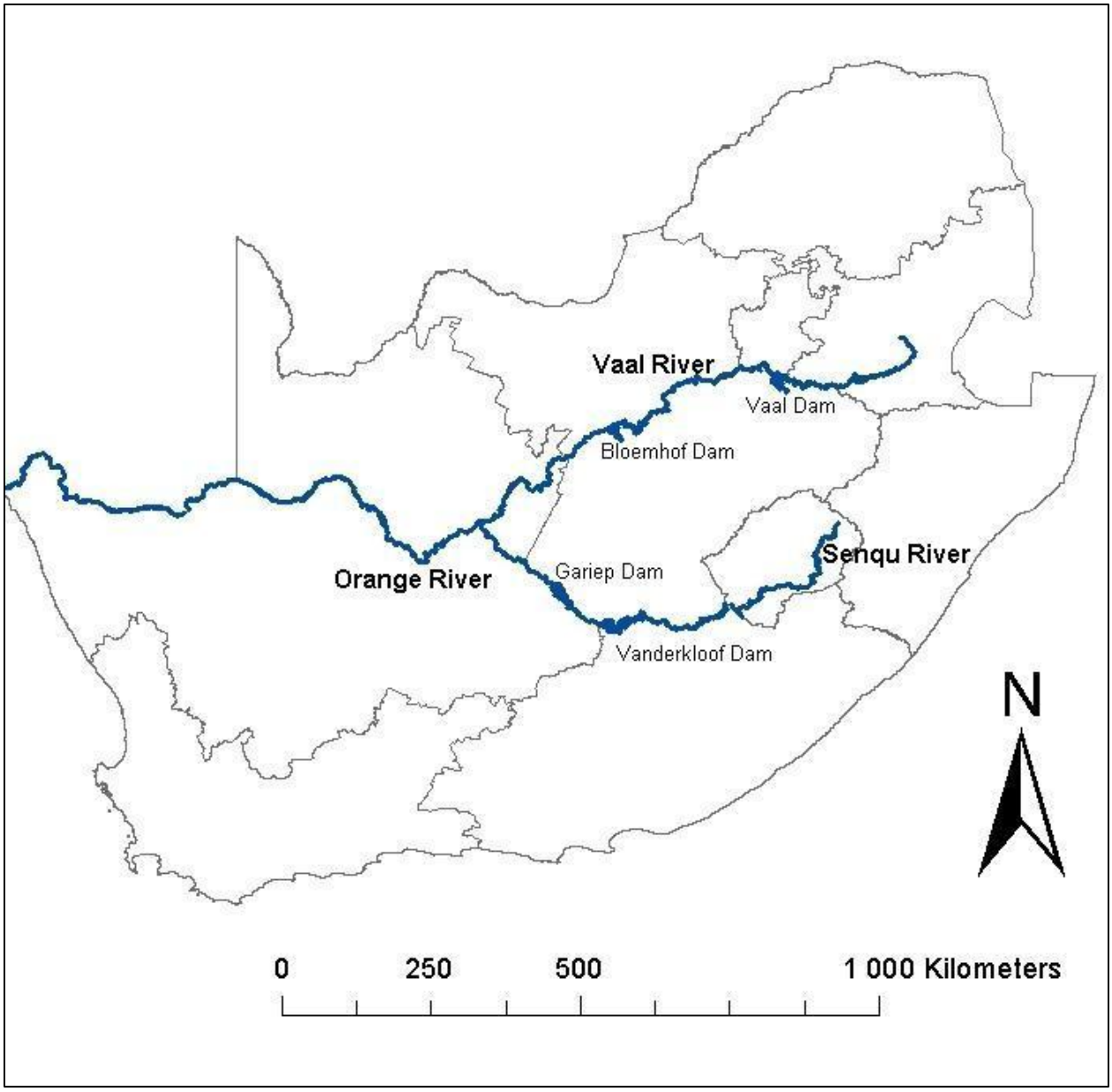

Figure 1: Map of South Africa showing the Orange-Vaal River system

The pollution results in an increase in the pH, salinity, dissolved nutrients (nitrates, sulphites, phosphates and chlorides) and heavy metals (arsenic, cobalt, cadmium, copper and zinc) (Van Wyk, 2001) of the water. The increase in nutrients leads to eutrophication. As the Vaal River flows towards the Orange-Vaal confluence in the Northern Cape, the water quality deteriorates dramatically (WRC, 2009). Tributaries of the Vaal River like the Blesbok Spruit (Roychoudhury & Strake, 2009), Riet Spruit and Suikerbosrand River (Chutter, 1963), Klip Spruit (Hancock, 1973) and the Klip River (Chutter, 1963; Hancock, 1973; McCarthy & Venter, 2006) are polluted by urban, industrial and agricultural activities and so indirectly pollutes the Orange-Senqu

system, more specifically the Lower Orange as it receives the combined pollution from the upstream systems (DWA, 2004c)

As agriculture and mining are widely practised alongside the Orange-Vaal system, the rivers and their tributaries are subject to pollution from industry. The focus of this study will be on the presence of persistent organic pollutants (POPs) and heavy metals as the contaminants in question.

2.2 Pollutants

2.2.1 Persistent Organic Pollutants (POPs)

POPs are harmful organic chemical substances (industrial chemicals and pesticides) that remain intact for long periods of time in the environment (UNEP, 2011; Stockholm Convention, 2011), due to their long half-life times in soils, sediment and biota (Jones & de Voogt, 1999). The POPs are polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), non dioxin-like polychlorinated biphenyls (non dl-PCBs) and dioxin-like polychlorinated biphenyls (dl-PCBs); Polybrominated diphenyl ethers and biphenyls (PBDE/PBB): hexabromodiphenyl ether , heptabromodiphenyl ether, tetrabromodiphenyl ether, pentabromodiphenyl ether and hexabromobiphenyl; Perfluorinated compounds (PFCs): perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride; the organo-chloride (OC) pesticides: aldrin, chlordane, chlordecone, dichloro diphenyl trichloroethane (DDT) and it's degradation products, dieldrin, endosulfan, endrin, heptachlor , Heptachloroepoxide, α-hexachlorocyclohexane and β-hexachlorocyclohexane (α- and β-HCH), hexachlorobenzene (HCB), lindane, mirex, pentachlorobenzene and toxaphene (Stockholm Convention, 2011).

PCBs are products of industry, found in transformers and used as hydraulic fluids and flame retardants. PCBs and PCDD/Fs are by-products of combustion from various industrial processes including the combustion of waste (Ross, 2004; Carpenter, 2006; Costa *et al*., 2008). PBDEs are a group of chemicals used a flame retardants in industrial and commercial products (Costa *et al*., 2008), these chemicals were produced at larger volumes before being banned and quantifiable levels are found in wildlife and humans (Darnerud, 2003). OC pesticides were used as a cost effective pest control, but due to their toxic effect on biota and ability to persist in the environment they were banned in a large number of countries from the 1970's to the present (Dallas & Day, 2004; Stockholm Convention, 2011). PFCs are produced by a wide range of

industrial processes, they are found in domestic and commercial products. They are used as water repellents, in upholstery, leather, clothing and carpets, as paints, waxes, polishes in the domestic and commercial environments. Industrial uses are as additives, surfactants, lubricants, coatings, emulsifiers and wetting agents (Post *et al*., 2012).

POPs are mainly lipophillic and hydrophobic (Jones & de Voogt, 1999), with the exception of PFOS which is both lipophillic and hydrophilic (Völkel *et al*., 2008). In aquatic environments these chemicals tend to particulate to solids, notably organic matter and avoid being in aqueous phase. Being lipophillic they partition with the lipids of an organism and get stored in the fatty tissue, instead of infiltrating the aqueous cytoplasm of the organisms' cells. Some POPs also have the inclination to turn into their gaseous form (Jones & de Voogt, 1999).

Due to their chemical properties POPs are easily transported by air and water currents and can have an effect in areas where they are not generated (US EPA, 2009), for example, the presence of PCBs, lindane and endosulfan have been noted in the Arctic, transported there by means of long range atmospheric transport (LRAT) (Halsall, 2004). These molecules have the ability to move between different locations by repeated evaporating and deposition over time and this is called the "grasshopper effect". The grasshopper effect or hopping differs from LRAT in the way that it has multiple depositions and not only a single emission-deposition event. This happens when a molecule deposits in an area and is later volatilised back into the air, where air currents transport it to a different area (Gouin *et al*.,2004). At the Faroe Islands (near Iceland) DDT and PCBs were found in high quantities in porpoise blubber (3 500-5 000 µg/kg ww and 8 000-12 000 µg/kg ww, respectively) (Fromberg, 1999).

POPs were widely used after the Second World War, where synthesised chemicals were commercially used in industry, agriculture and pest control (US EPA, 2009), until the Stockholm Convention on Persistent Organic Pollutants banned and/or limited the production and use of these chemicals (US EPA, 2009; UNEP, 2011; Stockholm Convention, 2011). In May 2002, South Africa signed the agreement and became a party to the Convention. In September 2004 the Stockholm Convention went into force and as a signed party, South Africa was obligated to reduce and ban the use of the listed POPs, abide by the other objectives of the convention and to promote research on POPs (Bouwman, 2004).

POPs bio-accumulate (the net accumulation of contaminants within an organism, from water, air and solid sources) (Newman, 2010) in the fatty tissue of living organisms, occurring in higher

concentrations at higher levels in the food chain (e.g. aquatic predators, Berglund *et al*., 2005) due to bio-magnification (the increase of contamination from one trophic level to the next attributable to accumulation of contaminant from food) (Newman, 2010). They cause adverse effects in humans and wildlife. POPs can induce cancer, increase allergic reactions, damage to the central and peripheral nervous systems. These chemicals can also cause disruption of the immune-, reproduction- and endocrine systems and can cause death (UNEP, 2011; Stockholm Convention, 2011).

The toxicological effects of the dioxin-like (dl) compounds (PCDD/Fs and dl-PCBs) are expressed in toxic equivalency. This value is based on the comparative toxic effect of each dl-compound. Equivalence values are factors of toxicity and are called toxic equivalent factors (TEFs). 2,3,7,8-Tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD) is the most toxic of the dl-compounds and is assigned a TEF value of 1.0, therefore the TEF values of the toxic PCDD/Fs and dl-PCBs are proportionate to 2,3,7,8-TCDD, relative to their toxicity. The toxicity of these compounds is expressed by multiplying the concentration of the chemical by its TEF value. The product is called the toxic equivalent quotient (TEQ) (Van den Berg *et al*., 2006; Newman, 2010). The TEQ of a mixture is determined by adding the individual TEQs (McKay, 2002) and can be used to assess the risk these compounds pose to humans and the environment (Yao *et al*., 2002).

Hydrophobic organic substances have a tendency to accumulate in the sediment of polluted rivers, where they persist and pose a danger for benthic fauna (Dmitruk *et al*., 2008) and their predators (Kidd *et al*., 2001). In winter, when metabolic demand increases, accumulated PCBs in the fatty deposits of arctic char (*Salvelinus alpinus*) migrate to the vital organs. With the high energy demand of the brain and liver, these the organs receive the highest PCB concentrations (Letcher *et al*., 2010). This migration to vital organs may have a negative effect on these organs due to the increasing concentrations as winter progresses.

2.2.2 Heavy metals

The term “heavy metal” refers to any metallic chemical element that has a density higher than 3.5 g/cm^3, has a high atomic mass (Duffus, 2002) and is toxic at low concentrations. Examples of these elements include cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu) and arsenic (As). Heavy metals are natural elements that cannot be degraded or destroyed. As trace elements, some heavy metals such as Cu, selenium (Se) and zinc (Zn) are essential to maintain the metabolism in living organisms. However, in higher concentrations, these elements can have toxic effects. Heavy metals are dangerous because they bio-accumulate and are not metabolised and excreted easily (Duffus, 2002; Dallas & Day, 2004).

Heavy metals occur in surface water in colloidal, particulate and dissolved form. Dissolved concentrations are generally low. Colloidal and particulate metals are found as oxides, hydroxides (Carvalho *et al*., 1999), sulphides (Danscher, 1981) or silicates or in the sediment, adsorbed by clay silica or organic matter (Rauret, 1997; Enguix González *et al*., 2000). The solubility of heavy metals is influenced by the pH of the water, temperature, salinity and the redox potential (Dallas & Day, 2004; Enguix González *et al*., 2000). When heavy metals are bio-available they will accumulate in the biota.

The main sources of metals in aquatic environments are geological weathering, agricultural run-off, industrial effluents and acid mine drainage (direct discharge and leaching). Heavy metals are used in and discharged from major industries like ferrous and non-ferrous metal works, tanning and textile plants, pulp/paper mills, petro-chemical- herbicide- and pesticide manufacturers (Dallas & Day, 2004).

2.2.3 Effects of pollutants on fish

It was found that Cd, Cu, chromium (Cr), Hg, nickel (Ni) and Zn accumulate in the heart, muscle, liver, gills and kidneys of fish (Amundsenat *et al*., 1997; Van Aardt & Booyens, 2004; Van Aardt & Hough, 2007; Farombi *et al*., 2007; Van Dyk *et al*., 2007).

Pollutants often show their effects on the histological level (Roberts, 1989; Hinton & Laurén, 1990). Morphological changes have been seen in fish tissue exposed to POPs and heavy metals); for example oocyte lesions were found in the black goby (*Gobius niger*) that was exposed to PCBs and DDT (Louiz *et al*., 2009). Testicular oocytes have been found in the African sharptooth catfish (*Clarias gariepinus*) exposed to DDE (Pieterse *et al.*, 2010) and in the

Mozambique tilapia, (*Oreochromis mossambicus*) exposed to DDT (Barnhoorn *et al*,. 2010). Cell degeneration and granulomas were observed in the livers and kidneys of the Mayan Catfish, *Ariopsis assimilis,* that were exposed to various POPs including PCBs, α- and β-HCH, HCB, DDT, heptachlor, aldrin, dieldrin and endosulfan (Noreña-Barroso *et al*., 2004). The livers of *Oreochromis mossambicus* exposed to Cd and Zn showed hyaline droplet degeneration, congested blood vessels, cellular swelling and increased vacuolation associated with lipid accumulation (Van Dyk *et al*., 2007). Gills are often damaged or changed with exposure to toxicants. The number of cells in the primary lamellae of the gills increased (hypertrophy) in Mosquitofish (*Gambusia holbrooki*) that had been exposed to inorganic mercury (Jagoe *et al.*, 1996). Secondary lamellae aneurisms (telangiecstasia) and hypertrophy of the epithelial cells have been found in tigerfish (*Hydrocynus vittatus*) exposed to DDT (McHugh *et al.*, 2011).

2.2.4 Effects of metals and POPs on humans

Pollution is mostly caused by anthropogenic activities (Dallas & Day, 2004). Humans are also exposed to these pollutants and can be affected by them.

People consuming contaminated fish are potentially at risk (Du Preez *et al.*, 2002). This was seen by Kidd *et al.* (2001) in Lake Malawi, where the concentrations of DDT increased from the producers (algae and phytoplankton) through primary consumers (invertebrates and molluscs) to the final predators (omnivorous- and piscivorous fish). Humans, whose diet is rich in fish, are particularly at risk. By determining the levels of pollutants in fish consumed by humans, extrapolation to human health and the performance of human health risk assessment can be done (Du Preez *et al.*, 2002). It was found that *O. mossambicus* and *C. gariepinus*, from a South African urban aquatic ecosystem, were unsafe for human consumption, because they were contaminated with metals, PCBs, DDT, dichloro diphenyl dichlorethane (DDD), dichlordiphenyl dichloro ethelyne (DDE), HCH and endosulfan above the recommended guidelines (Marchand, 2009).

Humans are exposed to heavy metals and POPs by ingestion, dermal absorption or inhalation (Ross, 2004; Martin & Griswold, 2009). The risk of exposure increases if the person lives close to an industrial area. Improper disposal of these chemicals can cause an increased chance of exposure to humans and pollution to the environment (Meneses *et al*., 2004; Costopolou *et al*., 2006; Martin & Griswold, 2009). Occupational safety- and environmental safety regulations are set in place to protect individuals against these pollutants (ISTAS, 2012; OSHA, 2012). Some

metals are used in the body for essential processes at low concentrations, but can become very toxic as the concentrations increase (Dallas & Day, 2004). The POPs can be grouped together: the PCBs, PCDD/Fs and the organo-chlorine (OC) pesticides.

There are a wide range of effects that heavy metals and POPs have on human health, affecting biochemical and physiological pathways. Some heavy metals, like Fe, Ni, Mn, Cd and Tl disrupt the metabolic pathways and physiological processes. The production of dopamine in the brain can be inhibited by high concentrations of Mn (Tran *et al*., 2002; Dallas & Day, 2004) and PCBs (Carpenter, 2006). PCBs can also stimulate an increase in insulin secretion (Carpenter, 2006). Nickel is known to inhibit citric acid cycle enzymes like cytochrome oxidase, to disrupt the cycle and inhibit electron transport (Dallas & Day, 2004; Garret & Grisham, 2005). Thallium interferes with the energy metabolism, by binding onto riboflavin and other flavin co-factors. Cadmium decreases metallothionein regulation by binding onto this protein (Dallas & Day, 2004; Nordberg *et al*., 2009). Metallothionein controls Ni and Zn concentrations in the body by binding to them and making them bio-unavailable, until they can be excreted. However, in the presence of Cd, metallothionein becomes less available for dealing with Ni and Zn, causing their levels to increase in the body. (Dallas & Day, 2004) PCDD/Fs and OC pesticides have the ability to cause immunological defects in humans (Bertazzi *et al*., 2001; Costopolou *et al*., 2006).

Pollutants that act on the endocrine system are referred to as endocrine disrupting chemicals (EDCs). These chemicals disrupt the balance of the endocrine system and have the potential to adversely affect human- and wildlife health (Bonefeld-Jorgensen, 2004; Gouronti *et al*., 2008). PCBs (Gourounti *et al*., 2008), PCDD/Fs (Kogevinas, 2001; Gourounti *et al*., 2008), and OC pesticides (Bonefeld-Jorgensen, 2004; Gourounti *et al*., 2008; Gerić *et al*., 2012) have been found to have ED effects. PCBs disrupt the sex steroid hormonal systems (Carpenter, 2006). If a person was to be exposed to and had accumulated PCBs and PCDD/Fs, his/her thyroid system can be disrupted (Kogevinas, 2001). Hormonal imbalance has also been found in humans exposed to OC pesticides like DDT (Gerić *et al*., 2012).

Extensive damage to important organs can occur due to heavy metal contamination and exposure to POPs. Kidney damage has been seen with Cd (Nordberg *et al*., 2009), Cu, Se, Pb, Ni (Drazniowsky *et al*., 1985), Hg (WHO, 2012) and PCB contamination (Carpenter, 2006). Liver damage has been associated with poisoning of PCBs (Carpenter, 2006), Cr (Kurosaki *et al*., 1995), Cu (ATSDR, 2012) Se (Vinceti *et al*., 2010) and Hg (Barcelos *et al*., 2011). The dioxins and furans, and PCBs have dermal toxic properties, creating hyper-pigmented areas on

the body (Carpenter, 2006; Costopolou *et al*., 2006). Several heavy metals like Se, Pb, Cu and Ni cause disruptions and damage to the circulatory system. Nickel at low concentrations is used in the production of red blood cells but has been reported to cause cardiac damage at high concentrations (HPA, 2009a). Lead (Dallas & Day, 2004) can inhibit the production of haeme and therefore cause anaemia. Lead also has the potential risk of causing cardiovascular disease (Poręba *et al*., 2011). PCBs (Carpenter, 2006) and OC pesticides (Khanki, 2007) also have the potential to cause heart disease. Cadmium, Hg and Pb cause oxidative stress in blood, damaging the erythrocytes (Turkez *et al*., 2012). The central nervous system is also affected by pollutant chemicals. Cobalt, Cu (Dallas & Day, 2004), Hg and Se (Vinceti *et al*., 2010) can cause brain damage, and nerve damage can be caused by Pb (Patrick, 2006) and Se (Vinceti *et al*., 2010). The POPs can act as neurotoxins, affecting the brain and nervous system. PCBs (Carpenter, 2006), PCDD/Fs (Bertazzi *et al*., 2001, Costopolou *et al*., 2006) and the OC pesticides (Bonefeld-Jorgensen, 2004; Gerić *et al*., 2012) have been found to adversely alter the nervous system. PCBs can cause memory loss and severe headaches (Schantz, 1996) and OC pesticides can result in neurological diseases like Parkinson's- and Alzheimer's (Khanki, 2007). The reproductive systems in humans are affected by the POPs (Bonefeld-Jorgensen, 2004; Costopolou *et al*., 2006). Males have a risk of lower seminal quality and females of lowered fertility when exposed to OC pesticides (Gerić *et al*., 2012).

Pollutants not only affect the tissue and organs, but can also act on a cellular level and sub-cellular level. Genotoxicity is defined as damage by a physical or chemical agent to genetic material. These effects occur at a molecular level where toxicants and/or their metabolites (e.g. metals that form free radicals and other oxidising agents through metabolic reactions) act on the genetic material (Newman, 2010). According to Newman (2010), metals can destabilize DNA and so change normal functionality, by binding to the heterocyclic bases or phosphate groups. An example is Cu that can bind between the nitrogen bases (of the DNA), competing for the hydrogen bonds, and destabilizing the structure. Magnesium destabilizes the DNA structure by binding onto the phosphate groups and altering the DNA backbone. Heavy metals like Se (at high concentrations) (Dallas & Day, 2004), Hg (in both organic and inorganic form) (Dallas & Day, 2004; Barcelos *et al*., 2011), Cr (Dallas & Day, 2004; Jamova & Valko, 2011), Cu, Co (Jamova & Valko, 2011) and Pb (Poręba *et al*., 2011) are genotoxic at different concentrations. PCBs and PCDD/Fs can bind to the receptors to express the CYP1A1 gene. This gene encodes for the cytochrome P450 1A1 enzyme, which breaks down xenobiotics like PCBs and

PCDD/Fs. The metabolism of these substances can generate reactive oxygen, that damage DNA and cause mutations on the genetic material (Gourounti *et al*., 2008).

Carcinogenesis includes the chemical, biochemical, and molecular events that lead to the large number of effects on cell growth collectively known as cancer (Hodgson, 2004), thus a carcinogen is a substance that can cause cancer (Newman, 2010). A number of metals and POPs have shown to be carcinogenic in humans. Carcinogens mainly act on the DNA to alter the function, which can cause uncontrolled growth of cells. These cells can form benign or malignant tumours (Newman, 2004). Examples of cancer from metal exposure through all venues are: skin cancer from As and U; lung cancer from As, Cd, Cr and Ni; kidney cancer from Ni and Hg. Uranium can cause bone cancers involving the tissue and/or the bone marrow. Males are prone to prostate cancer when exposed to Cd (Cope, 2004; Dallas & Day, 2004; Martin & Griswold, 2009). The POPs are also known carcinogens: PCBs can be a stimulus for brain-, breast-, lung-, pancreatic cancer and malignant melanomas (Carpenter, 2006); the dioxins and furans can cause liver-, rectal-, testicular-, endometrial-, breast and lung cancer (Kogevinas, 2001; Costopolou *et al*., 2006); and liver-, breast-, pancreatic-, testicular-, prostate-.,and endometrial cancer from OC pesticides (Bonefeld-Jorgensen, 2004; Khanki, 2007; Gerić *et al*., 2012)

Pollutants such as metals and POPs that are teratogens cause foetal or embryonic malformations (Branch, 2004; Newman, 2010). According to Karnofsky's law, any agent will be teratogenic if it is present at concentrations or intensities to cause cell toxicity in the developmental stage (Bantle, 1995; Newman, 2010). The dose of the teratogen influences the nature of the effect. Low doses cause growth retardation, and higher doses might cause malformations (Rogers & Kavlock, 2008; Newman, 2010). Effects caused earlier in development tend to be more deleterious than those later. This is because early damage affects cells that will differentiate and become involved with a wider range of tissues and organs (Bantle, 1995; Newman, 2010). The Minamata disease occurred in the 1950's when organic mercury was discharged into the Minamata Bay. The Hg transferred into marine food sources (fish and clams) and poisoned hundreds of people (Cope, 2004; Newman, 2010). This disease causes asymmetrical skulls, macrocephali (abnormally large heads), depressed optical region on the skull, poor muscle coordination, poor speech- and walking skills, mental retardation and a lowered intelligence quotient (IQ) (Newman, 2004). Other heavy metals that act as teratogens are Pb and Cr. Lead can cause damage to the reproduction systems during development and

cause spontaneous miscarriages (Martin & Griswold, 2009). These spontaneous abortions have also been associated with OC pesticide and PCDD/Fs contamination (Gerić *et al*., 2012). PCBs and PCDD/Fs have teratogenic properties, e.g. PCBs effects on the foetus include abnormal calcification of the skull, a lowered IQ, growth impairment (Schantz, 1996), hyper-pigmentation and lowered birth mass (Ross, 2004; Carpenter, 2006). The dioxins and furans can retard the neurological development of a foetus, cause dental defects and congenital malformations in the body (Bertazzi *et al*., 2001; Kogevinas, 2001; Costopolou *et al*., 2006).

Two of the more recently added POP groups, the PBDEs and PFCs have quantifiable amounts in wildlife and humans, but little is still known of the health implications they have. The health effects of these chemicals have been tested on animals, (Darnerud, 2003; Völkel *et al*., 2008; Butenhoff *et al*., 2009). The most common studied PFC, perflourooctane sulfonate (PFOS) (Butenhoff *et al*., 2009; HPA, 2009b) and PDBEs (McDonald, 2002; Völkel *et al*., 2008) act as neurotoxins and as weak carcinogens, affecting the liver and thyroid.

The presence of POPs and heavy metals in the environment are of great concern to humans and wildlife as they adversely affect not only the health but can be a mortal threat. This makes it very important to investigate the levels and distribution these chemicals in the South African environment, but specifically the aquatic environment. Knowing the levels of pollutants in fish consumed by humans allows for the extrapolation to human health and the performance of human health risk assessment (Du Preez *et al.*, 2002).

2.3 Indicators and bio-markers

An indicator is a parameter or a parameter derived value which provides information and describes the state of an area or the environment (OECD, 2003). In the case of this study, the state referred to is the overall health of the ecosystem. The most common usage of the term bio-marker has been for biochemical, physiological or histological indicators of either exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level (Bernet *et al*., 1999; Hanson *et al.,* 2006; Nikalje *et al.,* 2012).

The biomarker approach is applied as an early warning or proactive tool, to measure the effect of toxicants before serious permanent damage is done in an ecosystem because changes in the organism is generally detectable before adverse effects are seen in higher levels in the biological organization (Newman, 2010).

2.3.1 Sediment quality and indices as indicators

The study of sediment quality by evaluating the concentrations of pollutants is necessary as it helps assess the potential toxicity of the system (Chakravarty & Patgiri, 2009). Numerous studies have been undertaken to investigate environmental health, by studying the concentrations of pollutants in the sediment (Angulo, 1996; Atgin *et al*., 2000; Meybeck *et al*., 2004; Niewoudt *et al*., 2009; Varol, 2011). Pollutants in aquatic ecosystems generally exist in low levels in water (Öztürk *et al*., 2009) and mainly accumulate in the sediment (Praveena *et al*., 2008; Öztürk *et al*., 2009). Sediment has a long residence time in the aquatic systems, therefore they are ideal for the assessment of pollutants in rivers (Saha *et al*., 2001; Varol, 2011). Sediments are important sources for organic and inorganic pollutants, due to their variable physical and chemical properties (Praveena *et al*., 2008). They play a functional role in the mobilization of contaminants in aquatic systems under favourable conditions (Öztürk *et al*., 2009). In riverine communities, the population is directly and indirectly exposed to sediment and so the pollutants, and are at risk of contamination (Miller *et al*., 2004). Thus it is important to evaluate the pollution status of the sediment (Praveena *et al*., 2008).

This evaluation is done with a variety of indices, each calculating a different endpoint. Indices are sets of aggregated and measured parameters or indicators (OECD, 2003). The comparison of metal pollution between study areas is calculated by the metal pollution index (MPI) (Usero *et al*., 1996; Roychoudhury & Strake, 2009; Ameh & Akpah, 2011), and the pollution load index (PLI) (Angulo, 1996; Chakravarty & Patgiri, 2009; Varol, 2011). The metals accumulated in the environment above the area's natural concentrations is calculated with the enrichment factor (Ef) (Nash & Gries, 1995; Roychoudhury & Strake, 2009; Rogan Šmuc *et al*., 2009; Varol, 2011) and the degree of pollution caused by this enrichment is expressed with the geo-accumulation index (Igeo) (Ruiz, 2001; Praveena *et al*., 2008; Mohuiddin *et al*., 2010). The ecological toxicity risk that the sediment potentially has is calculated with the sediment quality guideline index (SQG-I) (Fairey *et al*., 2001), which incorporates the concentrations of the metals, POPs, and their guidelines. Similarly, the quality of the sediment is calculated with the sediment quality index (SQI) (Marvin *et al*., 2003).

2.3.2 Fish as indicator organisms of long term exposure

Fish could serve as ideal bio-indicators of aquatic health as they represent every trophic level in an aquatic food pyramid (Kidd *et al*., 2001). A bio-indicator is a group or an individual organism that is used to describe the quality of an ecosystem, depending on their abundance or well-being (Gerhardt, 2002). Disturbances in the lower levels will affect the apex predators, as they feed on prey in lower levels of the food chain. The abundant and well-studied in SA, sharptooth catfish (*Clarias gariepinus*) is an opportunistic bottom-feeder. Sharptooth catfish are omnivorous and formidable predators. They have also been known to be intentional detritus feeders (Skelton, 2001). The sharptooth catfish is a hardy and resilient fish, surviving harsh conditions (Skelton, 2001), thus creating the potential for long term bio-accumulation. This makes *C. gariepinus* an ideal species to use in this study as it is present on all trophic levels and is abundant in southern Africa. They are not the ideal species to use as a bio-indicator of ecological integrity of the river system because of its resilience, but they are ideal to show long term exposure to pollutants.

Generally, less is known about the effects of pollution in the Orange River than the Vaal River. The latter is subjected to a lot of research as one of South Africa's most polluted rivers. In understanding the effects and levels of the pollutants (POPs and heavy metals) in the sharptooth catfish in the Orange-Vaal system, efforts can be made to determine the sources of the pollution and contribute to the conservation of more sensitive indigenous species.

2.3.3 Histology as indicator

Histology can be used as an indicator or bio-marker of the effects of anthropogenic contaminants (Nikalje *et al.,* 2012). Histological changes in animals tissues are powerful indicators of prior exposure to environment stressors and are the net result of adverse biochemical and physiological changes in an organism (Hinton & Laurén, 1990; Palanismay *et al*., 2011). It is a useful method to detect and assess the degree of pollution, to reveal the overall health of a population or eco-system (Bernet *et al*., 1999; Velmurugan *et al*., 2007; Mohamed, 2009).

Histological assessments have been widely used to evaluate the health of fish. One of the great advantages of using histological assessments in environmental monitoring is that it allows examining specific target organs. Furthermore, the alterations found in these organs are normally easier to identify than functional alterations, and serve as warning signs of

deteriorating animal health (Hinton & Laurén, 1990; Camargo & Martinez, 2007). Such alterations which occur in fish living in polluted environments are in most cases described as pollutant-associated rather than pollutant-induced (Schwaiger *et al*., 2007).

The gills, kidney, and liver are primary target organs for fish histology and have proven to be indicative of aquatic pollution (Hinton & Laurén, 1990; Bernet *et al*., 2004; Van Dyk *et al*., 2007; Marchand, 2009; Pieterse *et al.*, 2010; McHugh *et al.*, 2011; Marchand *et al*., 2012).

Fish gills are in direct and permanent contact with the environment (Hinton & Laurén, 1990; Bernet *et al*., 1999; Velmurugan *et al*., 2007) and play an important role in physiological functions like respiration, osmoregulation and excretion (Palanismay *et al*., 2011). Fish livers are regarded as the main site of storage, bio-transformation and excretion of pollutants (Hinton & Laurén, 1990; Velmurugan *et al*., 2007). The liver plays an important role in the metabolism and excretion of xenobitics, and is also a site for vitellogenin production. Vitellogenin is induced by endogeneous oestrogens and is normally only detectable in females. Vitellogenin can be induced in males by an increasing number of man-made compounds which mimic oestrogens, and this makes the liver of additional interest for environmental impact studies (Bernet *et al*., 1999). The fish kidney is important for the maintenance of a stable internal environment with respect to water and salt, excretion and, to a certain extent, the metabolism of xenobiotics (Hinton & Laurén, 1990; Bernet *et al*., 1999; Velmurugan *et al*., 2007).

The use of these bio-indicators (sediment and fish) and the quantitative, and qualitative assessments (indices and histology) enables the investigation of both the biotic and abiotic matrices in the aquatic environment.

3. Materials and Methods

3.1 Site selection

Sites were chosen according to their location on the rivers and the pollution sources in the area, to compare different pollution sources and to be able to compare pollution load in the upper, middle and lower reaches of the Vaal River (Braune & Rogers, 1987). Three sites were chosen on the Vaal River and two sites on the Orange River. Sediment sampling was undertaken up- and downstream from each of these fish sampling sites.

The Standerton site (Sdn) (26 51.690'S; 29 31.141'E) is the most upstream site in the Vaal River (Figure 2) and represented the upper reaches of the river. Although this site already has its share of pollution sources such as mining and agricultural activities (Braune & Rogers, 1987; Van Wyk, 2001; WRC, 2009) this site was selected to be able to compare the level of increased pollution downstream in the Vaal River.

Parys (Ps) (26 54.958'S; 27 23.926'E) is the second site in the middle reach of the Vaal River (Figure 2), downstream of Gauteng, where the major pollution sources are mining activities and industry from the Vaal Triangle area (Braune & Rogers, 1987; Van Wyk, 2001; WRC, 2009; Dikio, 2010).

Rooipoort (Rp) (28 33.688'S; 24 09.621'E) is the last site on the Vaal River and represents the lower reaches of the river up to the confluence with the Orange River (Figure 2). It can therefore be used to learn the effect the upper Orange River and its pollution load might have on the biota in the Orange River after confluence with Vaal. Mining, especially alluvial diamond mining is a major activity in the Rooipoort area (DWA, 2003; DWA, 2004a).

Boegoeberg (Bg) (29 02.533'S; 22 12.321'E) is a weir on the Orange River, upstream of the confluence (Figure 2). Mining and agriculture are the main sources of pollution (DWA, 2003; DAFF, 2011; DWA, 2011b; NCB, 2012). This site would indicate if pollution was diluted or concentrated to any extent.

The second site that was selected on the Orange River was at Aliwal North, just below Lesotho, (Upper Orange), This site would have acted as the equivalent of the Standerton site on the Vaal River; i.e. a reference for up-stream in the Orange River. However, only one fish was caught during sampling due to unforeseen flooding. Therefore this sample site was excluded.

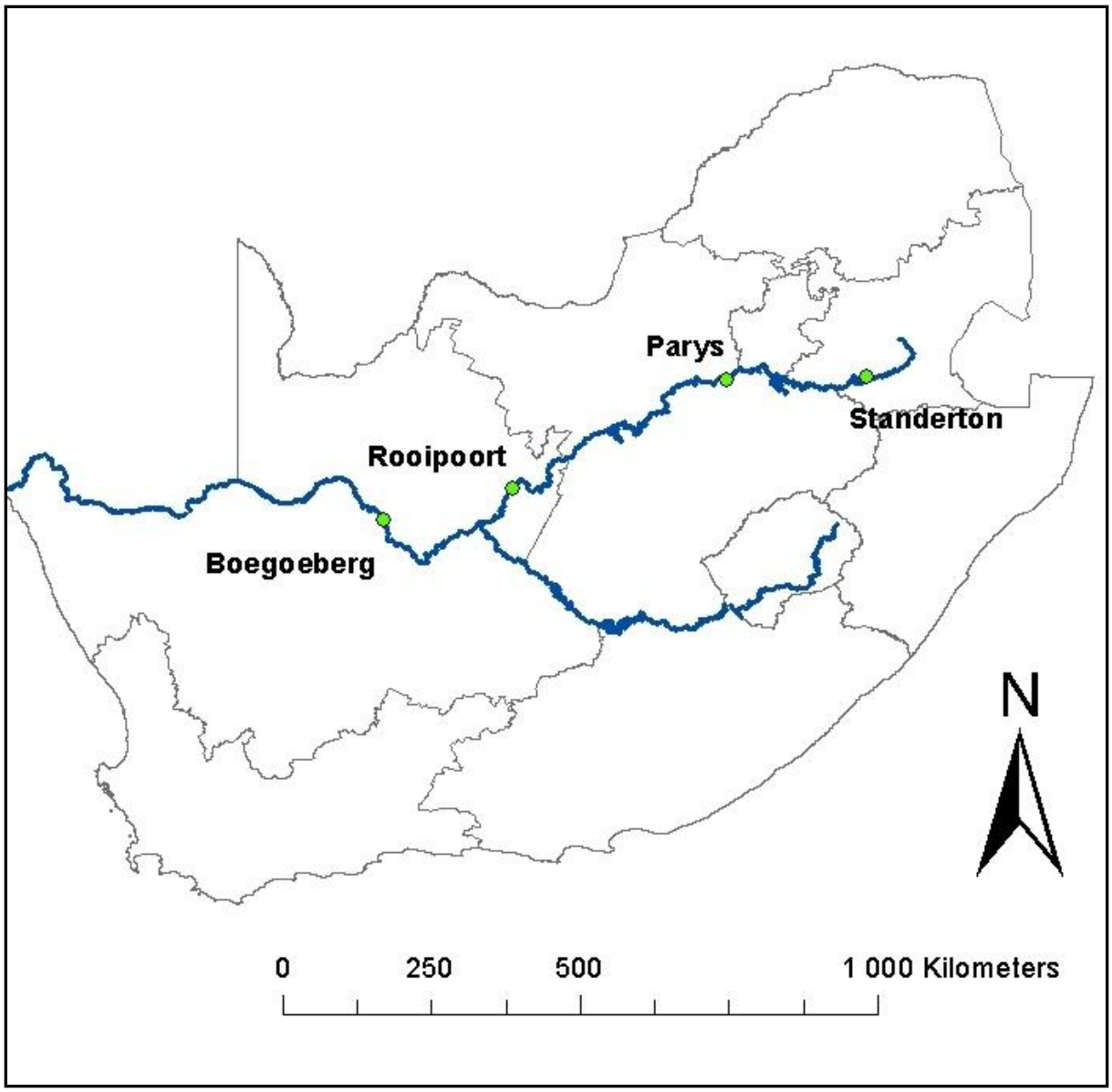

Figure 2: Map of South Africa indicating sampling sites on the Orange- and Vaal Rivers

3.2 Species sampled

Catfish (order Siluriformes) form one of the world's largest and abundant groups of fish types, consisting of 31 families, 400 genera and over 2 200 described species (Hecht *et al*., 1988; Skelton, 2001). Many have 3 to 4 pairs of barbels around their mouths and do not have scales although some, mainly from South America, have bony plates along their bodies (Skelton, 2001). These fishes are a valued food sources and are ideal aquaculture species, such as the sharptooth catfish (*Clarias gariepinus*) (Hecht et al., 1988; Skelton, 2001), blunt tooth catfish (*Clarias ngamensis*) (Skelton, 2001), and vundu (*Heterobranchus longifilis*) (Coulibaly *et al*., 2007). One of the families belonging to this order is the Clariidae family, or air breathing catfish,

that are indigenous to Africa and Asia. They are known for their hardiness and are easily recognised by their helmet like bony head and dorsal-ventrally flattened bodies with long anal- and dorsal fins along the length of their bodies (Hecht *et al*., 1988; Skelton, 2001). The sharptooth catfish (*Clarias gariepinus*), chosen as the indicator species for this study, belongs to the Clariidae family. Full identification of this species is as follows (ITIS, 2010):

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Super class:	Osteichthyes
Class:	Actinopterygii
Order:	Siluriformes
Family:	Clariidae (Bonaparte, 1846)
Genus:	*Clarias* (Scopoli, 1777)
Species:	*Clarias gariepinus* (Burchell, 1822)

Clarias gariepinus is found throughout Africa and parts of Asia. This species is distributed as far north as the Nile River and as far south as the Orange- and Umtamvuna systems. It was translocated to the Eastern- and Western Cape. It is also found in Israel, Lebanon and Turkey (Skelton, 2001; Froese & Pauly, 2010) and in South America (Froese & Pauly, 2010).

Clarias gariepinus is a dorsal-ventrally flattened fish; the body is compressed towards the tail and depressed at the head (Figure 2). The dorsal fin extends from behind the head to near the base of the caudal fin. The anal fin extends from the base of the anus to the base of the caudal fin. The pectoral fins have a spine with barbs on the outer edge only (Figure 2). This fish's fins are mainly of soft rays (Skelton, 2001). The eyes are in a super-lateral position and are relatively small (Froese & Pauly, 2010). The head has 4 pairs of long filamentous barbels (Figure 2). Both jaws are armed with broad bands of fine sharp teeth. Skin colour varies from black to light brown, often marbled in shades of olive green and grey. The ventral sections of the head and abdomen are white and sometimes have a red flush on edges of the fins, especially during spawning. *Clarias gariepinus* can attain lengths up to 1.4 m SL (standard length which is the length from tip of snout to mid-base of caudal fin) (Skelton, 2001).

Figure 3: The sharptooth catfish (*Clarias gariepinus*) (Skelton, 2001)

The presence of an accessory breathing organ enables the catfish to breathe air by gulping air with its mouth. It does this when very active or during very dry conditions. *Clarias gariepinus* occur mainly in quiet waters such as lakes and pools but may venture into fast flowing rivers and into rapids. It is tolerant to extreme environmental conditions such as low oxygen concentrations and poor water quality (Froese & Pauly, 2010).

Clarias gariepinus is completely omnivorous. It preys and scavenges on any available organic food source including fish, birds, frogs, small mammals, reptiles, molluscs, crustaceans, seeds, fruit and even plankton. They may hunt in packs, herding and trapping small fish in shallower water. *Clarias gariepinus* breeds in the summer after rain showers when large sexually mature adults migrate to flooded shallow grassy borders of the dam or river (Skelton, 2001).

Clarias gariepinus was chosen as an indicator species because of its abundance in the Vaal River system, their hardiness and because they are an apex predators. The position on the food chain and its preference for bottom-dwelling in the river system makes it ideal to study bio-accumulation and bio-magnification.

3.3 Sampling methods

3.3.1 Sediment sampling

Sediment samples were collected from eight sites in the Orange-Senqu catchment. The samples were collected up- and downstream to get a collective representation of the sediment in the area. Pooled samples were prepared at each of the sites by combining surface sediments collected in a 5 m range, mixing it thoroughly before storing it in high density polyethylene (HDPE) bottles, pre-cleaned with acetone and hexane, according to USEPA Method 1613 (US EPA, 1994). The samples were protected from UV and microbial degradation by transporting at 4 °C and stored at -20 °C in the laboratory. Sediment samples were air dried, ground into finer particles and sieved (1mm mesh size) before being sent for analysis (Kralik, 1999).

3.3.2 Sampling of *Clarias gariepinus*

Sharptooth catfish (*Clarias gariepinus*) were sampled during the high flow season in summer. Ten fish per site were sampled using gill- (73-, 93-, 118- and 150 mm), seine-, and fyke nets. The fish was euthanized by severing the spinal cord before samples were taken for chemical analysis and histological purposes. Muscle fillet samples were collected for POPs and heavy metal analysis and selected organs were removed for histology. Samples for POPs analysis were stored in pre-cleaned foil (washed with acetone and hexane) according to EPA Method 1668B (US EPA, 2008) and heavy metal analysis samples were freeze-dried and stored in plastic bags (US EPA, 2000a). Due to finances, heavy metal data was possible for each fish, but for the POPs data only pooled samples could be generated. The analysis of POPs is very expensive and samples were sent to Germany to be analysed (cf. Material and Methods section 3.4). Male and female fish for every site were pooled separately, as it is expected that the female fish would have higher POPs levels due to their naturally higher lipid content (reserve energy for physiological and biochemical processes associated with reproduction) (Anthony *et al*., 2000). All the muscle samples were transported at 4 ºC and stored at -20 ºC.

3.4 Chemical analysis

Heavy metal analyses were done for both sediment and fish muscle. Results were obtained by using the revised International EPA 3050B method (US EPA, 1996). Samples were acid digested using nitric acid (HNO_3) and an inductively coupled plasma mass spectrometry (ICP-

MS) was used to determine the heavy metal levels within these samples. The analysis was done by Eco-Analytica, Potchefstroom, South Africa.

Organic samples were sent to an accredited POPs laboratory (Oekometrics, GmbH) in Germany for analysis. Results were obtained by using the following methods: For PCDD/PCDF and dioxin-like PCBs, the DIN 38414-S24 method (DIN, 2000; Oetken *et al*., 2005), for polybrominated diphenyl ethers and biphenyls the ISO 22032 method (ISO, 2004) and for the perfluorinated compounds and pesticides the DIN 38407-2 method (Neumann *et al*., 2002) was followed. High resolution gas chromatography-high resolution mass spectrometer (HRGC-HRMS) was used to determine the concentrations.

3.5 Sediment assessment

Indices were calculated to describe the quality of the sediment regarding a number of factors:

The enrichment factor (Eq. 1) of individual heavy metals evaluated elevation in levels above natural geology. This is determined by calculating the amount a heavy metal accumulated, compared to background levels of that same heavy metal.

$$Ef = \frac{C_{HM}/C_{Fe(Sample)}}{C_{HM}/C_{Fe(Background)}} \quad (1)$$

The heavy metal concentrations (C_{HM}) were normalised to the concentration of Fe (C_{Fe}), as Fe was the main geochemical carrier in the sediment studied (Nash & Gries, 1995; Roychoudhury & Strake, 2009; Rogan Šmuc *et al*., 2009).

The geo-accumulation index (Eq. 2) determined the degree of pollution by the enrichment levels of the individual metals at each site.

$$I_{geo} = \log_2 \frac{C_{HM(sample)}}{1.5 \times C_{HM(Background)}} \quad (2)$$

C_{HM} = the heavy metal concentrations

factor of 1.5 = includes the variation of the heavy metal concentration in the background levels due to lithogenic effects (Ruiz, 2001; Praveena *et al*., 2008; Mohuiddin *et al*., 2010).

The pollution effect of the total mixture of metals at each site was investigated by the metal pollution index (Eq. 3) and pollution load index (Eq. 5). The MPI is the geometric mean of the concentrations of the heavy metals measured at a site (C_{HM}) (Roychoudhury & Strake, 2009; Ameh & Akpah, 2011).

$$MPI = (C_{HM1}, C_{HM2}, C_{HM3,} C_{HM4} \ldots\ldots C_{HMn})^{\frac{1}{n}} \quad (3)$$

The PLI (Eq. 5) is the geometric mean of the contamination factor (Eq. 4), i.e. how many times the measured concentration ($C_{HM(Sample)}$) is higher than a background value ($C_{HM(Background)}$) (Chakravarty & Patgiri, 2009).

$$Cf = \frac{C_{HM(sample)}}{C_{HM(Background)}} \quad (4)$$

$$PLI = (Cf_1 \times Cf_2 \times Cf_3 \times Cf_4 \ldots . Cf_n)^{\frac{1}{n}} \quad (5)$$

Ecological risk was determined by calculating the sediment quality guideline index (Eq. 6) at each site.

$$SQG\text{-}I = \frac{\sum_{i=1}^{n} C_{HMi\ (Sample)}/C_{HMi\ (Threshold)}}{n} \quad (6)$$

The SQG-I is the arithmetic mean of how many times the measured concentration ($C_{HM(Sample)}$) of individual metals at a specific site were higher than a guideline level ($C_{HM(Threshold)}$) (Fairey *et al*., 2001). In this case, threshold sediment quality guideline values for New Zealand and Australia (ANZECC, 2000), the Netherlands and Canada (Friday, 1998) were used, since South Africa has not developed guidelines.

In addition, a sediment quality index, SQI (Eq. 7) described by Marvin *et al*. (2003), was calculated to incorporate the percentage of metals per site that did not meet guidelines and the magnitude of exceedance.

$$SQI = 100 - \frac{\sqrt{F_1^2 + F_3^2}}{\sqrt{2}} \quad (7)$$

This equation makes use of two elements; scope (Eq.7a) and amplitude (Eq.7b). There is a third element, namely frequency that can be used if there are groups and multiple sampling times. Frequency was not used in this study since there was only one sampling event. The SQI calculates a value out of 100, where 100 represents the highest sediment quality and 0 the worst.

Scope (Eq.7a) is the percentage of variables that did not meet sediment quality guidelines

$$F_1 = \left(\frac{\text{number of failed variables}}{\text{total variables}}\right) \times 100 \quad (7a)$$

Amplitude (Eq.7b) is the magnitude by which the failed variables exceeded the sediment quality guidelines.

$$F_3 = \left(\frac{\text{mdnc}}{0.01\text{mdnc}+0.01}\right) \quad (7b)$$

Where:

mdnc = Mean degree of non-compliance

$$\text{mdnc} = \sum_{i=1}^{p} \text{non-compliance}_i \quad (7c)$$

$$\text{non-compliance}_i = \left(\frac{\text{failed test value}_i}{\text{guideline}_i}\right) \quad (7d)$$

Failed test value = amount of samples not meeting guidelines

i = Individual guideline

p = Total amount of guidelines used

3.6 Histology based assessment

3.6.1 Tissue processing

Processing fish tissue for histology consists of fixation (during sampling), dehydration, clearing, infiltration, imbedding, sectioning, staining and lastly mounting (Culling, 1974; Gabe, 1976; Humason, 1979)

The target organs (gills, kidney, and liver) were removed. The same region of each of the organs was sampled for consistency and accurate comparison between individual fish and sites. A sample size of approximately 1 cm^3 was removed and fixed in 10% neutral buffered formalin (Humason, 1979; Van Dyk *et al*., 2007; Mlambo *et al*., 2009).

Following fixation, the samples were placed in biopsy-cassettes (Rotilabo) and washed in running water for 60 minutes. Tissues were dehydrated in a series of ethanol concentrations (30-, 50-, 70-, 80-, 90-, 100-, 100%) for 60 minutes per concentration. Samples were cleared using ethanol:xylene mixture (1:1) for 30 minutes and 5 minutes in 100% xylene. The tissues were infiltrated with paraffin wax at 60°C. This was a serial process consisting of paraffin wax:xylene (1:1) for one hour, then 100% wax, for another hour; 100% wax (not contaminated by xylene) overnight to allow complete infiltration (Gabe, 1976; Humason, 1979; Albas, 2001). The infiltrated samples were imbedded in paraffin blocks (2 cm x 3 cm x 1 cm) using the Slee paraffin wax imbedding machine.

The cooled wax blocks were sectioned (5 µm) using a microtome (Reicther-Jung 2050). Disposable microtome blades (Shandon LP MX 35 premium) were used and replaced after each organ (Albas, 2001; Van Dyk, 2006). The sectioned ribbons were stretched on a slide with heated Meyer's albumins solution (Gabe, 1976; Humason, 1979; Albas, 2001). The dried slides were dried overnight at 45°C. The slides were stained with haematoxilyn and eosin Y (Humason, 1979; Albas, 2001, Van Dyk, 2006) and mounted with Entellan® (Merck).

3.6.2 Histological assessment

A qualitative assessment of each organ was undertaken, to identify histological alterations (Van Dyk *et al*., 2009a; Pieterse *et al*., 2010). The alterations found were categorised into six reaction patterns or histological alterations types.

Circulatory disturbances is a pathological occurrence involving the flow of tissue fluid and blood (Bernet *et al*., 1999).

- Intercellular oedema — Stagnant tissue fluid, leaked from capillaries
- Aneurysm — Dilation of arterial blood vessels
- Haemorrhage — Blood leaking from blood vessels
- Hyperaemia — Congestion of blood in target organs due to arterial or venous processes (Bernet *et al*., 1999).

Progressive alterations are changes that lead to an increase of cell and tissue activity.

- Hypertrophy — The increase in cell volume or tissue without increasing the cell number
- Hyperplasia — The enlargement of the target organ or tissue by an increase of cells, without changing the size of the cells (Bernet *et al*., 1999).

Regressive alterations are processes that reduce the functionality by reduction or loss of an organ.

- Structural alterations — Change in tissue structures, cell shape and -arrangement
- Plasma alterations — Change in cellular plasma caused by hyaline droplets (granular degeneration), colloidal degeneration, degenerative fatty vacuolisation, glycogen droplets (glycogen degeneration) and thickening of connective fibres (hyaline degeneration)
- Nuclear alterations — Change in nuclear shape and chromatin structure (karyopyknosis, karyrorrhexis and karyorrlysis)
- Deposits — Intercellular accumulation of substances of degeneration
- Atrophy — The reduction of volume and number of cells, and intercellular substances
- Necrosis — Morphological state after cells and tissue have lost function (Bernet *et al*., 1999).

Inflammatory responses are changes and are often associated with other alterations, and are difficult to attribute to a single reaction pattern.

- Infiltration — Leukocytes penetrating blood vessel walls and infiltrating surrounding tissue
- Exudate — High protein containing fluid and cellular debris exuded from blood and lymph vessels
- Activation of reticuloendothelial system — Hypertrophy of the reticuloendothelial system, which includes endothelial cells and macrophages that line blood vessels (Bernet *et al*., 1999).

Neoplasia is the reaction pattern where tumours form. Tumours are cells that grow uncontrollably.

- Malignant tumours — Poorly differentiated cells that multiply rapidly. They invade and destroy resident organ tissue and can undergo metastasis
- Benign tumours — Differentiated cells which can displace and replace original organ tissue. These cells can resemble original organ cells (Bernet *et al*., 1999).

The six reaction patterns identified in the qualitative assessment were used in the semi-quantitative histological assessment, where a numerical value was given for each alteration (Marchand *et al*., 2008; Van Dyk *et al*., 2009b). The protocol from Van Dyk *et al*. (2009a) was adapted from Bernet *et al.,* (1999). An importance factor was allocated to represent the severity of the alteration according to Marchand *et al*. (2009) and Van Dyk *et al.* (2009a):

1 = Reversible alteration as the exposure ends

2 = Reversible alteration if the stressor is neutralised or removed

3 = Alteration is irreversible

A score value was given on the frequency of the occurrence of the alteration (Marchand *et al*., 2009; Van Dyk *et al*., 2009a):

0 = Absent

2 = Mild

4 = Moderate

6 = Severe

The results from the semi-quantitative assessment were used to calculate index values for each target organ (liver [I_L], kidney [I_K], and gills [I_G]) by multiplying the importance factor with the score value. These indices represent the histological reactions in the respective organs. The sum of these indices calculates the total fish index (I_{Fish}), which represents the overall histological response within the target fish (Van Dyk *et al*., 2009a; McHugh *et al*., 2011). The organ indices were classified in different classes of severity (Van Dyk *et al*., 2009a) which is based on the scoring system developed by Zimmerli *et al*., (2007).

Class 1 (index < 10)	Normal tissue structure with slight histological alterations
Class 2 (index 10–25)	Normal tissue structure with moderate histological alterations
Class 3 (index 26–35)	Pronounced alterations of organ tissue
Class 4 (index > 35)	Severe alterations of organ tissue

3.7 Limited human health risk assessment

Health risk assessments of chemical concentrations in fish tissue can provide the data to estimate the probability of health effects in humans consuming contaminated fish. The risk from the consumption of contaminated fish is predicted by evaluating the ability of a chemical contaminant to cause adverse effects over different exposure times at different concentrations (Heath *et al*., 2004).

The risk assessment process consists of four steps:

- Hazard identification,
- Dose-response,
- Exposure assessment,
- Exposure characterization (US EPAa, 2000)

Hazard identification assesses the likelihood that exposure to chemical under specific exposure conditions will pose a threat to human health (US EPA, 2000a; Heath *et al*., 2004). Information like physio-chemical properties of the chemical, routes of exposure, metabolic properties, toxicological effects, and chronic- and acute animal exposure studies are used in the identification. To create a hazard profile, databases such as Integrated Risk Information System (IRIS) are used, that contain the above information as well as related risk values and health effect endpoints (US EPA, 2000a; Heath *et al*., 2004; IRIS, 2012).

Dose-response is the assessment where the relationship between the dose and the likelihood- and magnitude of health effects is characterized. The dose-response dynamic is therefore the functional relationship between the exposure and the observed health effects. Hazardous chemicals can be grouped into groups with non-threshold effects (cancer risk) and threshold effects (non-cancer risk). The assessment of the toxicity of hazardous chemicals is usually done with animal toxicity data, as data for human exposure to most of the contaminants is unavailable (US EPA, 2000a; Heath *et al*., 2004; Newman, 2004).

In the exposure assessment, the intensity, magnitude, frequency, and duration of exposure is estimated or determined (US EPA, 2000a; Heath *et al*., 2004; Newman, 2004). In these assessments the exposures are determined for different sub-populations (e.g. children, adult, and elderly) by specific exposure pathways (US EPA, 2000a; Newman, 2004)

The data from the assessments is combined together in the risk characterization. The data is interpreted and a risk statement is created, in which the overall risk for individual and population health risks is described (Heath *et al*., 2004; Newman, 2004).

The human health risk of heavy metals and POPs, in terms of cancer risk (CR) and non-cancer risk or hazard index (HI), was calculated. These calculations could only be done for those metals and POPs that had reference data available. The US EPA's IRIS database was used as the reference (IRIS, 2012).

Hazard index (HI)

The Average Daily Dosage (ADD) was used for the calculation of non-carcinogenic risk

$$ADD = \frac{C_m \times IR_m \times ED}{BM \times ED} \quad (8)$$

Where:

ADD = Average Daily Dose (mg/kg/day)
C_m = Average concentration of pollutant in food substance (mg/kg)
IR_m = Average intake rate (kg/day)
ED = Exposure duration (days)
BM = Body mass (kg) (Heath *et al*., 2004)

$$HI = \frac{ADD}{RfD} \quad (9)$$

Where:

HI = Hazard Index (non-carcinogenic risk) (dimensionless)

ADD = Average Daily Dose (mg/kg/day)

*RfD = Reference Dose (mg/kg/day) (Heath *et al*., 2004).

* The Reference Dose is pollutant specific, value can be found on international databases, e.g. IRIS (2012).

Cancer risk (CR)

The Lifetime Average Daily Dose (LADD) was used in the calculation of carcinogenic risk

$$LADD = ADD \times \frac{ED}{LT} \quad (10)$$

Where:

LADD = Lifetime Average Daily Dose (mg/kg/day)

ADD = Average Daily Dose (mg/kg/day)

ED = Exposure duration (days)

LT = Expected lifetime (days) (Heath *et al*., 2004).

$$CR = Sf \times LADD \quad (11)$$

Where:

CR = Cancer risk (dimensionless)

*Sf = Slope factor (mg/kg/day)

LADD = Lifetime Average Daily Dose (mg/kg/day) (Heath *et al*., 2004).

* The Slope factor is pollutant specific, value can be found on international databases, e.g. IRIS (2012).

3.8 Statistical assessment

Descriptive statistics, i.e. means, standard deviations and covariance of analysis were calculated with MS Excel. Software programmes SPSS 20 and Statistica 10 were used for statistical analysis.

For small samples sizes the Shapiro-Wilk test was used to determine normality of data distribution. If data were not distributed normally, Box-Cox analyses (Statistica10) were done to select the appropriate log transformations. Distribution was investigated again. If the data set was still not normally distributed non-parametric tests were used. The Kruskal-Wallis analysis of variance (ANOVA) was used to determine the significant difference between the means of three or more groups, and the Mann-Whitney U test was used when two groups were compared.

Levene's test was used to evaluate the homogeneity of the variances found in the groups. For parametric testing, ANOVAs were used to compare the means of all the groups. If the variances between two groups were uneven, Games-Howell test was used to determine statistical significant differences between the groups, and Tukey-B test was used in the case of equal variances.

4. Results

4.1 Introduction

The results from the assessment of heavy metals and POPs present in the sediment and fish from selected sites in the Orange River (Boegoeberg [Bg]) and Vaal River (Standerton [Sdn]; Parys [Ps] and Rooipoort [Rp]) are reported in this chapter. The concentrations of POPs (section 4.2.1) and heavy metals (section 4.2.2) were identified in the sediment and indices (4.2.3) were calculated to describe the quality of the sediment. Chemical analysis of the fish tissue is shown in section 4.4, reporting on the levels of POPs (4.4.1) and heavy metals (4.4.2). Histological results (photomicrographs [4.5.1] and the semi-quantitative assessment results [4.5.2]) are explained in section 4.5, and a limited human health risk assessment was performed on the exposure through diet (section 4.6)

4.2 Chemical analysis of sediment

4.2.1 Persistent organic pollutants in sediment

The levels of the POPs were very low and could be quantified only in some instances. Those below the level of instrumental detection are presented as half the detection limit (½LOD) and are grey scaled in tables 1 and 2.

Table 1: Concentrations of PCDD/Fs and dl-PCBs measured in the sediment from sites in the Orange- and Vaal Rivers (See Appendix Table 16 for corresponding TEF values)

	Boegoeberg (ng/kg)	(ngTEQ/kg)	Rooipoort (ng/kg)	(ngTEQ/kg)	Parys (ng/kg)	(ngTEQ/kg)	Standerton (ng/kg)	(ngTEQ/kg)
2,3,7,8-TCDD	0.03	0.05	0.03	0.05	0.03	0.05	0.05	0.05
1,2,3,7,8-PeCDD	0.03	0.05	0.03	0.05	0.03	0.05	0.05	0.05
1,2,3,4,7,8-HxCDD	0.03	0.005	0.03	0.005	0.03	0.005	0.05	0.005
1,2,3,6,7,8-HxCDD	0.03	0.005	0.08	0.005	0.03	0.005	0.05	0.005
1,2,3,7,8,9-HxCDD	0.03	0.005	0.19	0.005	0.03	0.005	0.06	0.006
1,2,3,4,6,7,8-HpCDD	0.08	0.0015	0.57	0.0015	0.76	0.0076	0.75	0.0015
OCDD	0.48	0.00021	2.25	0.00015	5.40	0.00162	0.70	0.00021
ΣPCDDs (ngTEQ/kg)		0.12		0.12		0.12		0.12
2,3,7,8-TCDF	0.03	0.005	0.05	0.005	0.07	0.007	0.10	0.010
1,2,3,7,8-PeCDF	0.03	0.002	0.07	0.002	0.03	0.002	0.03	0.002
2,3,4,7,8-PeCDF	0.03	0.015	0.03	0.015	0.03	0.015	0.03	0.015
1,2,3,4,7,8-HxCDF	0.03	0.005	0.04	0.005	0.03	0.005	0.03	0.005
1,2,3,6,7,8-HxCDF	0.03	0.005	0.04	0.005	0.03	0.005	0.03	0.005
1,2,3,7,8,9,-HxCDF	0.03	0.005	0.12	0.005	0.03	0.005	0.03	0.005
2,3,4,6,7,8-HxCDF	0.03	0.005	0.03	0.005	0.03	0.005	0.03	0.005
1,2,3,4,6,7,8-HpCDF	0.08	0.00015	0.14	0.00015	0.08	0.00015	0.08	0.00015
1,2,3,4,7,8,9-HpCDF	0.08	0.00015	0.08	0.00015	0.08	0.00015	0.08	0.00015
OCDF	0.25	0.00015	0.25	0.00015	0.25	0.00015	0.25	0.00015
ΣPCDFs (ngTEQ/kg)		0.042		0.042		0.044		0.047
PCB 77	1.50	0.0003	1.50	0.0003	5.00	0.0005	1.50	0.0003
PCB 81	0.05	0.00003	0.05	0.00003	0.30	0.00009	0.05	0.00003
PCB 126	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
PCB 169	0.05	0.003	0.05	0.003	0.05	0.003	0.05	0.003
PCB 105	1.50	0.00009	1.50	0.00009	7.00	0.00021	1.50	0.00009
PCB 114	0.50	0.00003	1.00	0.00003	0.50	0.00003	0.50	0.00003
PCB 118	5.00	0.00030	2.75	0.00030	23.00	0.00069	5.00	0.00030
PCB 123	0.50	0.00003	2.75	0.00003	0.50	0.00003	0.50	0.00003
PCB 156	0.50	0.00003	0.50	0.00003	3.00	0.00009	0.50	0.00003
PCB 157	0.50	0.00003	0.50	0.00003	0.50	0.00003	0.50	0.00003
PCB 167	0.50	0.00003	0.50	0.00003	0.50	0.00003	0.50	0.00003
PCB 189	0.50	0.00003	0.50	0.00003	0.50	0.00003	0.50	0.00003
Σdl-PCBs (ngTEQ/kg)		0.0139		0.0139		0.0147		0.0139
ΣTEQs (ngTEQ/kg)		0.173		0.173		0.183		0.179

Table 2: Concentrations of non dl-PCBs, PBDEs, PFOS, and OC insecticides measured in the sediment from sites in the Orange- and Vaal Rivers

	Boegoeberg (ng/kg)	Rooipoort (ng/kg)	Parys (ng/kg)	Standerton (ng/kg)
PCB 28	25	25	25	25
PCB 52	25	25	25	25
PCB 101	25	25	25	25
PCB 138	25	25	25	25
PCB 153	25	25	25	25
PCB 180	25	25	25	25
Σnon dl-PCBs	150	150	150	150
TetraBDE	25	25	25	25
PentaBDE	25	25	25	25
HexaBDE	25	25	25	25
HeptaBDE	25	25	25	25
HexaBB	50	50	50	50
ΣPBDE	150	150	150	150
ΣPFOS	0.50	0.50	0.50	0.50
α-HCH	0.25	0.25	0.25	0.25
β-HCH	0.25	0.25	0.25	0.25
γ-HCH (Lindane)	0.25	0.25	0.25	0.25
HCB	0.25	0.25	0.25	0.25
Heptachlor	1.00	0.25	1.00	1.00
Aldrin	1.00	1.00	1.00	1.00
Dieldrin	1.00	1.00	1.00	1.00
Endrin	1.00	1.00	1.00	1.00
Heptachloroepoxide	1.00	1.00	1.00	1.00
Chlordane	1.00	1.00	1.00	1.00
o,p'-DDE	0.25	1.00	0.25	0.25
p,p-DDE	0.25	0.25	0.25	0.25
o,p'-DDD	0.25	0.25	0.25	0.25
p,p-DDD	0.25	0.25	0.25	0.25
o,p'-DDT	0.25	0.25	0.25	0.25
p,p-DDT	0.25	0.25	0.25	0.25
Mirex	1.00	0.25	1.00	1.00
Pentachlorobenzene	0.25	1.00	0.25	0.25
Chlordecone	1.00	0.25	1.00	1.00
Toxaphene	1.00	1.00	1.00	1.00
ΣOCpesticides (ng/kg)	12.75	12.75	12.75	12.75

Rooipoort, Parys and Standerton had quantifiable levels of PCDDs (above half LOD). Rooipoort had the highest levels of 1,2,3,6,7,8-hexachlorinated dibenzo-*p*-dioxin (HxCDD) and 1,2,3,7,8,9-HxCDD. The highest levels of 1,2,3,4,6,7,8-HpCDD and OCDD were determined for Parys. Standerton had the highest levels of 2,3,7,8-TCDF, while Rooipoort had the highest levels of the other of the polychlorinated dibenzofurans. Parys is the only site with levels of PCBs above ½LOD, with PCB 118 the highest (Table 1). Parys had the highest TEQ values for the dioxin-like compounds followed by Standerton (Table 1). The non-dioxin-like PCBs, PBDEs, PFOS and pesticide values were the same for all sites because they were all too low to quantify (Table 2).

4.2.2 Heavy metals in the sediment

Iron was the element with the highest levels in the sediment, followed by Mn (Table 3). Rooipoort had the highest iron levels followed by Standerton, Boegoeberg and Parys respectively. Rooipoort had the highest concentrations of Ni, Zn, As, Se, Ag and U. Standerton had the highest levels of Cr, Co, Cu, and Pb.

Table 3: Heavy metal concentrations in the sediment from sites in the Orange- and Vaal Rivers

(mg/kg)	Boegoeberg	Rooipoort	Parys	Standerton
Cr	0.79	3.35	1.4	3.5
Mn	8.4	30	12	29
Fe	495	0.895	200	810
Co	0.39	0.8	0.43	0.93
Ni	1.35	4.05	1.5	3
Cu	0.805	1.45	0.72	1.7
Zn	1.25	2.15	1.3	2.1
As	0.115	0.25	0.11	0.17
Se	0.066	0.46	0.43	0.47
Ag	0.11	0.37	0.11	0.14
Pt	0.004	0.001	0.001	0.001
Au	0.015	0.004	0.002	0.002
Cd	0.003	0.005	0.002	0.003
Hg	0.037	0.02	0.014	0.01
Pb	0.185	0.405	0.15	0.57
U	0.016	1.019	0.015	0.044

4.2.3 Sediment indices

Indices were calculated to describe the quality of the sediment. These include the enrichment factor (Ef) (Table 4), geo-accumulation index (Igeo) (Figure 4), metal pollution index (MPI) and pollution load index (PLI) (Figure 5), sediment quality index (SQI) (Figure 6), and the sediment quality guideline index (SQG-I) (Figure 7).

Selenium and Ag had the highest enrichment factors at all sites, followed by Cd and Au. Parys had the highest enrichment factors for Se and Ag. Boegoeberg had the highest Cd and Au enrichment factor, and Parys showed severe enrichment for Ni. Uranium had a high enrichment factor at Rooipoort showing severe enrichment. As can be seen from the number of coloured cells as well as the intensity of the grey scale, Parys had the most enriched sediment and Boegoeberg had the least enriched sediment.

Table 4: Enrichment factors (Ef) for heavy metals in the sediment from sites in the Orange- and Vaal Rivers

	Boegoeberg	Rooipoort	Parys	Standerton
Cr	1.19	3.3	6.18	3.81
Mn	0.99	1.96	3.52	2.1
***Fe**	1	1	1	1
Co	2.1	2.38	5.73	3.06
Ni	4.53	7.52	12.46	6.15
Cu	3.51	3.5	7.78	4.53
Zn	1.5	1.43	3.86	1.54
As	3.59	4.31	8.49	3.24
Se	49.62	191.28	800.16	215.95
Ag	124.81	232.18	308.9	97.07
Cd	184.09	24.5	67.96	12.97
Pt	1.56	1.66	2.73	1.16
Au	40.67	12.33	38.61	6.81
Hg	0.98	0.45	0.95	0.51
Pb	0.68	0.82	1.36	1.28
U	0.39	14.06	0.93	0.67

*Fe was the normaliser (see section 3.6)

Interpretation of Ef scale	
1<	natural enrichment
<3	minor anthropogenic enrichment
3-5	moderate enrichment
5-10	moderately severe enrichment
10-25	severe enrichment
25-50	very severe enrichment
50+	extremely severe enrichment

The geo-accumulation (Igeo) for most of the metals fell in the “unpolluted” category in terms of that specific metal’s measured concentration. The Igeo values for Se, Ag and Cd indicated “highly to very highly polluted” in all four sites (Figure 4). The highest Igeo value for Se was at Standerton > Rooipoort > Parys > Boegoeberg. Silver had the highest Igeo at Rooipoort,

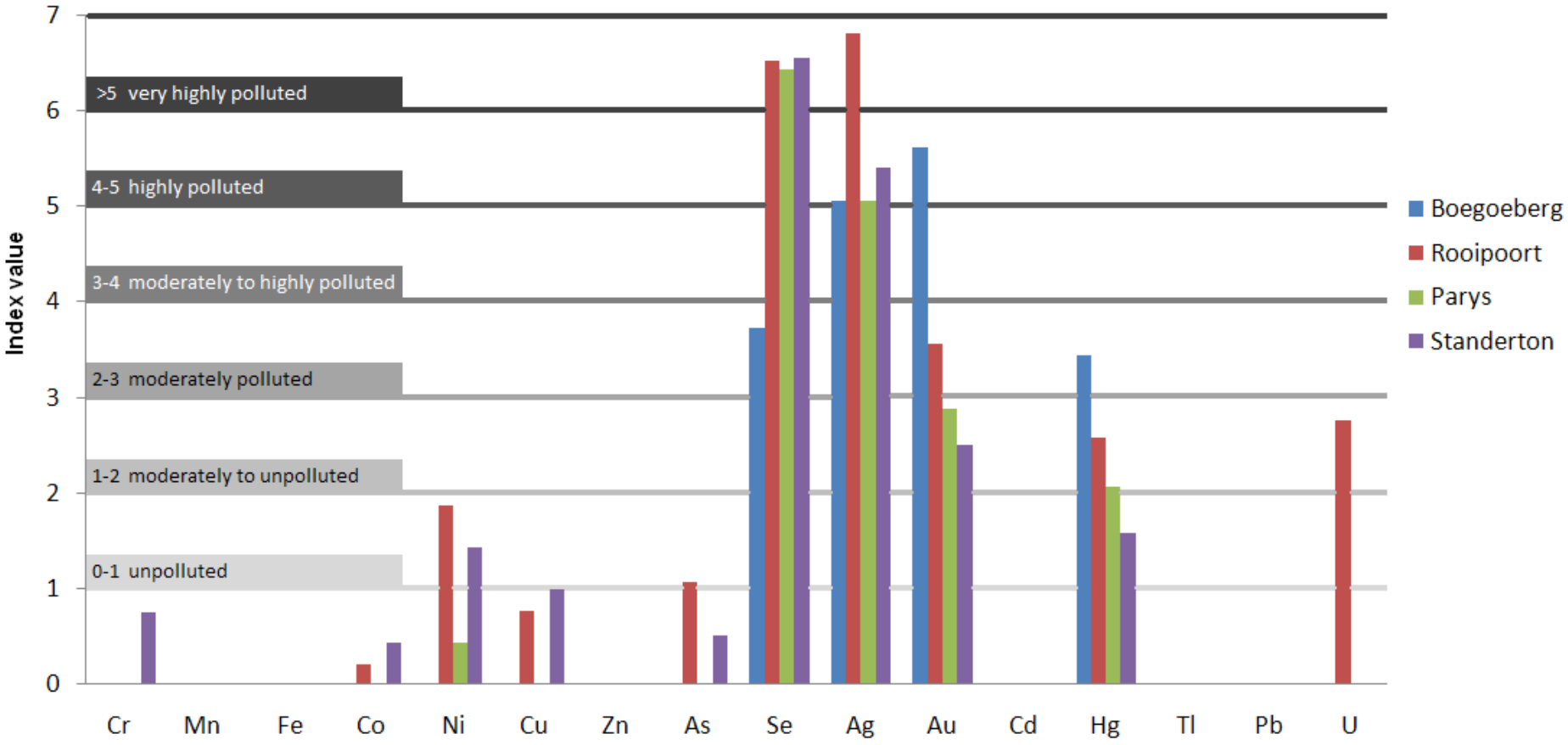

Figure 4: The geo-accumulation index values for heavy metals in the sediment from sites in the Orange- and Vaal Rivers (See Appendix Table 17 for Igeo values)

Standerton, Boegoeberg and Parys respectively. Cadmium had the highest Igeo Boegoeberg. According to the Igeo, gold showed an increase in pollution from Standerton < Parys < Rooipoort < Boegoeberg. Of all four sites, uranium had a high Igeo value only at Rooipoort..

The metal pollution index (MPI) and pollution load index (PLI) both showed the same overall pattern: the pollution as determined by both indices decreased as follows Rooipoort > Standerton > Boegoeberg > Parys.

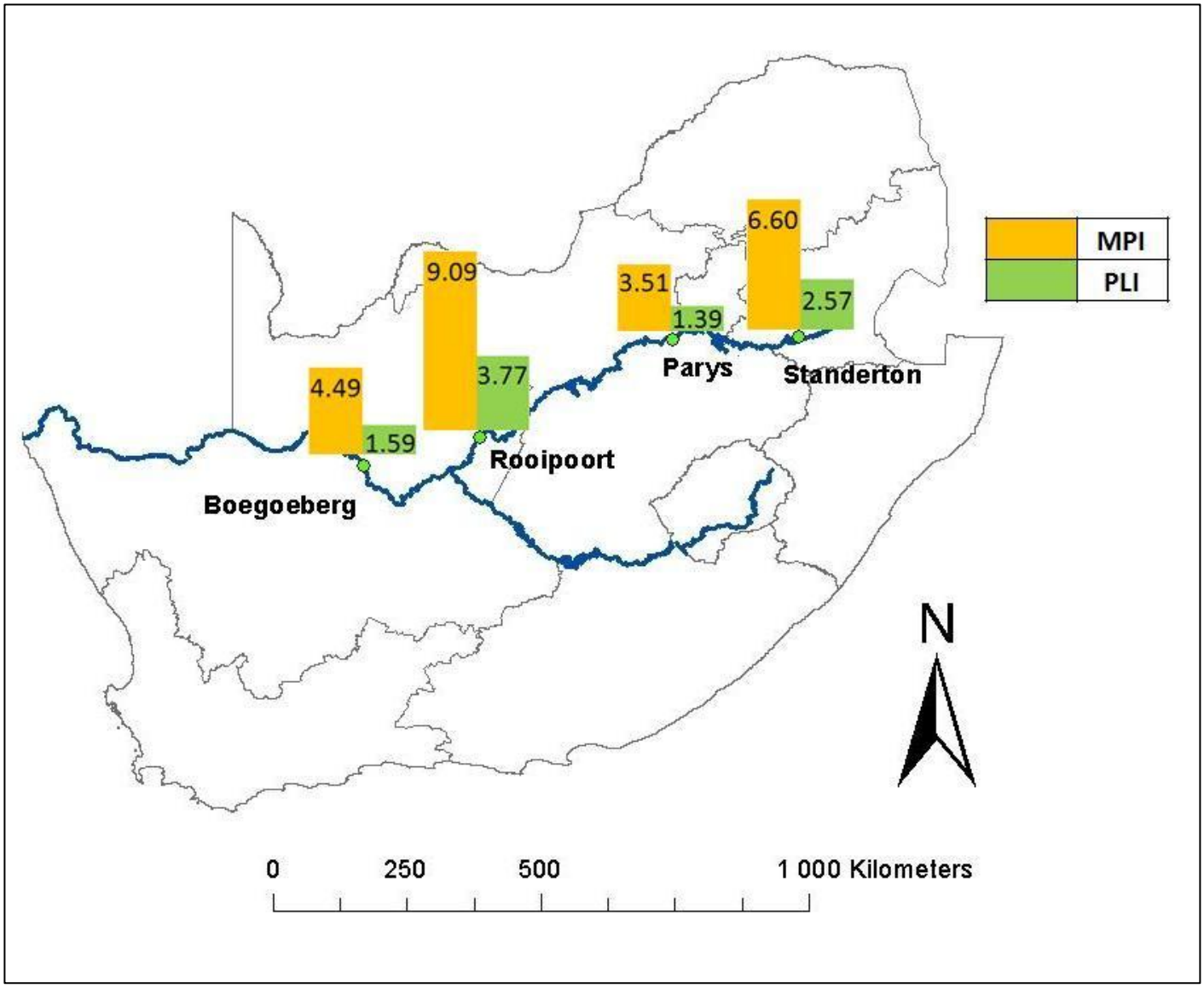

Figure 5: The metal pollution index (MPI) and pollution load index (PLI) for sediment from sites in the Orange- and Vaal Rivers

The sediment regarding the POPs showed to be in excellent quality due to the very low levels of the POPS. For this reason only influence of the heavy metal on sediment quality is presented in Figure 6.

Results showed that Rooipoort had poor sediment quality, whereas Standerton had marginal quality. The rest of the sites had fair quality scores for their sediment (Figure 6)

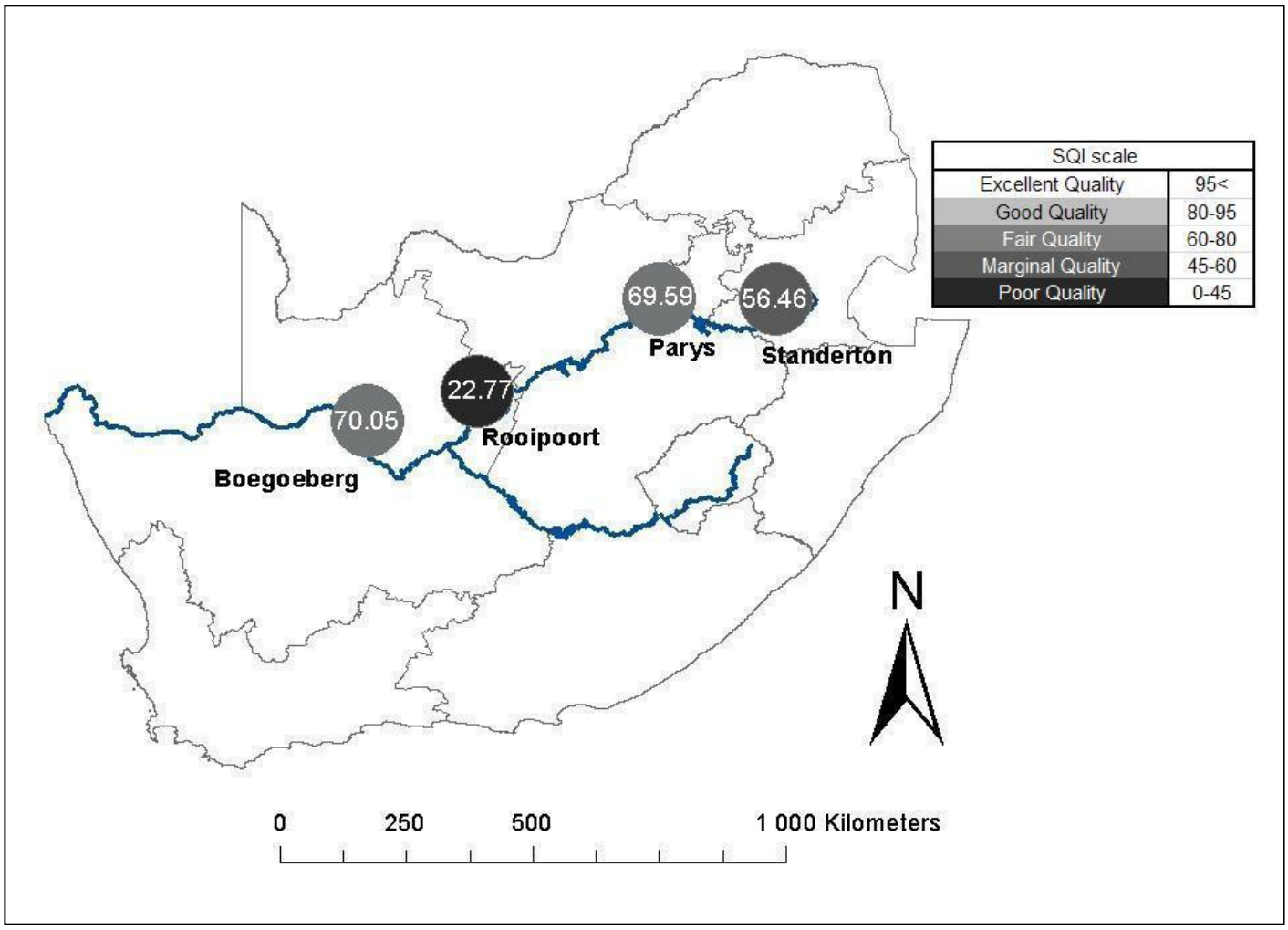

Figure 6: The sediment quality index values for heavy metals in the sediment from sites in the Orange- and Vaal Rivers

Results showed that Rooipoort sediment had a high probability of being toxic to biota according to the SQG-I, whereas the rest of the sites had a moderate probability of being toxic to biota.

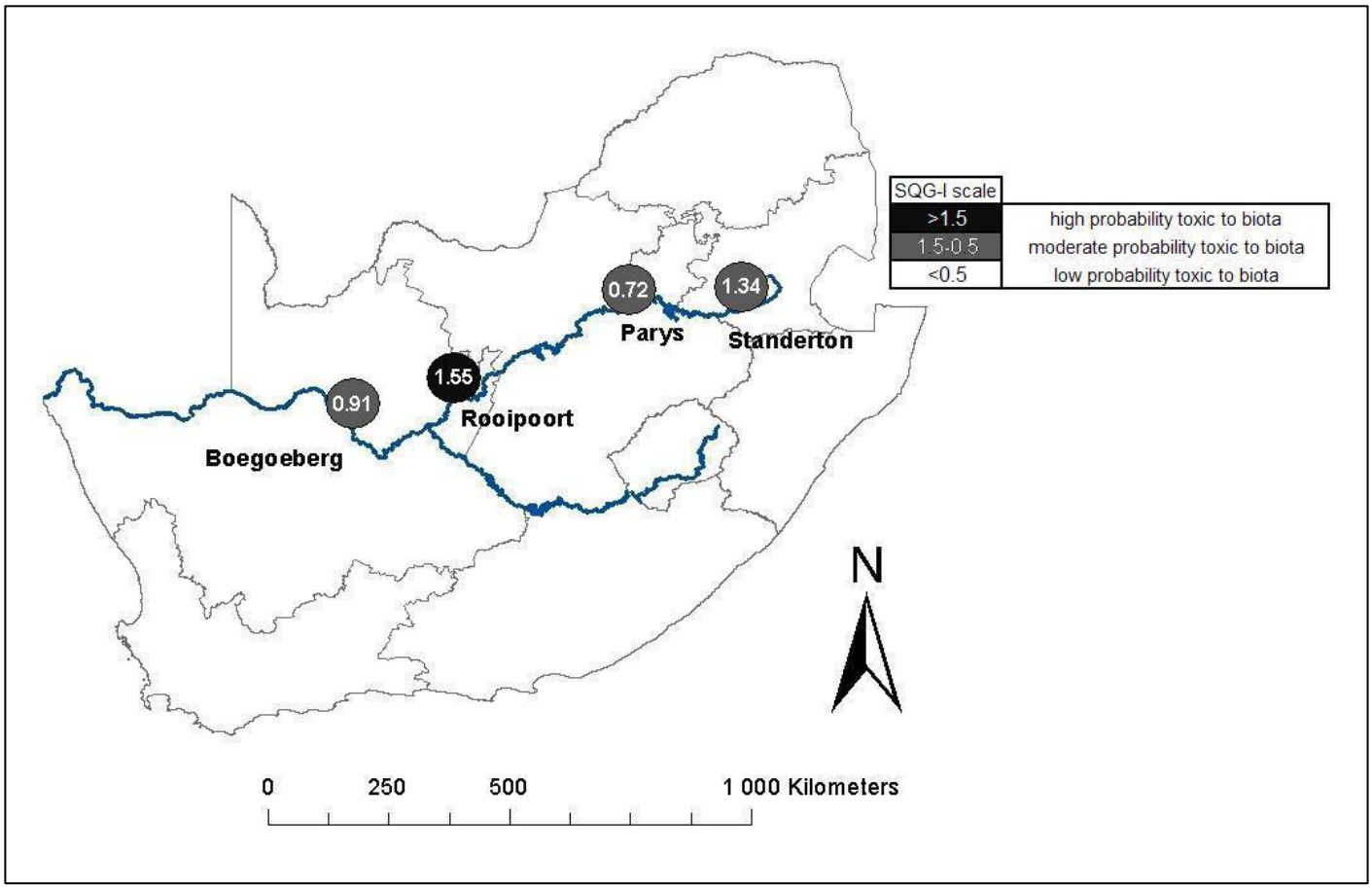

Figure 7: The sediment quality guideline index values for heavy metals in the sediment from sites in the Orange- and Vaal Rivers

4.3 General information of fish sampled

The morphometric information given in table 5 might help explain differences in concentrations of pollutants in the fish from different sites and genders. More females might explain higher chemical concentrations since female fish have more fat and thus have a higher capacity to store more lipophillic chemicals (Anthony *et al*., 2000).

Table 5: Sex ratio, mean mass, mass range and standard length range of *Clarias gariepinus* sampled from sites in the Orange- and Vaal Rivers

	Boegoeberg (n = 10)	Rooipoort (n = 10)	Parys (n = 10)	Standerton (n = 10)
Sex ratio (F:M)	5:5	3:7	6:4	4:6
Mean mass (g)	2 350	5 950	4 117	3 995
Mass range (g)	1 300-3 280	2 520-12 380	3 520-4 700	2 600-6 400
Range standard length (mm)	530-720	670-1070	660-770	630-930

4.4 Chemical analysis of fish

4.4.1 Persistent organic pollutants in *Clarias gariepinus*

The fish caught at each site were pooled according to gender. The results are presented separately for males and females at each site. There was no significant difference between the genders of the pooled samples ($p = 0.751$). The POPs below the level of instrumental detection are presented as half the detection limit (½LOD) and are grey scaled in tables 6 and 7.

Table 6: Concentrations of PCDD/Fs measured in *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

	Boegoeberg male (ngTEQ/kg)	Boegoeberg female (ngTEQ/kg)	Rooipoort male (ngTEQ/kg)	Rooipoort female (ngTEQ/kg)	Parys male (ngTEQ/kg)	Parys female (ngTEQ/kg)	Standerton male (ngTEQ/kg)	Standerton female (ngTEQ/kg)
2,3,7,8-TCDD	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,7,8-PeCDD	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,4,7,8-HxCDD	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,6,7,8-HxCDD	0.03	0.03	0.03	0.03	0.03	0.07	0.03	0.03
1,2,3,7,8,9-HxCDD	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,4,6,7,8-HpCDD	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
OCDD	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ΣPCDDs	0.48	0.48	0.48	0.48	0.48	0.52	0.48	0.48
2,3,7,8-TCDF	0.03	0.03	0.03	0.03	0.03	0.11	0.03	0.03
1,2,3,7,8-PeCDF	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.03
2,3,4,7,8-PeCDF	0.03	0.03	0.03	0.03	0.03	0.24	0.03	0.03
1,2,3,4,7,8-HxCDF	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,6,7,8-HxCDF	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1,2,3,7,8,9,-HxCDF	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
2,3,4,6,7,8-HxCDF	0.03	0.03	0.03	0.03	0.03	0.12	0.03	0.03
1,2,3,4,6,7,8-HpCDF	0.15	0.15	0.15	0.15	0.15	0.08	0.15	0.15
1,2,3,4,7,8,9-HpCDF	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
OCDF	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ΣPCDFs	0.69	0.69	0.69	0.69	0.69	1.03	0.69	0.69
PCB 77	1.5	1.5	1.5	1.5	1.5	10.3	1.5	1.5
PCB 81	0.25	0.25	0.25	0.25	0.6	1.6	0.25	0.25
PCB 126	0.25	0.25	0.25	0.25	1.2	5.6	0.25	0.25
PCB 169	0.25	0.25	0.25	0.25	0.25	1.2	0.25	0.25
PCB 105	2.5	2.5	2.5	2.5	46	203	2.5	2.5
PCB 114	2.5	2.5	2.5	2.5	2.5	17	2.5	2.5
PCB 118	15	15	15	15	152	487	15	15
PCB 123	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
PCB 156	2.5	2.5	2.5	2.5	27	99	2.5	2.5
PCB 157	2.5	2.5	2.5	2.5	8	19	2.5	2.5
PCB 167	2.5	2.5	2.5	2.5	17	67	2.5	2.5
PCB 189	2.5	2.5	2.5	2.5	2.5	15	2.5	2.5
Σdl-PCBs	34.75	34.75	34.75	34.75	261.05	928.2	34.75	34.75
ΣTEQs	35.92	35.92	35.92	35.92	262.22	929.75	35.92	35.92

Table 7: Concentrations of non dl-PCBs, PBDEs, PFOS, and OC pesticides measured in *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

	Boegoeberg male (ng/kg)	Boegoeberg female (ng/kg)	Rooipoort male (ng/kg)	Rooipoort female (ng/kg)	Parys male (ng/kg)	Parys female (ng/kg)	Standerton male (ng/kg)	Standerton female (ng/kg)
PCB 28	25	25	25	25	167	407	25	25
PCB 52	25	25	25	25	123	206	25	25
PCB 101	25	25	25	25	166	324	25	25
PCB 138	25	35	132	25	440	1 210	45	25
PCB 153	45	40	200	25	604	2 300	45	25
PCB 180	25	25	122	25	280	962	25	25
Σnon dl-PCBs	170	175	529	150	1780	5 409	190	150
TetraBDE	25	25	25	25	112	168	25	25
PentaBDE	25	25	25	25	25	103	25	25
HexaBDE	25	25	25	25	25	25	25	25
HeptaBDE	25	25	25	25	25	25	25	25
HexaBB	50	50	50	50	50	50	50	50
ΣPBDE	150	150	150	150	237	371	150	150
ΣPFOS	0.250	1	1	1	1	1.7	2.6	1.2
α-HCH	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
β-HCH	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
γ-HCH (Lindane)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
HCB	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Heptachlor	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aldrin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dieldrin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Endrin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Heptachloroepoxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chlordane	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
o,p'-DDE	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
p,p-DDE	1.1	1.6	18	9.3	4	7.4	1.4	1.7
o,p'-DDD	0.25	0.25	0.25	0.5	0.25	0.5	0.25	0.25
p,p-DDD	0.25	0.25	0.9	0.8	0.25	0.8	0.25	0.25
o,p'-DDT	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
p,p-DDT	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mirex	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Pentachlorobenzene	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chlordecone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Toxaphene	1	1	1	1	1	1	1	1
ΣOCpesticides (ng/kg)	8.6	9.1	26.15	17.6	11.5	15.7	8.9	9.2

Results showed that Parys had the highest TEQ value for dioxin-like compounds (Figures 8 & 9), females having a higher value than the males. Boegoeberg females, both sexes from Rooipoort and Parys' males had elevated PFOS values (Figure 11). Higher values of PFOS were found in the females of Parys and both sexes at Standerton. The highest value of PFOS was for males from Standerton. Rooipoort's males had the highest pesticide value followed by Rooipoort's females, Parys' females and then Parys' males (Figure 12).

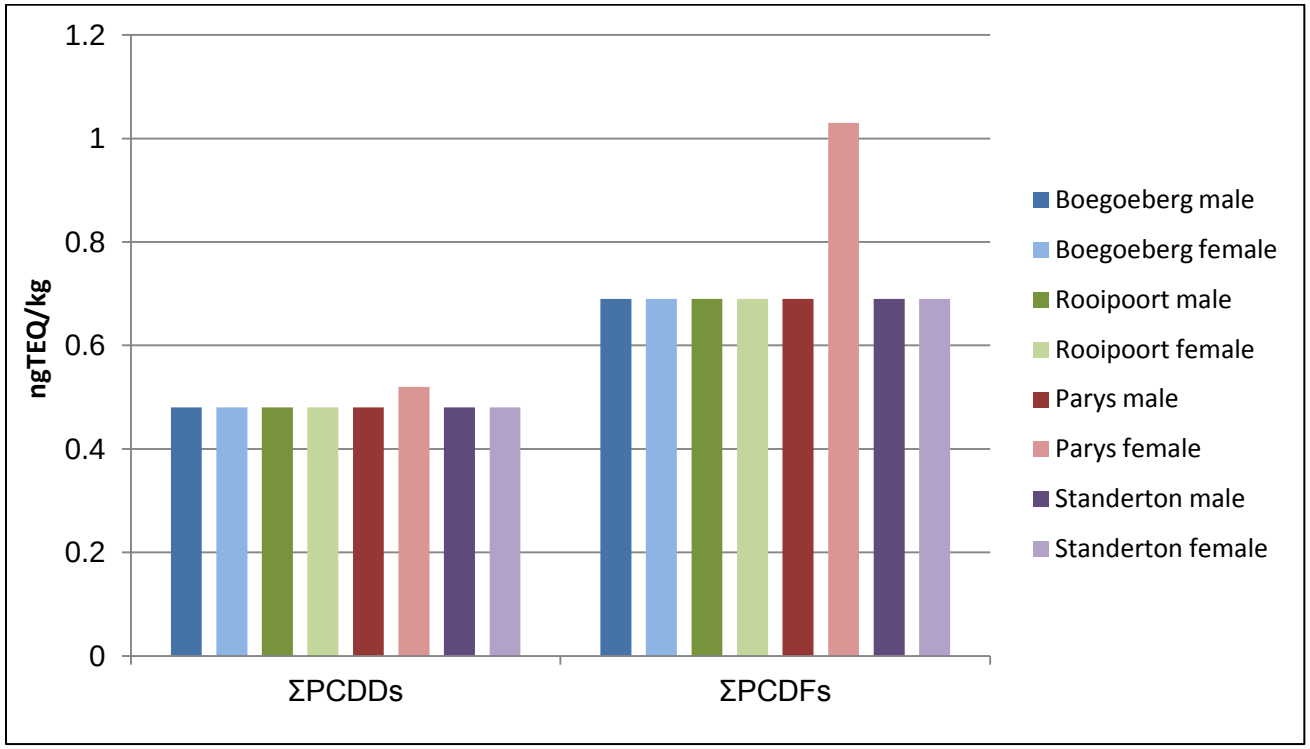

Figure 8: The sum of PCDDs and PCDFs in *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

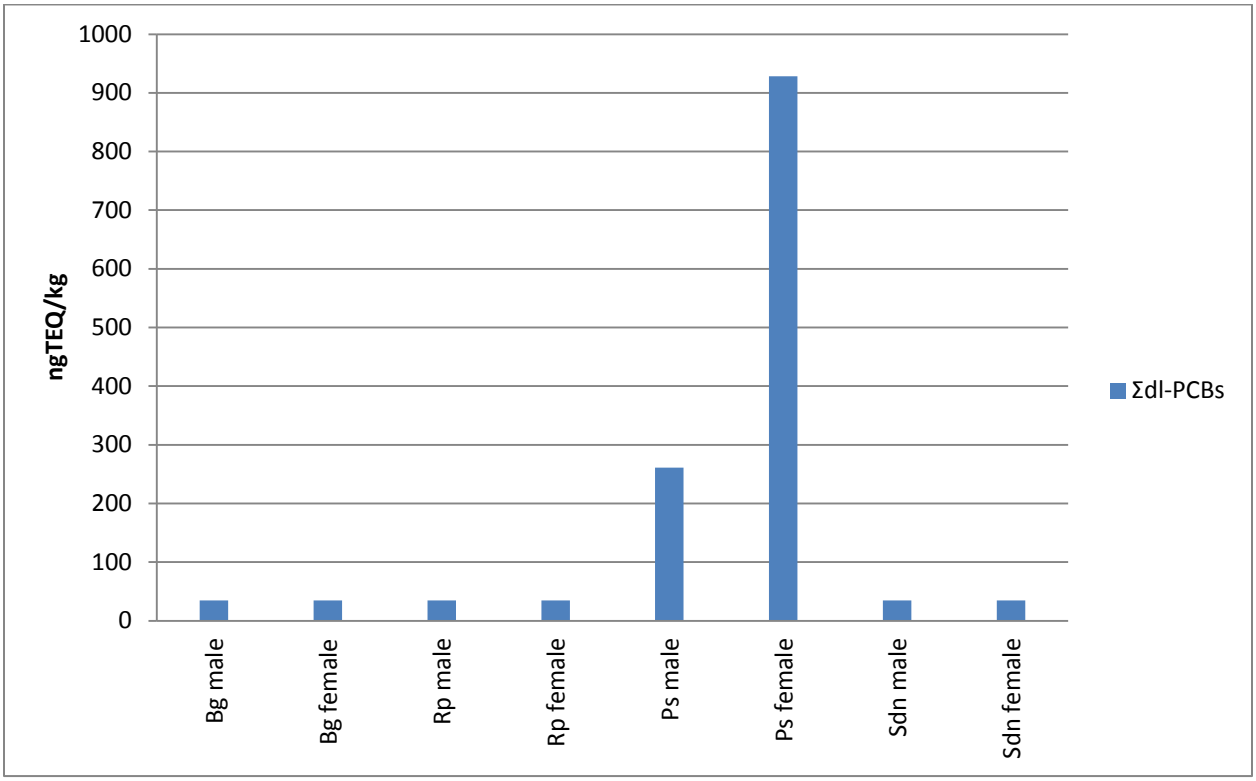

Figure 9: The sum of the dl-PCBS in *Clarias gariepinus* from sites (Boegoeberg [Bg], Rooipoort [Rp], Parys [Ps] and Standerton [Sdn]) in the Orange- and Vaal Rivers

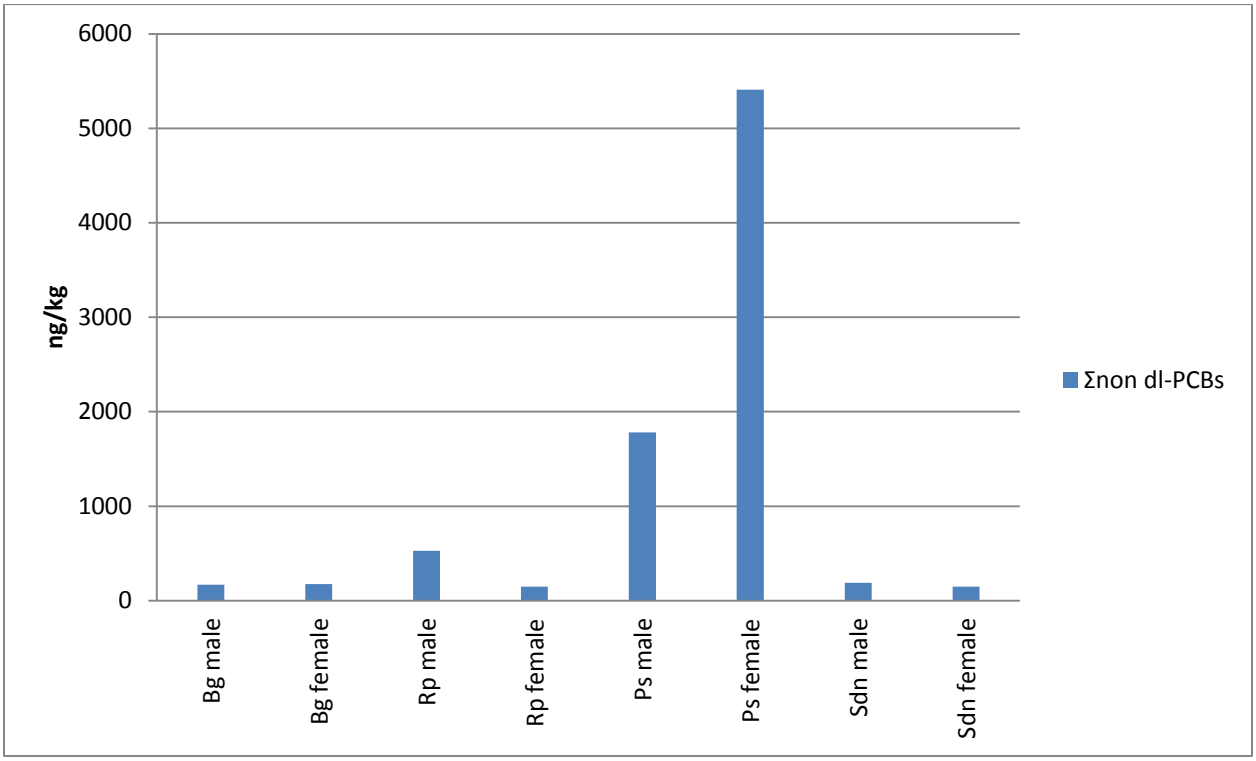

Figure 10: The sum of the non dl-PCBs in *Clarias gariepinus* from sites (Boegoeberg [Bg], Rooipoort [Rp], Parys [Ps] and Standerton [Sdn]) in the Orange- and Vaal Rivers

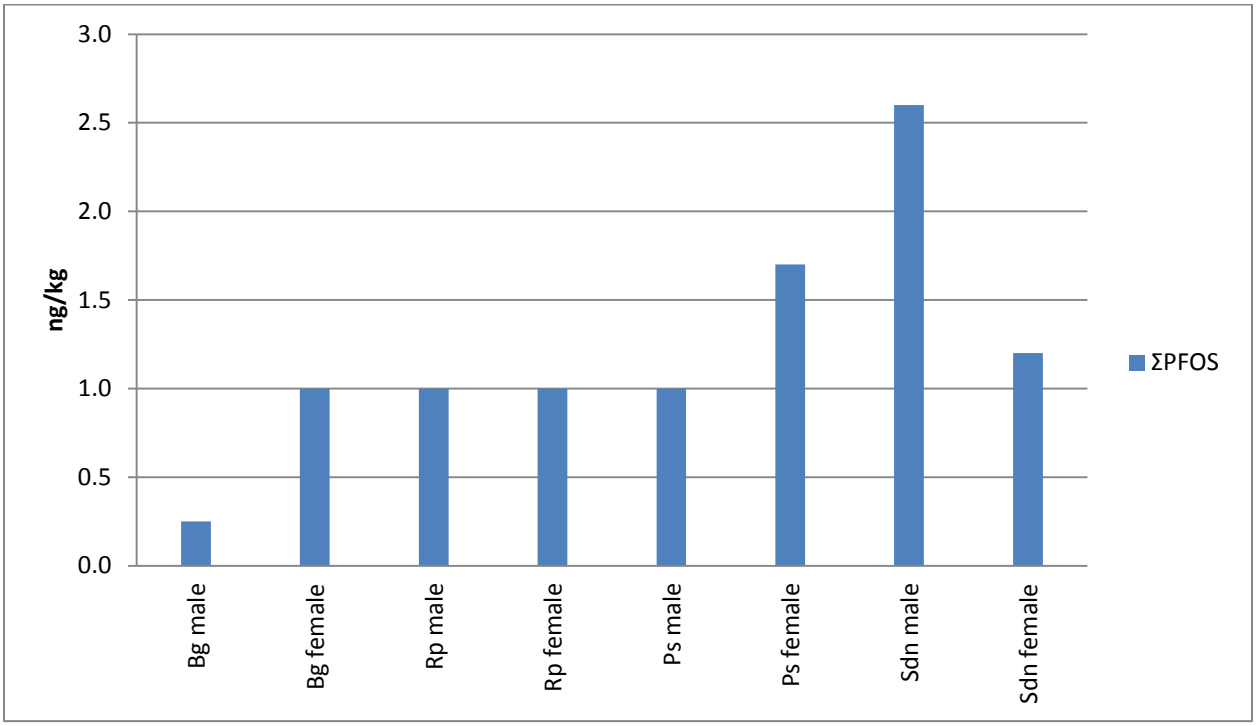

Figure 11: The sum of PFOS in *Clarias gariepinus* from sites (Boegoeberg [Bg], Rooipoort [Rp], Parys [Ps] and Standerton [Sdn]) in the Orange- and Vaal Rivers

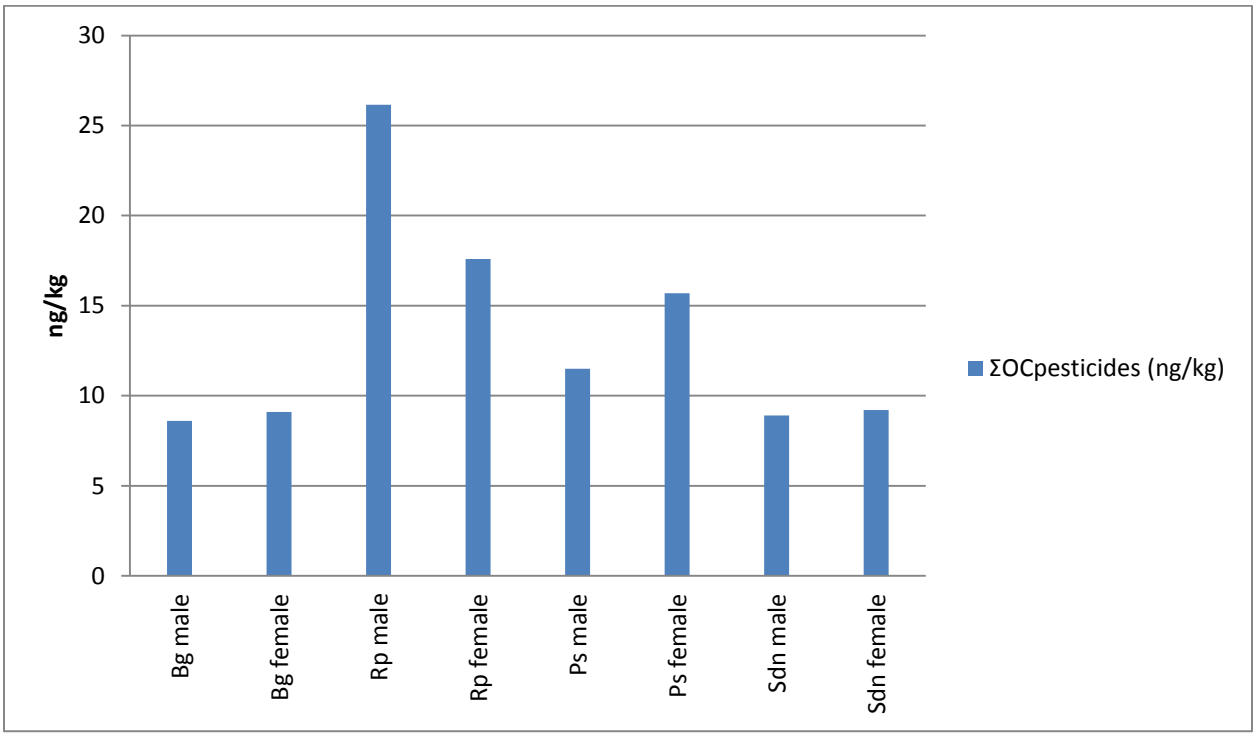

Figure 12: The sum of the OC pesticides in *Clarias gariepinus* from sites (Boegoeberg [Bg], Rooipoort [Rp], Parys [Ps] and Standerton [Sdn]) in the Orange- and Vaal Rivers

4.4.2 Heavy metals in *Clarias gariepinus*

The fish caught were individually analysed for heavy metals in the fillet tissue (See Appendix Table 18 and Figure 27 for metal concentrations from all sites).

Boegoeberg samples had the highest metal concentrations, namely: Ag (4.65±0.675 mg/kg) (Figure 13), Co (0.00392±0.0024 mg/kg), Cu (0.063±0.342 mg/kg), Cd (0.002301±0.0024 mg/kg), Pt (0.01984±0.023 mg/kg), Pb (0.01636±0.0112 mg/kg), U (0.01913±0.0234 mg/kg) (Figure 14), Mn (0.056±0.0054 mg/kg), Ni (0.0337±0.0082 mg/kg), Au (0.2527±0.259 mg/kg), Hg (0.0725±0.016 mg/kg) (Figure 15). The fish from Parys had the highest levels of Fe (1.73±0.27 mg/kg) (Figure 13), Se (0.161±0.026 mg/kg) (Figure 14), and Zn (0.689±0.17 mg/kg) (Figure 15). Chromium (0.252±0.011 mg/kg) (Figure 13) and As (0.049±0.003 mg/kg) (Figure 15) levels were the highest in Standerton fish.

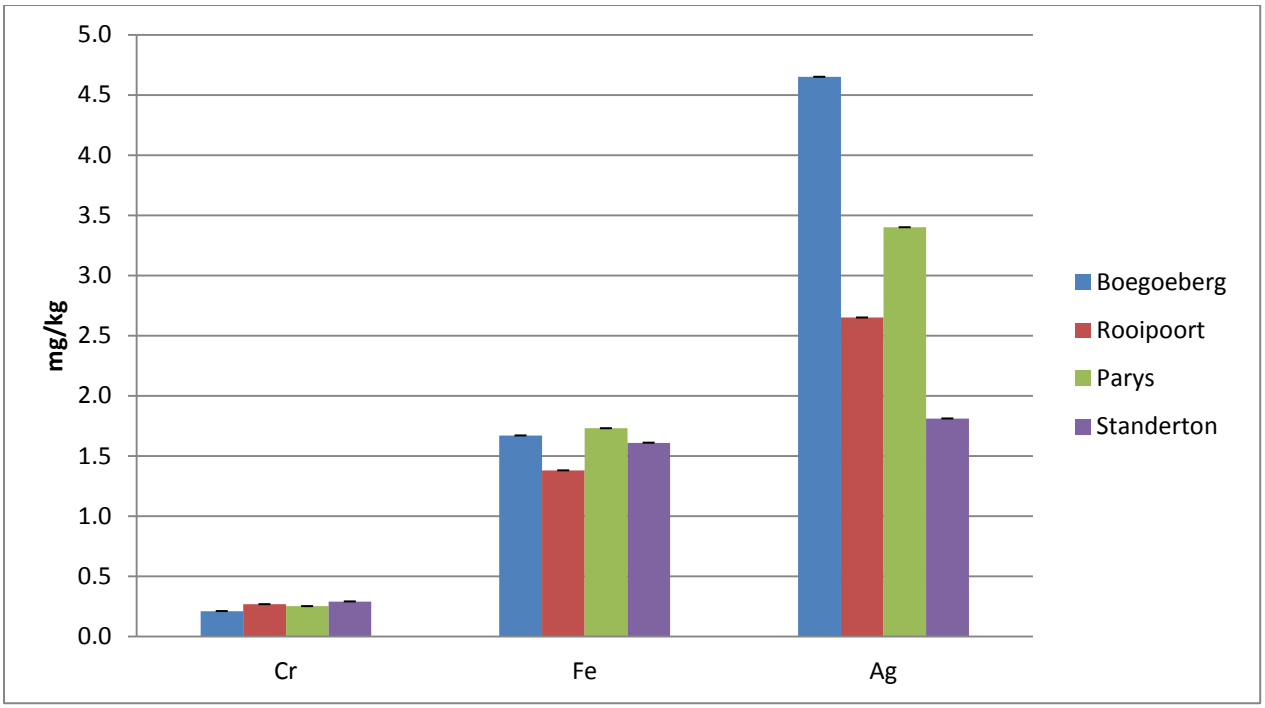

Figure 13: Mean concentrations of Cr, Fe, and Ag in *Clarias gariepinus* (n = 10) from sites in the Orange- and Vaal Rivers

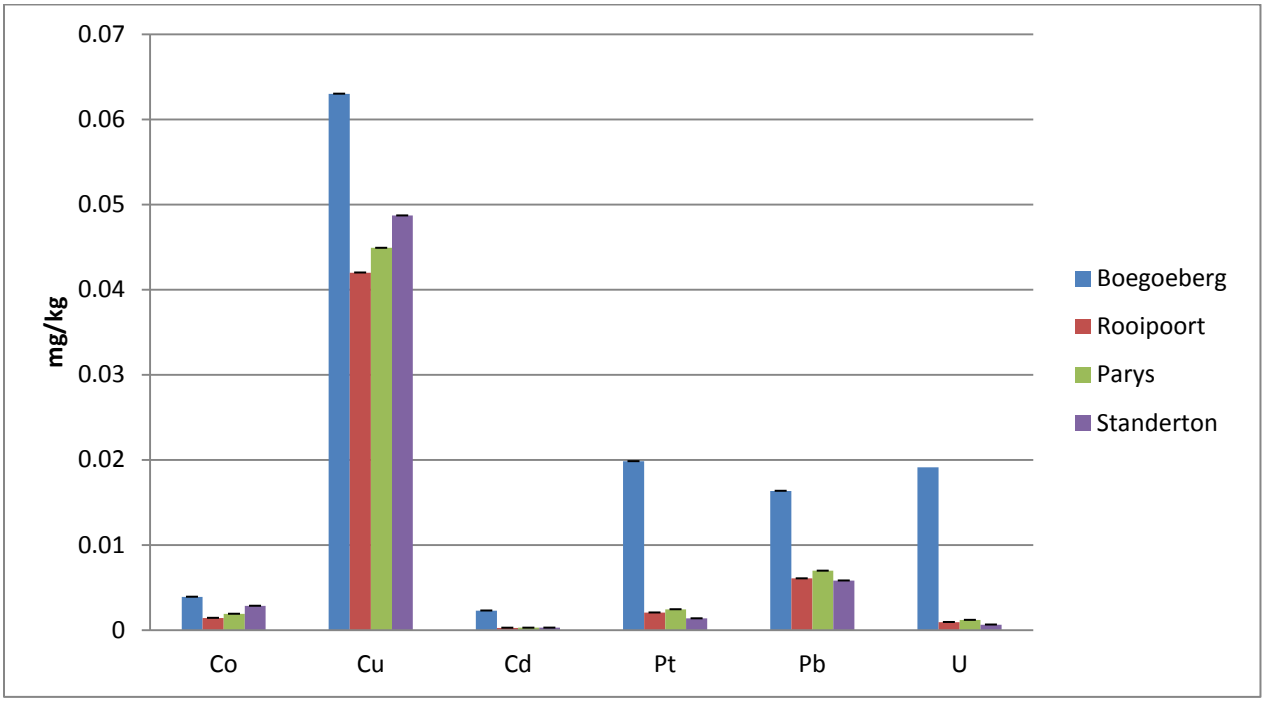

Figure 14: Mean concentrations of Co, Cu, Cd, Pt, Pb and U in *Clarias gariepinus* (n = 10) from sites in the Orange- and Vaal Rivers

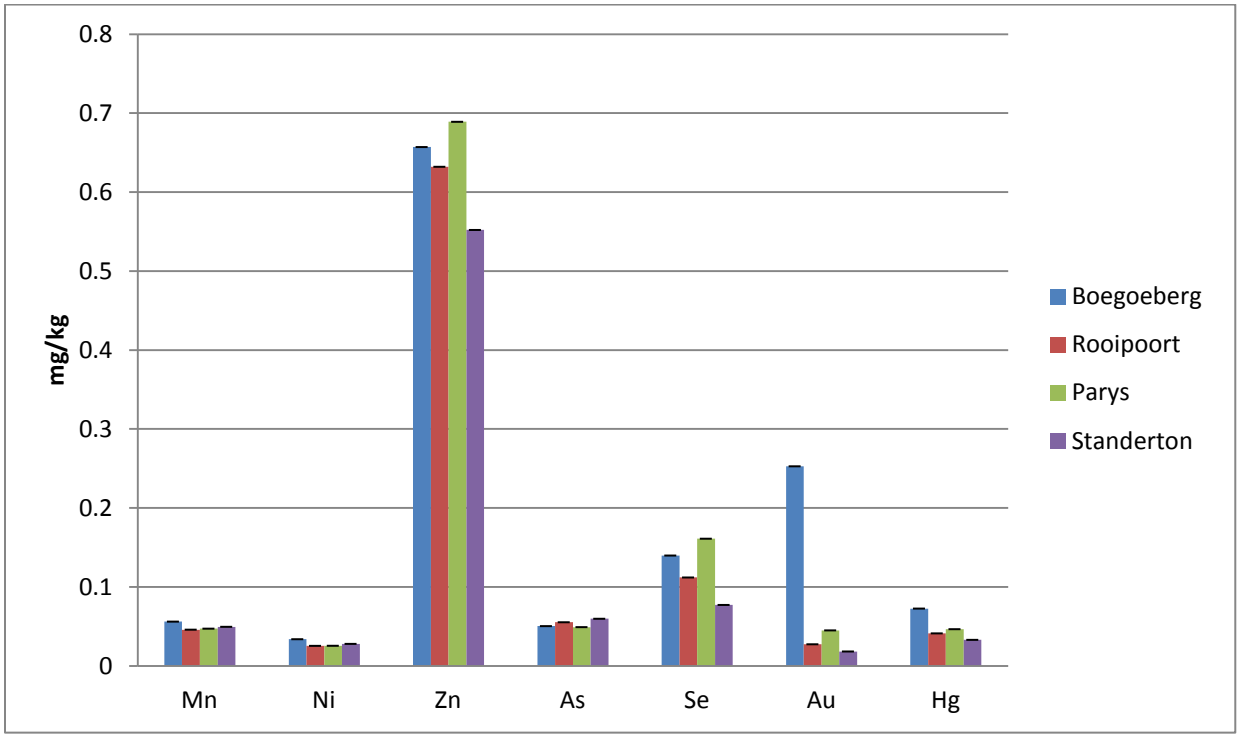

Figure 15: Mean concentrations of Mn, Ni, Zn, As, Se, Au, and Hg in *Clarias gariepinus* (n = 10) from sites in the Orange- and Vaal Rivers

4.5 Histology results

4.5.1 Qualitative histology results

The histological findings for the liver and the kidneys were categorised into two main categories, non-pathological and pathological phenomena, but all alterations in the gills mentioned here were regarded as pathological.

Gills

Histological alterations observed in the gills of the fish sampled were circulatory disturbances, regressive- and progressive responses. Circulatory disturbance were aneurysms on the secondary lamellae (telangiecstasia) (Figure 16) (Table 8). Branching of the lamellae is an example of a regressive alteration and was also the only regressive alteration found in this study (Table 8). Increase in cells or hyperplasia is a progressive alteration and was noted in chloride cells, mucous cells and epithelial cells (Figure 17) (Table 8).

Table 8: Qualitative histology results for the gills of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

Histological alteration	**Boegoeberg** n = 10	**Rooipoort** n = 10	**Parys** n = 10	**Standerton** n = 10
Telangiecstasia	5	6	8	7
Branching of secondary lamellae	1	-	-	-
Branching of primary lamellae	-	-	3	-
Monogean parasites	3	1	1	3
Chloride cell hyperplasia	4	6	-	-
Mucous cell hyperplasia	-	-	-	3
Epithelial hyperplasia	2	3	4	5

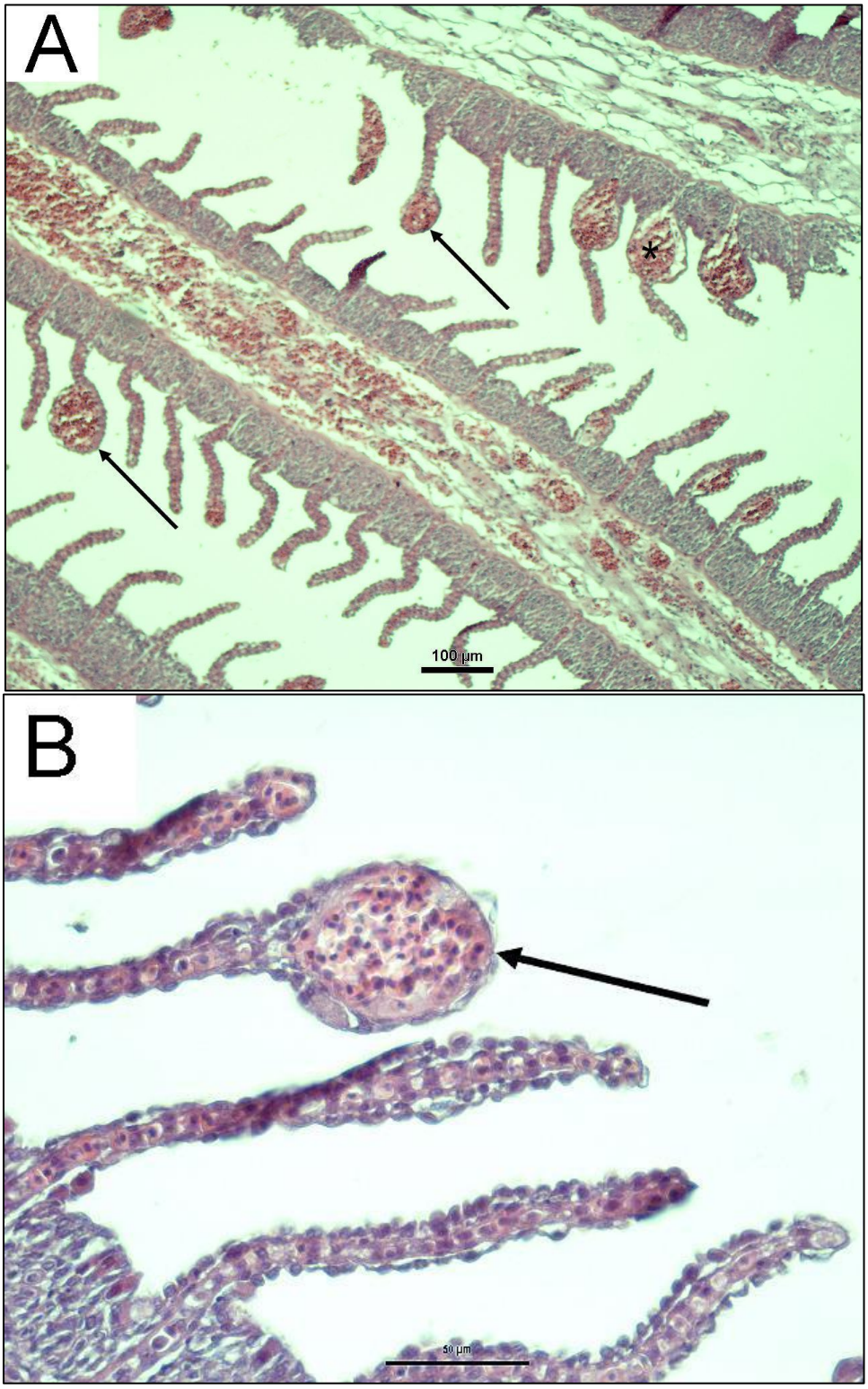

Figure 16: Telangiecstasia and aneurysms found in the gills of *Clarias gariepinus* from all sites. Telangiecstasia (arrows [A+B]) and aneurysims (asterix) on the secondary lamellae of the gill

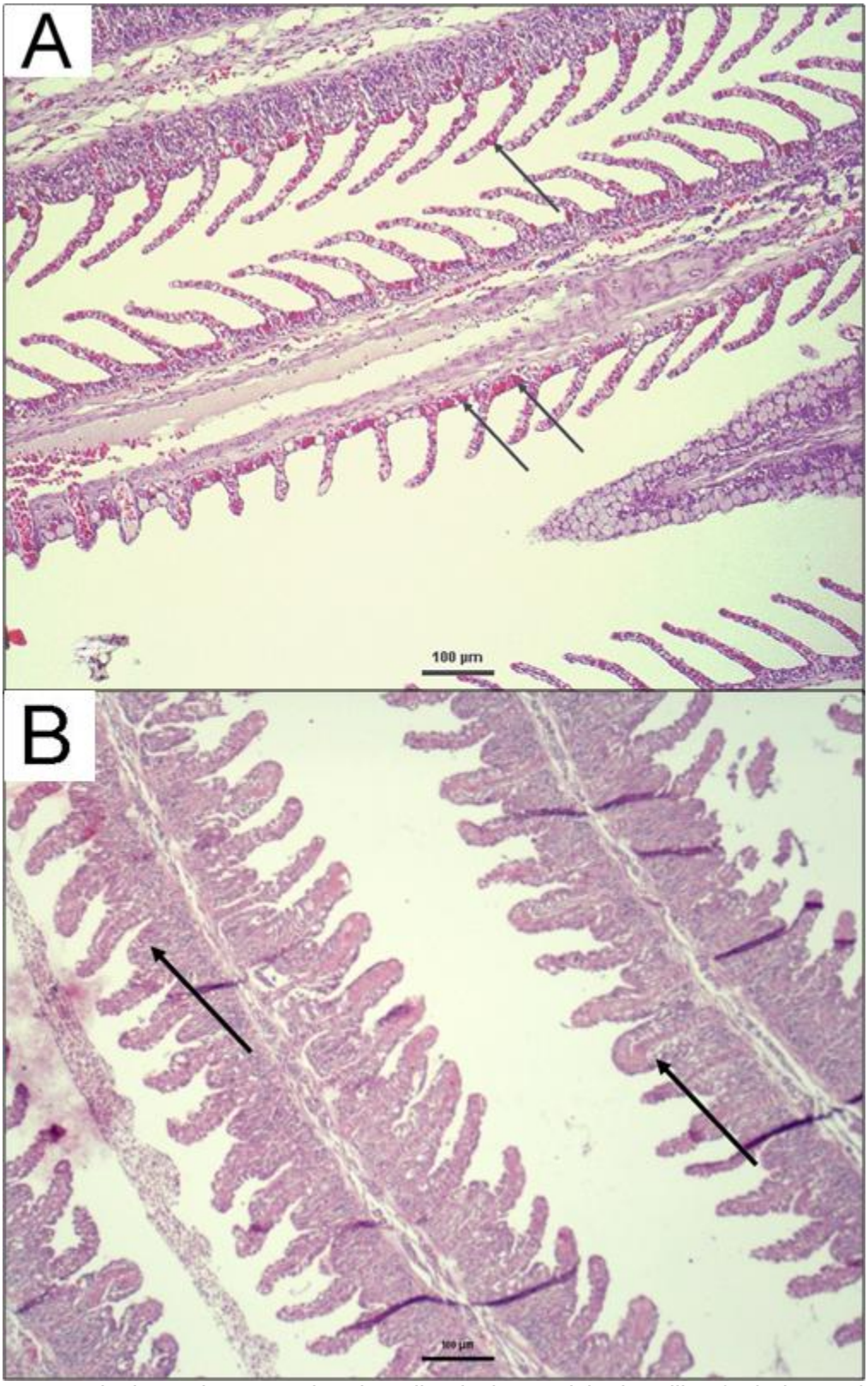

Figure 17: Hyperplasia on the secondary lamellae in (arrows) in the gills of *Clarias gariepinus.* A = hyperplasia of chloride cells (Boegoeberg and Rooipoort), B = hyperplasia of gill epithelium (all sites)

Liver

Non-pathological alterations

The cytoplasmic characteristics observed for the fish were random, meaning that no trend of a specific characteristic associated with a particular site. The hepatocyte cytoplasm differed between the individual fish. In 58% of the livers the majority of hepatocytes contained granular cytoplasm. Only 20% of the livers had clear cytoplasm in their hepatocytes. One fish from Rooipoort (female) had an equal amount of hepatocytes with either granular or clear cytoplasm, distributed equally in the liver. Other cytoplasmic characteristics observed were dense eosinophillic cytoplasm (one fish, from Rooipoort), clumped cytoplasm (two fish each from Parys and Standerton), and basophilic cytoplasm (three fish from Standerton).

Melano-macrophage centres (MMCs) are areas concentrated with heterogeneous materials including melanin, hemosidirin and lipofuschin in the liver tissue of fish (Agius, 1985; Van Dyk *et al*., 2011). MMCs are often associated with normal histology (Bruslé & Gonzàlez, 1996; Van Dyk *et al*., 2011) (Table 9), but an increase in both size and number could be from toxicant exposure (Van Dyk *et al*., 2011). The increase in MMCs is believed to be involved in the destruction, detoxifying and recycling of endogenous and exogenous compounds (Camargo & Martinez, 2007). Melano-macrophage centres (MMCs) were abundant, 82 % of all the livers sampled had MMCs present, ranging from small and few, to large centres spread densely across the liver. Parys and Rooipoort had the highest amount of MMCs.

Perivascular- and peribilary inflammation was seen in some of the fish (Table 9), but this inflammatory response was not pathological.

Pathological alterations

Regressive alterations in the liver included vacuolation, intercellular deposits and hyalinization. Vacuolation in the form of glycogen-like vacuoles (Figure 18), fatty change (fat degeneration) as well as micro-(Figure 19) and macrovesicular steatosis (Figure 20) were noted in fish across all four sites (Table 9).

Glycogen is the main form of energy storage in fish (Miranda *et al*., 2008) therefore it is expected to find glycogen-like vacuoles in the livers. Fish under stress usually have less glycogen in their hepatocytes (Hinton & Lauren, 1990), but glycogenolysis can occur following an acute chemical stress (Camargo & Martinez, 2007). Fish at Boegoeberg had the most glycogen-like vacuolation.

Fatlike vacuolation is the accumulation of excess fat in a cell (Van Dyk *et al*., 2007). Steatosis (a form of fat vacuolation) is when fatlike vacuoles occupy the hepatocytes and push the nuclei to the periphery of the cell (Van Dyk *et al*., 2011) (Figures 19 & 20). Micro-vesicular steatosis refers to many small vacuoles that fill the hepatocytes, and macro-vesicular steatosis refers to one or two large vacuoles. The occurrence of fatty vacuoles in the livers of *Clarias gariepinus* are thought to be normal, but the amount and severity of this alteration is more at polluted sites (Wolf & Wolfe, 2005; Van Dyk *et al*., 2009a; Van Dyk *et al*., 2011). The accumulation of fat in the livers could be from lipid peroxidation (Wolf & Wolfe, 2005), but also toxicant induced.

Focal areas of change were found in one fish (female) from Rooipoort, and two fish (both female) livers from Standerton. The Rooipoort liver had a focus of clear hepatocytes between dominant granular hepatocytes and a focus of macrovesicular steatosis (Table 9). Clear cell foci were seen in one of the fish from Standerton, in addition to an eosinophillic focus (Figure 21). The other fish had foci of vacuolation (micro- and macrovesicular steatosis) (Table 9) and foci of clear, smooth, and eosinophillic cytoplasms. These foci also had different degrees of intercellular deposits and vacuolation (Figure 22).

Hyalinization was only seen in one fish (male) from Standerton (Figure 23). Hyalinization is the appearance of eosinophillc spherical droplets in the cells. It is often associated with degeneration of hepatocytes (Boornman *et al*., 1997; Wolf & Wolfe, 2005). The appearance of hyaline droplets can be due to the disruption of protein synthesis in the liver (Van Dyk *et al*., 2007). Progressive changes were seen in only one fish from Parys in the form of hypertrophy of the hepatocytes (Figure 22) (Table 9).

Table 9: Qualitative histology results for the livers of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

Histological alteration		**Boegoeberg** n = 10	**Rooipoort** n = 10	**Parys** n = 10	**Standerton** n = 10
Vacuolation	Glycogen-like vacuolation	6	2	-	4
	Fatty change vacuolation	1	-	1	1
	Microvesicular steatosis	-	-	-	1
	Macrovesicular steatosis	-	1	-	1
Perivascular inflammation		4	5	4	4
Hypertrophy		-	-	1	-
Melano-macrophage centres		6	9	10	8

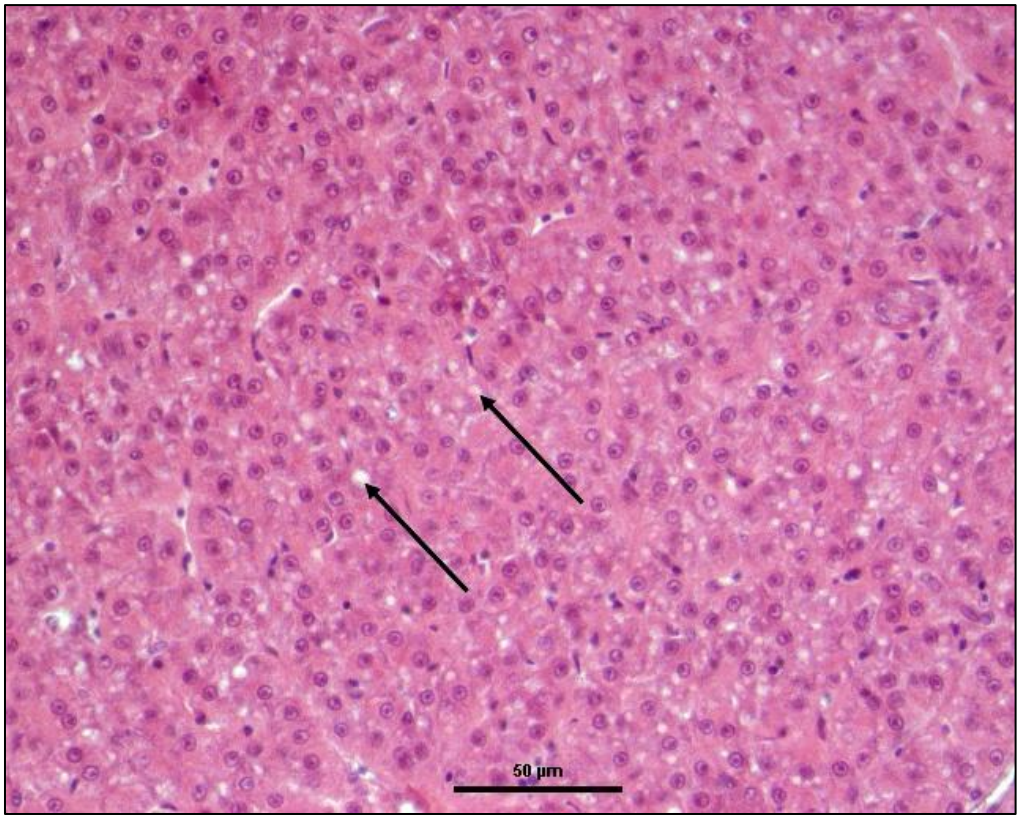

Figure 18: Glycogen-like vacuolation (arrows)in the liver of *Clarias gariepinus* (Boegoeberg, Rooipoort and Standerton)

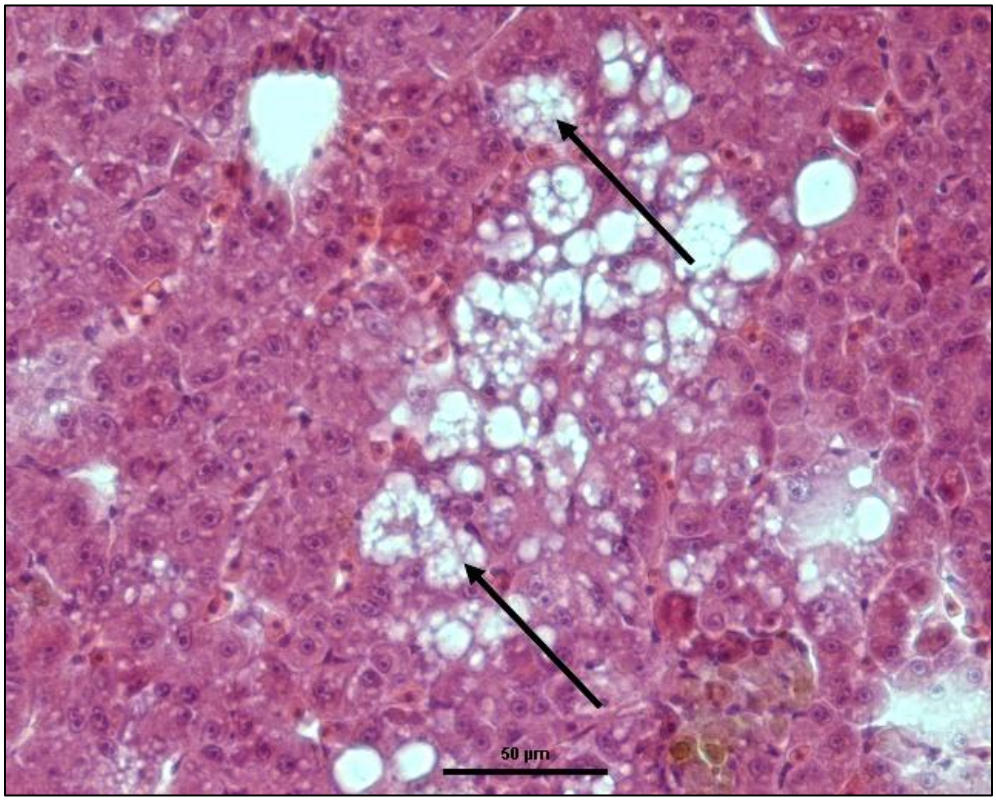

Figure 19: Microvesicular steatosis (arrows) in the liver of *Clarias gariepinus* (Standerton)

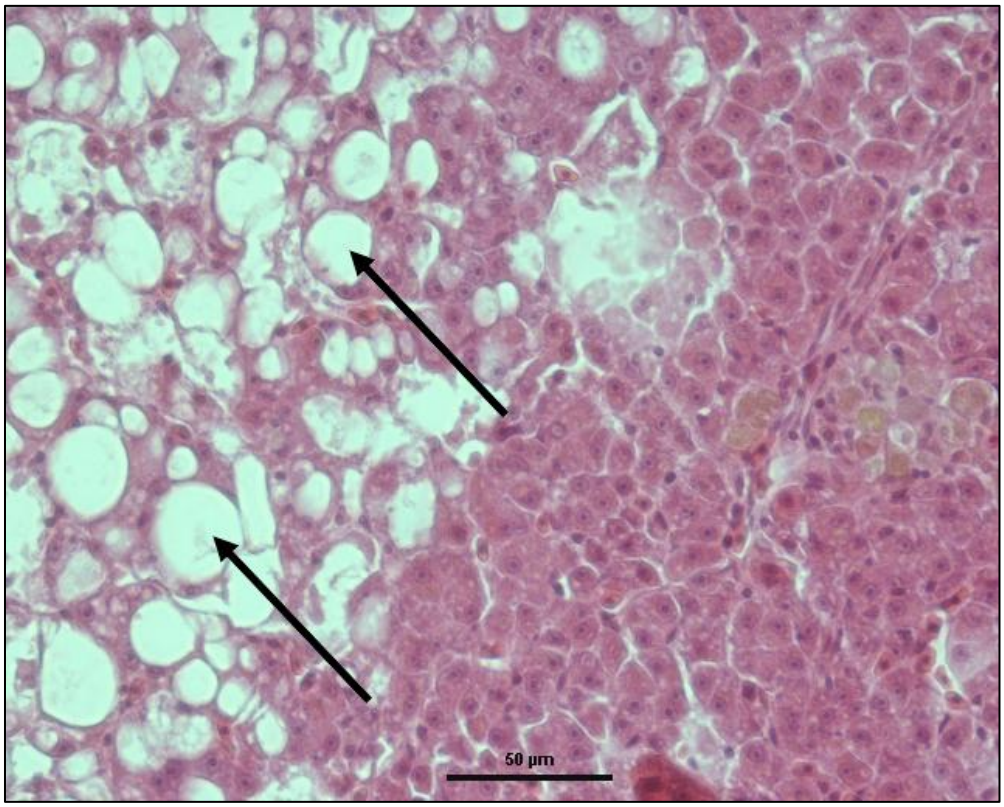

Figure 20: Macrovesicular steatosis (arrows) in the liver of *Clarias gariepinus* (Standerton and Rooipoort)

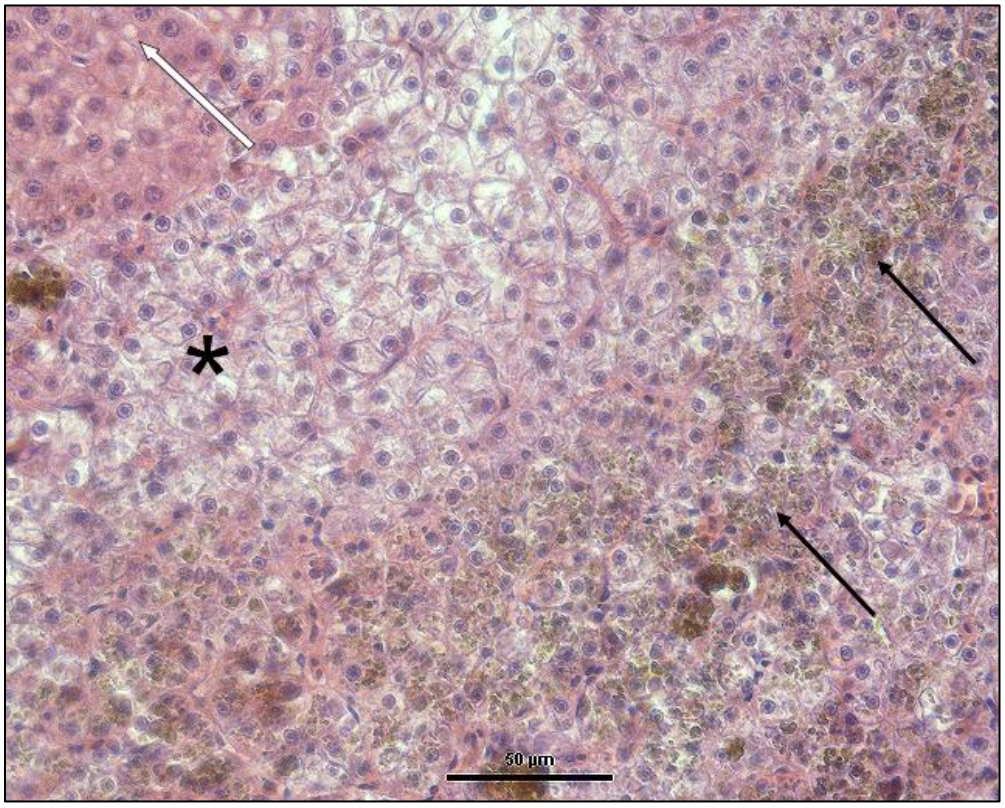

Figure 21: Foci of clear granular hepatocytes (asterix), intercellular deposits (black arrows) and vacuolated eosinophillic hepatocytes, in the liver of *Clarias gariepinus* (female fish from Standerton)

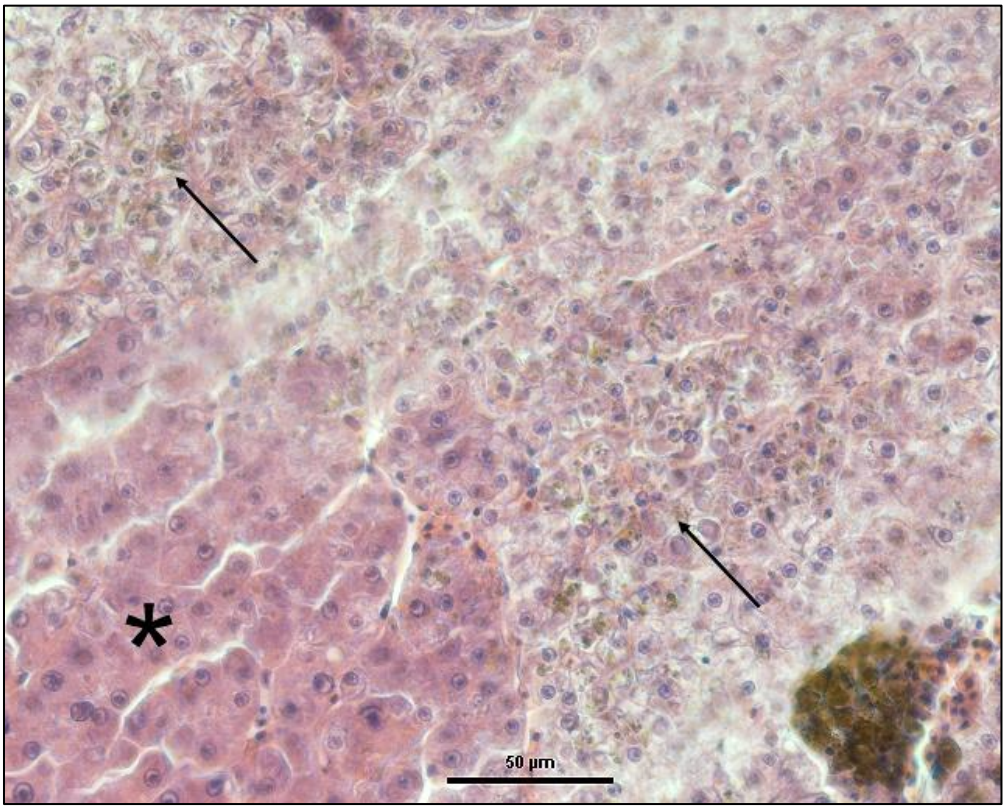

Figure 22: Foci of clear granular hepatocytes with intercellular deposits (arrows) and hypertrophy of eosinophillic hepatocytes (arrows), in the liver of *Clarias gariepinus* (female fish from Standerton)

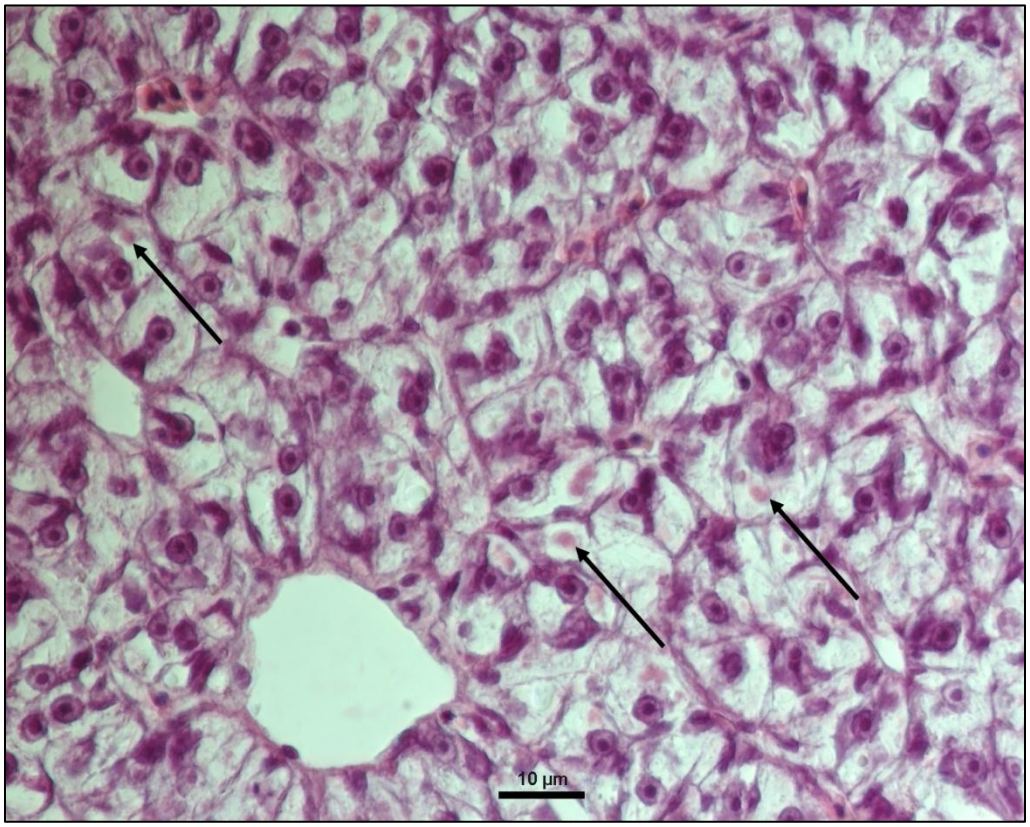

Figure 23: Hyalinization of hepatocytes (arrows) in the liver of *Clarias gariepinus* (male fish from Standerton)

Kidney

Non-pathological alterations

Melano-macrophage centres occurred in the kidneys (93% of the kidneys). These MMCs were predominantly medium sized and sparsely distributed throughout the kidneys. One fish each from Standerton (female), Parys (male) and Rooipoort (male) had perivascular adipose tissue (Table 10). Another fish from Parys had a corpuscle of Stannius, which is an enclosed area of endocrine tissue that excretes the glycoprotein teleocalcin, that regulate calcium concentrations (Groman, 1986) (Table 10).

Pathological alterations

The pathological alteration that was most frequently observed in the kidneys was hyaline droplet degeneration (Figure 24) and to a lesser extent, vacuolation of the renal tubules (Table 10). Hyaline droplet degeneration is a result of loss of plasma protein due to glomerular filtration disorders, which leads to the reabsorption by the tubular epithelial cells. These reabsorbed proteins appear as hyaline intracellular droplets (Van Dyk *et al*., 2009a).

Table 10: Qualitative histology results for the kidneys of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

Histological alteration	**Boegoeberg** n = 10	**Rooipoort** n = 10	**Parys** n = 10	**Standerton** n = 10
Hyaline droplet degeneration	2	7	5	3
Vacuolation of renal tubules	1	-	-	-
Adipose tissue	-	1	1	1
Corpuscle of Stannius	-	-	1	-

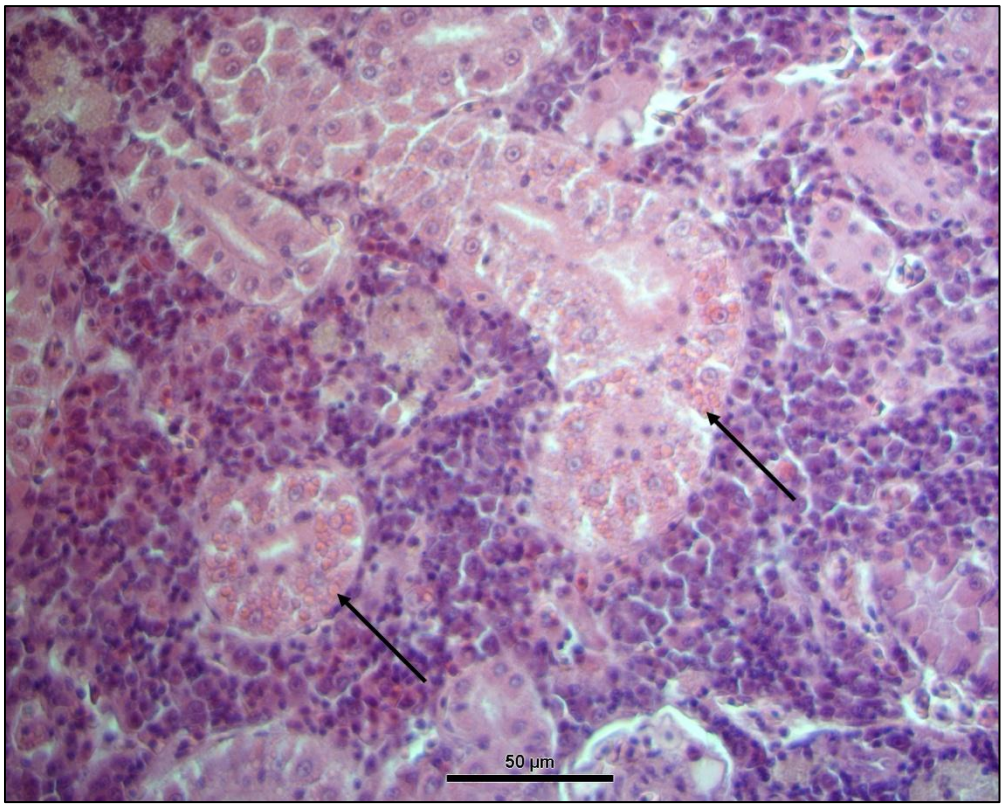

Figure 24: Hyaline droplet degeneration of the renal tubules (arrows) in the kidney of *Clarias gariepinus* (all sites)

4.5.2 Semi-quantitative histological results.

The semi-quantitative histological assessment showed that the mean organ indices of the sites fell within the requirements of class 1 (normal tissue structure with slight histological alterations) and class 2 (normal tissue structure with moderate histological alterations) (Table 11) (cf. Materials and Methods section 3.5.2). The mean gill index (I_G) values for all the sites were in class 1. Fish from Standerton and Rooipoort had the highest I_G value (not statistical significant). The mean liver index (I_L) values for all four sites fell in class 1. Standerton fish had the highest value and was significantly greater than those from Parys, which had the lowest I_L value. All the mean kidney index (I_K) values also fell into class 1. Boegoeberg fish was significantly smaller ($p = 0.023$) than Rooipoort, which had the greatest value for I_K. For all the sites, the mean fish index (I_{Fish}) fell into class 2. Standerton had the highest I_{Fish} value, followed by Rooipoort ($p = 0.806$), Boegoeberg ($p = 0.133$) and then Parys ($p = 0.77$), none of the sites was statistical significantly different from one another.

Table 11: Mean organ indices and medians for semi-quantitative histology of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

	Boegoeberg n = 10	**Rooipoort** n = 10	**Parys** n = 10	**Standerton** n = 10
I_G	3.6 ± 2.07	5.2 ± 3.16	3.8 ± 3.05	5.2 ± 4.13
Median	4	6	3	6
I_L	5.2 ± 4.44	5.2 ± 3.01	3.4 ± 1.65	9 ± 7.13
Median	5	4	3	7
I_K	2.6 ± 1.65	4.6 ± 2.12	3.6 ± 2.63	3.2±2.15
Median	2	5	4	2
I_{Fish}	11.4 ± 5.08	15 ± 5.44	10.8 ± 4.92	17.4 ± 8.54
Median	11	18	11	14

4.6 Limited human health risk assessment

For the human health assessment, four scenarios were chosen to show the risk of the consumption of *Clarias gariepinus* at the sample sites (Table 12). The POPs health risk assessment was very low and is therefore not reported. Only the heavy metals that showed risk are presented shown

Table 12: Scenarios for human health risk calculations for the consumption of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

Scenario 1	**Scenario 2**
85 kg adult eating 400 g fish/day for 10 years	40 kg child eating 400 g fish/day for 10 years
Scenario 3	**Scenario 4**
85 kg adult eating 400 g fish/day for 5 years	40 kg child eating 400 g fish/day for 5 years

(adapted from Du Preez *et al*., 2002; Marchand, 2009)

Hazard index (HI) calculations were categorised using the following guidelines. A HI value smaller than 0.1 shows no hazard and a value between 0.1 and 1 has a low hazard risk. If the HI is between 1.1 and 10 there is a moderate hazard risk and if it is greater than 10 the hazard is high (Lemly, 1996).

Boegoeberg possessed the highest HI values, followed by Parys, Rooipoort and then Standerton (Figure 25). The relative HI for the elements at each of the sites, showed a similar pattern. It is the exact value that differed from site to site. In every site the HI-value showed a decrease of Ag Sc2 > Hg Sc2 > Ag Sc1 & Sc4> HgSc1 & Sc4> Ag Sc3> Hg Sc3 > As Sc2 > As Sc1 & Sc4 > Cr Sc 2 > As Sc3 > Cr Sc1 & Sc4 > Cr Sc3. Silver and Hg had the highest HI independent of the site or scenario (Sc). Arsenic and Cr followed the pattern set by Ag and Hg. Selenium was not included in this pattern because of its very small HI-value in most instances. The scenario with the highest HI-value, was Sc2, followed by Sc1 and Sc4 (which were equal) and then the lowest, Sc3 (Figure 25).

The element with the smallest hazard, was Se. It had a low hazard only at two sites, Parys and Boegoeberg, and then only for Sc1 and Sc3. The HI values of selenium for Sc1, Sc3 and Sc4 showed a low hazardous risk for all the sites. Selenium also showed low risk at Rooipoort and Standerton for Sc2, while Boegoeberg and Parys showed moderate hazardous risk (Figure 25). Chromium had low risk values at all the sites for Sc3. Sc1, Sc2 and Sc4 had moderate risk for chromium. Arsenic had moderate risk at all sites for all four scenarios (Figure 25). Mercury and Ag also showed moderate risk at all the sites for Sc3 and at Standerton for Sc1 and Sc4. Mercury also had moderate risk at Parys and Rooipoort for Sc1 and Sc4 (Figure 25). Boegoeberg showed high hazardous risk in terms of Hg HI values for Sc1, Sc3 and Sc4. Sc2 had high hazardous risk for both Hg and Ag, for all the sites (Figure 25). Silver poses a high risk at Boegoeberg, Rooipoort and Parys for Sc1 and Sc4 (Figure 25).

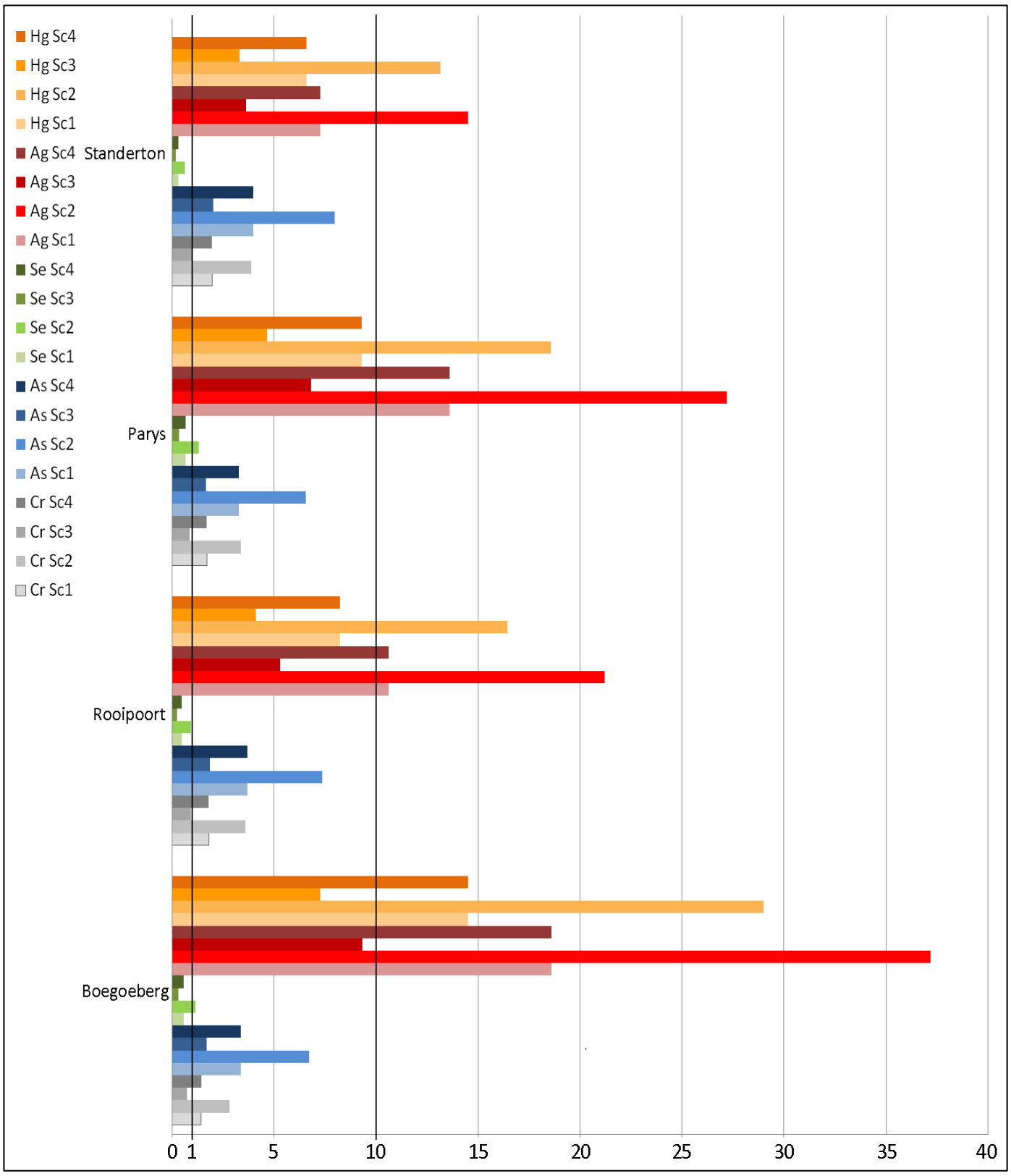

Figure 25: Heavy metal hazard index results for human consumption of *Clarias gariepinus* from sites in the Orange- and Vaal Rivers

5 Discussion and Interpretation

5.1 Introduction

In this chapter the results of the sediment analysis and indices (5.2.1), as well as the fish chemical analysis (5.2.2) will be discussed and the histological alterations in the fish (5.3), and then the possible risk they have on human health (5.4). The sediment is the abiotic matrix of the environment that was investigated. Sediment is a useful matrix because it serves as a trap for pollutants (Saha *et al*., 2001; Praveena *et al*., 2008; Öztürk *et al*., 2009; Varol, 2011) and is therefore an indicator of recent and current pollution. Due to bio-magnification the fish sampled represent the biota living within this environment and contaminants in the fish would be an indication of how much of the environmental pollution accumulated in the fish which occurs at the top of the aquatic food chain. In addition to knowing the levels of the pollutants in the fish, the alterations that these pollutants might have caused in the target organs were studied through histology. A limited human health risk assessment was also performed to investigate what the possible risk, if any, there would be on human health, should they consume these fish.

The heavy metals showed good quantifiable results, whereas the POPs data was mostly below the limit of detection (Table 1 and 2). The discussion therefore focuses on the heavy metals and reference is made to the POPs in only one or two instances, where the POPs levels are above the limit of detection.

5.2 Sediment- and fish chemical analysis

5.2.1 Sediment chemical analysis and indices

South Africa does not currently have sediment quality guidelines, so this presents a challenge for interpretation. For this reason international guidelines were selected for comparison (Table 13). The concentrations of the metals in the sediment were used to calculate various indices to compare sample sites taking natural geology into consideration.

The two elements with the highest concentrations at all four sediment sites were Fe and Mn (Table 3) and this might be due to the geology of the areas. This assumption is supported by the low enrichment factors for these elements: In three of the sites the Ef were in the unpolluted category and at Parys, in the unpolluted to slightly polluted category (Table 4). Boegoeberg is located in the Namaqua-Natal geological Supergroup, which consists of gneiss and pegmatite. These rocks have high levels of Fe and to a lesser extent Mn (McCarthy & Rubige, 2005) explaining why both Fe and Mn did not show any enrichment (due to anthropogenic activity) (Table 4). Rooipoort is situated in the Transvaal Supergroup geological area that mainly

consists of dolomite and chert rock types (McCarthy & Rubige, 2005). The minerals in these rocks are richer in Mn than in Fe, which was reflected in the levels for the sediment of this site (Table 3). Parys and Standerton lies in the Karoo Supergroup (sandstone, shale and basalt), which has naturally high Fe and Mn levels (McCarthy & Rubige, 2005), which is also seen in the data (Table 3). The levels of Mn and Fe were so high compared to that of the other elements that they were measured in g/kg while the rest were in mg/kg.

Silver, Au, Se, Cd, and Hg had low levels in the sediment when compared to the other elements (Table 4), but played important roles in the indices. The enrichment factor of Ag was high at all the sites and showed "extreme anthropogenic enrichment" (Table 4). This shows that the Ag levels found in the sediment have the potential to be a pollutant which was proven by its Igeo-values which varied between "highly polluted" and "very highly polluted" at the different sites (Figure 4). Although Parys had the highest Ag Ef its corresponding Igeo value was second highest compared to those of other sites (Figure 4). Rooipoort however had the second highest enrichment factor for Ag, but had the highest Igeo value (6.8) (Figure 4), classifying that site as "very highly polluted". Silver can enter a system from wastewater treatment plants in sewage effluent (Ratte, 1999), and from the silver plating- and photographic industries (Ratte, 1999; Dallas & Day, 2004).

Selenium had high enrichment factors at all four sites. The sites at Rooipoort, Parys and Standerton had their enrichment categorised as "extremely severe" and Boegoeberg as "very severe enrichment" with the Ef value at Parys was as much as four times higher than any of the other sites (Table 4). The Igeo shows that Parys, Rooipoort and Standerton are "very highly polluted" by this element (Figure 4), but for Boegoeberg only "moderately to highly polluted". Although Parys had the highest Ef value for Se, the highest Igeo value was calculated for Standerton. Selenium is a common pollutant from industry including steel works, food processing plants and motor vehicle plating (Dallas & Day, 2004) which might explain its high Ef value at Parys, which is downstream of the industrial hub of Gauteng.

Cadmium calculated an extreme enrichment at Boegoeberg and Parys (Table 4), but the corresponding Igeo values showed that these enrichments have no pollution potential, scoring "unpolluted" (Figure 4). Boegoeberg and Parys both had a "severe enrichment" of Au. The Igeo for Au showed that Boegoeberg had "very high pollution" from Au but Parys only showed "moderate pollution" (Figure 4). It was expected that the sites upstream from Boegoeberg and Rooipoort would have higher pollution values for Au, as the gold mining regions of the Witwatersrand is closer to the Parys and Standerton sites on the Vaal River (AngloGold-Ashanti, 2006).

Mercury had a low Ef of all the sites, indicating “natural enrichment” (Table 4), but the Igeo showed the opposite. The Igeo indicated that the Hg level at Boegoeberg is “moderately to severely polluting” that site (Figure 4). The Igeo for Hg increased moving downstream, behaving similarly to Au.

Rooipoort was the only site that had a high Ef value for uranium. The rest of the sites scored “natural enrichment” (Table 4). The “severe enrichment” score (Table 4) for Rooipoort corresponds with the high Igeo value calculated for this site (Figure 4). The source of the U that calculated the “moderately polluted” Igeo value, can most probably be from the mines from the uranium rich areas in the North-West Province (US DOI, 2007) upstream from Rooipoort, or from the uranium mines of the Northern Cape (Rooiland, 2011).

From the indices values for Ag, Au, Hg, and Se, these four elements individually played a large role in the pollution in the river system, especially at the Rooipoort and Standerton sites.

The conflicting information presented by the Ef and its corresponding Igeo might be explained by using Fe as the normaliser in the calculation of the Ef. If a different element were selected as normaliser, the Ef might have looked differently. In general, however, if an element had a high Ef value, it would predict a high Igeo value. Cadmium was one exception: it showed extreme enrichment, but its Igeo categorised as “unpolluted”. Mercury was another exception: it showed no Ef but high Igeo.

A factor contributing to the limited validity of both the Ef and Igeo indices is the fact that both uses a background value for the element, in other words, a concentration of the elements due to its natural occurrence. Since these values are lacking for most parts of South Africa (and for most of the world) the background values used in these calculations were that of the “internationally” calculated values for the earth’s upper crust (Nash & Gries, 1995; Ruiz, 2001; Praveena *et al*., 2008; Roychoudhury & Strake, 2009; Rogan Šmuc *et al*., 2009; Mohuiddin *et al*., 2010; Varol, 2011). It is therefore possible, that once the true background values at each of the study sites become available, the Ef and Igeo would change.

Biota are usually exposed to a mixture of pollutants and it is therefore necessary to also learn the possible pollution of the total mixture. The mixture effect was calculated with indices like the MPI and PLI. Both these indices uses the geometric means of the concentrations of the elements, but the PLI’s concentrations are in terms of their background values (cf. Materials and Methods section 3.6). This means, again, that the PLI has an uncertainty built in. In spite of this uncertainty, the MPI and PLI results showed the same pattern between the sites, i.e. Rooipoort > Standerton > Boegoeberg > Parys (Figure 5). For this reason only the MPI results

is discussed further. The high MPI value of Rooipoort corresponds with the results found with the previously discussed indices. Rooipoort had predominantly the highest Igeo values of all the sites that indicated pollution from individual elements.

Rooipoort is also the most downstream site in the Vaal River that could explain why it would have the highest MPI of the three sites in the Vaal River, i.e. if one assumes all upstream pollution is carried downstream and there is no dilution or breakdown/metabolisation of the contaminants. This assumption of downstream transport of pollution does not hold for the Standerton site that had a higher MPI than Parys. Standerton, which is the farthest site upstream, was expected to be the least polluted but first its Igeo values and then its MPI value shows high pollution. Parys had the lowest MPI of all the sites (Figure 5). Because Parys is downstream of Gauteng, it was expected to be more polluted than Standerton, but the Vaal River has two large impoundments between these two sites. The Vaal Dam and Vaal Barrage act as massive silt and sediment traps (Chutter, 1967; Chutter, 1969) and possibly because of this, the pollution will peak at the Barrage and a decrease at downstream sites will be seen (van Streenderen *et al*., 1987). Igeo values were calculated using metal levels measured at the Vaal Barrage and Vaal Dam by Groenewald (2000). The Igeo values were higher at the Barrage than at the Vaal Dam, supporting the downstream transport theory.

Boegoeberg had the second lowest MPI (Figure 5), which might be caused by the dilution from the waters of the Orange River that joined upstream from this site, provided that its pollution load is smaller. This dilution effect was noted by Chutter (1963), where the water from the Vaal River Barrage diluted the incoming pollutants from the Suikerbosrand- and Klip Rivers. A similar dilution effect was reported by Totten and co-authors (1969) between the Ottawa- and Auglaize Rivers in Ohio, USA. The industrial pollution in the Ottawa River was diluted after its confluence with the Auglaize River (Totten *et al.,* 1969).

The sediment quality index showed the quality of the sediment in terms of whether the measured levels exceeded their guideline levels and with how much the guideline was exceeded (cf. Materials and Methods section 3.6). As with its high Ef, Igeo and MPI, the Rooipoort site had the worst sediment quality (Figure 6). The SQI scored Rooipoort as “poor” who according to Marvin *et al* (2003) means that the sediment quality is almost always impaired because most measurements exceed their guideline levels and do so with considerable amount. This site had eight elements exceeding the guideline levels: Ni, Se, Hg, Cr, Mn, Cu, As, and U levels compared to the seven (Cr, Mn, Co, Ni, Cu, Se, and Hg) of Standerton (Table 13). The amount of exceedance of Rooipoort’s elements was also more than that of Standerton’s

elements (Table 13) and therefore its SQI was categorised as “marginal quality” (Figure 6). The sediment quality scores are supported by the results obtained for the Ef, Igeo and MPI.

The Boegoeberg and Parys sites both had the best sediment quality scores with a grading of “fair” (Figure 6), which meant that the sediment quality is only occasionally impaired by deviating measurements (Marvin *et al*., 2003). The elements that exceeded the guidelines at Boegoeberg were Hg, Ni, Cu, and Se. At the Parys site Cr, Ni, Cu, Se, and Hg exceeded the guidelines (Table 13).

Of the selected study sites in the Vaal River, Rooipoort is the furthest downstream. Again the downstream transport of pollution, can concentrate the metals at the site. The SQI at Parys could be affected by the trapping of pollutants at the Vaal Barrage and thus Standerton has a higher SQI.

Table 13: Selected sediment quality guidelines and the metals that exceed their guidelines

Element	**Threshold value** (mg/kg)	**Boegoeberg** (mg/kg)	**Rooipoort** (mg/kg)	**Parys** (mg/kg)	**Standerton** (mg/kg)
Cr	26 [a]	19.8	83.8	35	87.5
Mn	460 [a]	210	750	300	725
Co	20 [b]	9.8	20	10.8	23.3
Ni	18 [c]	33.8	101.25	37.5	75
Cu	16 [a]	20.1	36.25	18	42.5
Zn	123 [b]	31.25	53.75	32.5	52.5
As	5.9 [b]	2.9	6.25	2.75	4.25
Se	0.083 [d]	1.7	11.5	10.75	11.75
Cd	0.569 [b]	0.06	0.1225	0.045	0.0775
Hg	0.174 [b]	0.9125	0.5	0.35	0.25
Pb	35 [b]	4.625	10.125	3.75	14.25
U	2.5 [e]	0.3875	25.5	0.375	1.1

Guidelines: [a] NZ-Australia (ANZECC, 2000), [b] Netherlands (Friday, 1998), [c] Canada (Friday, 1998), [d] Hamilton, 2004 and [e] Sheppard *et al*., 2005.

Silver does not have sediment quality guidelines and therefore could not be included in these calculations, but due to its high Igeo value it can be speculated that if Ag had sediment quality guidelines, the SQI value at each of the sites would deteriorate.

The ecological risk at the sites were calculated using the sediment quality guideline index (SQG-I). The probability for sediment to be toxic to biota is the arithmetic mean of the ratio between the measured levels and their guideline levels. The SQG-I for Rooipoort had a high probability for biota toxicity (Figure 7). The previously calculated indices, Ef, Igeo, MPI and SQI all predicted that the SQG-I of Rooipoort should be the highest of all the sites. This high toxic probability is due to Ag (Igeo) (Figure 4), Ni, Se, Hg, Cr, Mn, Cu, As, and U (Table 13). The SQG-I for the other sites showed moderate probability for biota toxicity. Standerton had the second highest index value. This again coincides with the findings of the previous indices. Cr, Mn, Co, Ni, Cu, Se, and Hg would be the elements contributing to the toxic effect of the sediment at Standerton. Boegoeberg and Parys had the lowest index values (Figure 7), and on the moderate probability of toxic effects, the effects would be from Au, Ag (Figure 4), Hg, Ni, Cu, and Se (Table 13). Again the reason why Parys had a low SQG-I (and SQI for that matter) compared to Standerton, could be due to the trapping effect the Vaal Barrage and Vaal Dam have on the sediment as previously explained.

5.2.2 Fish chemical analysis and bio-accumulation factor

The levels of pollutants in the abiotic matrices measured are not necessarily bio-available to the biota. One of the methods to determine if the biota are not in osmotic equilibrium with the pollutants in the environment is to determine the bio-accumulation (BF) of these pollutants in the biota. In other words, how many times the pollutant levels in the biota are higher than in the surrounding environment. In aquatic environments the abiotic matrices where pollutants can be found, are in the sediment and water. There are different pathways through which pollutants can enter the biota. They can be absorbed through the skin and gills or can be ingested with food. *Clarias gariepinus* hunts and feeds mainly on the bottom of rivers and dams, in the sediment (Skelton, 2001). In this study, pollutants in the sediment only were analysed and therefore the BF only shows accumulation from the sediment at each site.

Ten fish (*Clarias gariepinus)* were caught at every site. Heavy metal analysis was possible for each fish however, due to the high cost of POPs sample analysis fish were pooled to generate the data for POPs. Male and female fish for every site were pooled separately, but there was no significant difference between the genders ($p = 0.751$).

POPs were quantified for the fish of mainly Parys (PCDFs, PCBs, PBDEs, PFOS and low level DDT-degradation products) and Rooipoort (few PCBs and DDT-degradation products). The female fish from Parys were the only fish that had accumulated levels of PCDFs and PCBs (Table 14). PBDEs were quantifiable only at Parys and the female fish had higher levels of this pollutant accumulated than the males (Table 14). Even though there was no significant

differences between the genders, female fish appear to have a naturally higher lipid content (Anthony *et al*., 2000), explaining the higher levels of lipophillic substances detected. Parys is downstream from the major industrial area in South-Africa which could be the origin of the PCBs, PCDFs and PBDEs measured. POPs are often bio-magnified in apex predators, which ingested contaminated food (Kidd *et al*., 2001; Bornman *et al*., 2007).

Although the catfish is one of the aquatic apex predators in the Orange-Vaal River system the levels of the industrial POPs were very low and mostly undetectable (Table 6) compared to that found for other freshwater fishes in international studies: Levels of PCBs and PCDFs as well as PBDEs were found in predatory fish e.g. PCBs and PCDFS in arctic char (*Salvelinus alpinus*) (2.7 ng/kg) (Letcher *et al*., 2010); wolf fish (*Hoplias malabricus*) (1,32-7.6 ng/kg) (Miranda *et al*., 2008); African catfish species (0.4-2.7 ng/kg) (Adu-Kumi *et al*., 2010); and PBDEs in brook- (*Salvelinus fontinalis*); brown- (*Salmo trutta fario*); lake trout (*Salvelinus namaycush*) (0.01-0.1 ng/kg) (Schmid *et al*., 2007).

No detectable levels of OC pesticides except for DDT degradation products were found in the fish: *p,p*'-DDE, was found at all the sites and *p,p*'-DDD only at Rooipoort and Parys (Table 7). Of all the sites where *p,p*'-DDE and *p,p*'-DDD were found, it accumulated in the fish of Rooipoort only (Table 14). The presence of *p,p*'-DDD and *p,p*'-DDE, shows historic use of DDT in the area. A high DDE/DDT ratio indicates old DDT inputs and that most of the DDT used, has degraded into *p,p*'-DDE and *p,p*'-DDD (Strandberg & Hites, 2001). Rooipoort is situated in an agricultural rich area, where DDT most probably was used before it was banned for agricultural use in 1976 (Bouwman, 2004). Areas in South Africa (Limpopo Province and northern Kwa-Zulu Natal) however still use DDT as malaria vector control (Sereda & Meinhardt, 2005; Urbach, 2007) and DDT and it's degradation products are present in the biota of these areas (Barnhoorn *et al*., 2009). DDT degradation products were found in catfish (*Clarias* spp and *Heterobranchus* spp) (0.02-0.04 ng/kg) and tilapia (*Tilapia zilli*) (0.01-0.07 ng/kg) of Ghana and represent the technical use of the DDT during last decade (Adu-Kumi *et al*., 2010). Tigerfish tissue from Pongolapoort Dam showed higher levels of *p,p*'-DDE (0.3 ng/kg) than DDT (0.07 ng/kg) (McHugh *et al*., 2011). Pongolopoort Dam is in the malaria zone, and DDT is sprayed as vector control (Sereda & Meinhardt, 2005). The higher DDE levels (along with the presecnce of DDT) indicate the frequent and long-term use of DDT in the Pongolapoort Dam area.

The upstream sites of the study (Standerton and Parys) measured more PFOS in the fish than downstream: Standerton male > Parys females > Standerton female> Parys male, Rooipoort male and female, and Boegoeberg female (Table 7). Consequently the bio-accumulation

patterns were the same where the Standerton fish and the females of Parys had the highest PFOS accumulation of all the sites (Table 14). PFOS is part of the perfluorinated compounds (PFCs), characterised by a lipophillic linear carbon chain, completely fluorinated and attached to a hydrophilic head. These properties ensure chemical- and thermal stability and a low surface energy (Völkel *et al*., 2008). Because of these properties, PFCs are used in industrial surfactants, emulsifiers and wetting agents (Post *et al*., 2012). Surfactants are agents that reduce the surface tension and increase the wetting properties and spreading of liquids. Surfactants are used in coal based thermal power station to reduce the drag of fly ash slurry in the disposal pipelines (Naik *et al*., 2009). An increase in PFOS levels was seen in the Ganges Rivers water by Yeung *et al.* (2009), at the Yamuna River confluence. The Yamuna River is one of the most severely polluted rivers in the world and flows past an industrialised area, receiving discharge from coal based thermal power plants, textile manufacturing plants, and tanneries (Yeung *et al*., 2009). Mpumalanga has seven major coal based power stations, three of which is close to Standerton (Figure 26) (Eskom, 2012 a; b; c; d). The Camden power station is situated outside Ermelo (Eskom, 2012e), and is close to the origin of the Vaal River (Braune & Rogers, 1987). The Majuba power station is between Amersfoort and Volksrust (Eskom, 2012f) and is 36 km South-East from Standerton fishing site and the Tutuka power station is 19 km North West, close to the Grootdraai Dam (Eskom, 2012g).

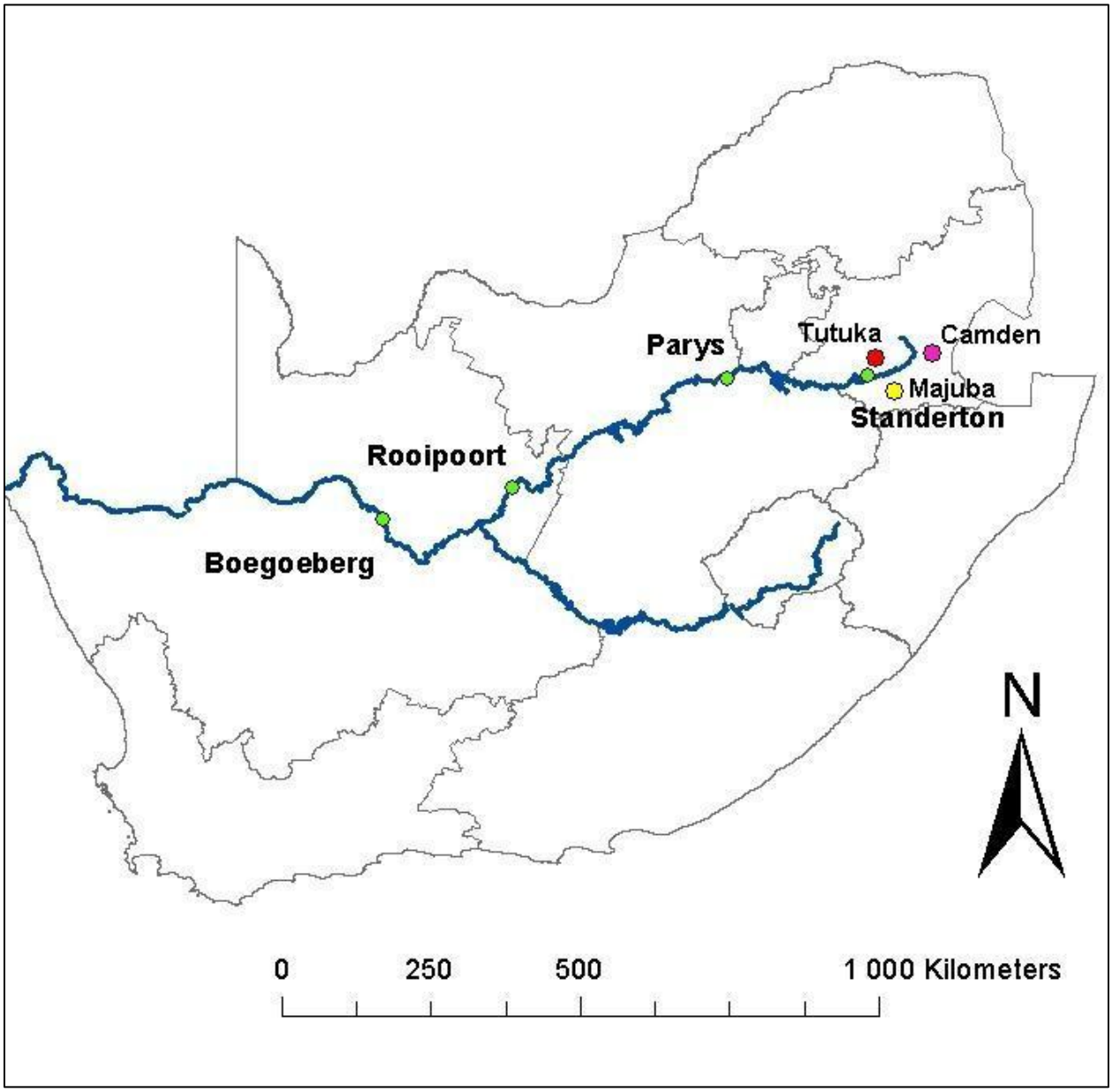

Figure 26: Map of South Africa indicating three coal based power stations near the Standerton sample site

Most of these power stations have effluent entering Vaal River tributaries up-stream of the Standerton sampling site. It is possible that the surfactants used in these South African coal based thermal power stations could be of PFCs origin and since these compounds were listed in the Stockholm Convention only in 2009 there are possibly high levels present in the South African environment. However, this needs further investigation and confirmation.

There is currently no evidence available that PFCs can be metabolised in living organisms (Völkel *et al*., 2008). This could possibly explain why we found PFOS in the fish, even if the power stations are aware of PFC restrictions and abandon use of the PFOS containing products – if this in fact what they had been using until recently. In a study by Giesy & Kannan (2001),

PFOS was found to bio-accumulate and bio-magnify in predators. The levels of PFOS detected in lake whitefish (0.13 ng/kg) and Chinook salmon (0.11 ng/kg), were well below the levels found in *Clarias gariepinus* in the present study (2.6 ng/kg) (Table 7). The levels in these North American fish were approximately 20 times lower than the catfish levels. This is cause for concern, firstly because of the high PFOS levels found in the Orange Vaal system fish. Secondly, the levels in other South African systems and fish are mostly unknown.

Table 14: Bio-accumulation factors (BF) of POPs in *Clarias gariepinus* from sites in the Orange- and Vaal Rivers. Factors higher than 1.5 are highlighted

	Boegoeberg		Rooipoort		Parys		Standerton	
	♂ n = 5	♀ n = 5	♂ n = 7	♀ n = 3	♂ n = 4	♀ n = 6	♂ n = 5	♀ n = 5
ΣTEQs (ng/kg)	0.7	0.7	0.7	0.7	1.2	4.3	0.6	0.6
ΣPBDE (ng/kg)	1.0	1.0	1.0	1.0	1.6	2.5	1.0	1.0
ΣPFOS (ng/kg)	0.5	2.0	2.0	2.0	2.0	3.4	5.2	2.4
Σpesticides (ng/kg)	0.7	0.8	2.2	1.5	0.9	1.3	0.7	0.8

The heavy metals measured in the fish were all below the quality guidelines set up by the European Union (EU, 2006) and as suggested by Wagner & Boman (2003) for human consumption (See Appendix Table 19 for quality guidelines values). The highest heavy metals measured in the fish were Ag > Fe > Zn > Cr.

The fish from Parys had the highest Fe and Zn levels followed by Boegoeberg (Figure 13 & 15). This was not the case for the sediment (Table 3). Fe did not accumulate in the fish (Table 15). Although the Igeo of Zn suggests that it is not causing pollution (Figure 4), there was enrichment at Parys (Table 4) therefore there are levels of Zn present that can enter the fish. The Zn measured in the fish, would most probably be metabolised (facilitated by metallothioneins) (Newman, 2010) and excreted because this metal did not accumulate according to the BF (Table 15).

The levels of Cr were the highest in fish from Standerton and then Parys (Figure 16). This was also seen in the sediment of these two sites (Table 3). The enrichment of Cr at these sites ("moderate" and "moderately severe") (Table 4) suggests that there is Cr available in the sediment for uptake by biota. Although Cr was detected in the tissue it appear not to have accumulated (Table 15).

Similar levels of Zn (16-29 mg/kg), Cu (0.8-2.4 mg/kg), Cd (0.03-0.5 mg/kg), Cr (0.18-0.7 mg/kg) and Pb (0.1-1 mg/kg) to that found in the present study were measured by Nnaji *et al.* (2007) in *Oreochromis niloticus* and *Synodontis schall* from the Galma River in Nigeria, which is polluted

by agriculture and industry. According to Skelton (2001), the *Synodontis* spp are bottom feeders, occupying the same niche as *C. gariepinus*. In South Africa, Van Aardt & Erdmann (2004), measured levels of Cd (3 µg/g), Pb (5 µg/g), Cu (15 µg/g) and Zn (50 µg/g) in *Labeo capensis*, from impoundments in the Mooi River in the North-West Province affected by mining activity. The levels measured by van Aardt & Erdmann (2004) in *Labeo capensis*, are similar to the catfish of this study, but *L. capensis* is herbivorous and detrivorous, thus lower on the food chain than catfish. It can be expected that the catfish from the impoundments investigated by Van Aardt & Erdmann (2004), would have even greater levels, due to bio-magnification, as *L. capensis* are prey of *C. gariepinus.*

The Ag in the fish tissue was very high, compared to the other elements (Figure 13-15). At Boegoeberg especially, the fish had a high concentration of Ag in their tissue and was the only site that showed accumulation of Ag (Table 15). Parys had the second highest Ag levels in the fish, followed by Rooipoort and Standerton. This does not correspond to the sediment levels (Table 3) or the Igeo values for Ag (Figure 4). It would be expected that Rooipoort would have had the highest Ag in the fish, because of its high Igeo ("very highly polluted") (Figure 4). Yamakazi *et al*. (1996) measured similar high levels (to this study's sites) of Ag in the different tissues (entrails, scales, gills and muscle) of *Carasius auratus langsdorfii*, *Gnathopogon elongatus*, *Tribolodon hakonensis* and *Zacco platypus*. The organs with the highest Ag levels were entrails >gills >scales >muscle. If these other organs had been investigated in this study, perhaps levels similar to those detected by Yamakazi *et al* (1996) would have been measured. These fish were from the Asakawa River that receives municipal and domestic waste water from Tokyo. Silver is a by-product of Zn, Pb, Cu and precious- and semi-precious gem mining activities, which are abundant in the Northern Cape (Mbedi, 2012b). These mines could possibly be the source of the silver found in the fish of Boegoeberg.

Boegoeberg also showed accumulation for Au (Table16). The Ef showed that Au is "very severely" enriched (Table 4) and the Igeo indicated that Boegoeberg is "very highly" polluted (Figure 4) by Au, even though the sediment levels were low (Table 3). The levels of Au in the fish were also low for all the sites except at Boegoeberg, where the Au levels were notably higher than the rest (Figure 16). The low levels of Au in the sediment and the higher levels measured in the fish, indicated accumulation of Au in *C .gariepinus*. Even though the BF indicated that Au accumulated in the fish of Boegoeberg, the levels detected in the tissue were too low to raise any concern

Table 15: Bio-accumulation factors (BF) of heavy metals in *Clarias gariepinus* from sites in the Orange- and Vaal Rivers. Factors higher than 1.5 are highlighted

	Boegoeberg	Rooipoort	Parys	Standerton
Cr	0.013	0.0	0.0	0.0
Mn	0.0	0.0	0.0	0.0
Fe	0.0	0.0	0.0	0.0
Co	0.0	0.0	0.0	0.0
Ni	0.0	0.0	0.0	0.0
Cu	0.0	0.001	0.0	0.0
Zn	0.0	0.012	0.0	0.0
As	0.0	0.009	0.0	0.0
Se	0.1	0.010	0.0	0.0
Ag	1.7	0.286	1.2	0.5
Cd	0.0	0.018	0.0	0.0
Pt	0.1	0.023	0.1	0.033
Au	4	0.223	1.0	0.2
Hg	0.1	0.082	0.1	0.1
Pb	0.0	0.001	0.0	0.0
U	0.1	0.000	0.0	0.0

5.3 Histological assessment

The aim of the histology based assessment was to identify pathological alterations in the target organs, which could be attributed to pollutants, measured in both the biotic- and abiotic analysis. Anatomical and physiological change of fish is modified according to their environment and temperature regulation. These two major ecological factors have significant control in dictating the events that follow pathological changes (Roberts, 1989). These changes are adaptive responses, due to exposure to toxicants, which can disrupt the normal function of biochemical and/or physiological processes. The adaptive response can be so intense that it can lead to lowered survival (Roberts, 1989; Hinton & Lauren, 1990), depending on the severity of that change. Even though alterations found in the fish are not toxicant specific (Schwaiger, *et al*., 2007), histological changes have been associated with certain pollutant exposures, among these are DDT and its degradation products, PCBs and PCDFs, PFOS, and heavy metals, all of which were found in the fish tissue of this study. These pollutants' presence may be considered as factors that mediated histological changes.

The OC pesticides consist of chlorinated hydrocarbons (e.g. DDT) and cyclodienes (e.g. aldrin, dieldrin and endrin). These pesticides were inexpensive to produce and very effective in pest control, making them cost-effective to use (Dallas & Day, 2004), but their strong potential to

enter freshwater systems and their high aquatic toxicity caused concern and they were banned in many countries since 1970 (Stockholm Convention, 2011). The effects of OC pesticides have been studied in humans (Cheung *et al*., 2007; Tsiplakou *et al*., 2010; Gerić *et al*., 2012) and wildlife (Kidd *et al*., 2001; Zaroogian *et al*., 2001; Glaser & Connolloy, 2002; Tricklebank *et al*., 2002; Bouwman *et al*., 2008; Adu-Kumi *et al*., 2010).

The pathological effects of DDT on reproduction of fish are well documented, but information on the effects of DDT on the liver, kidney and gills is scanty (McHugh *et al*., 2011). However DDT and its degradation products inhibit osmoregulation in the gills by inhibiting activate Na and K ions in the gills. This results in the inhibition of Na and K activated ATPase, which down regulates osmoregulation (Evans, 1987; Geeraerts & Belpaire, 2010). The result of this is an increase in chloride cells in the gills to compensate for the decrease in ion regulation (Evans, 1987). Fish from Rooipoort and Boegoeberg had chloride cell hyperplasia (Table 7 & Figure 17), and had *p,p*'-DDD levels in the fish (Table 7).

DDT and its degradation products have been found to cause degeneration in the kidneys of fish (Ayas *et al*., 2007). Although these authors do not identify the kidney degeneration as "hylaline droplet degeneration" *per se* it is visible on their photomicrographs (Figure 2 in Ayas *et al*., 2007). Although hyaline droplet degeneration is a natural process, it increases with toxicant exposures. This alteration was seen in most fish of the study, but Rooipoort had the most fish with hyaline droplet degeneration (Table 11) and is also the site with the highest *p,p*'-DDD levels in fish (Table 7).

PCBs were used as oils in transformers and in hydraulic systems and are formed as by-products of industrial combustion processes. PCDD/Fs are also products of industrial combustion (Ross, 2004; Carpenter, 2006; Costa *et al*., 2008). These chemicals were banned (specifically PCBs) and restricted due to their persistence and toxic effects (Stockholm Convention, 2011). PCBs and PCDD/Fs are still found in the environment (Marvin *et al*., 2003; Berglund *et al*., 2005; Dmitruk *et al*., 2008; Chakravarty & Patgiri, 2009; Nieuwoudt *et al*., 2009). Health of humans (Carpenter, 2006 Bertazzi *et al*., 2001; Costopolou *et al*., 2006, Kogevinas, 2001; Gourounti *et al*., 2008 Letcher *et al*., 2010) and wildlife (Louiz *et al*., 2009; Adu-Kumi *et al*., 2010 Glaser & Connolly, 2002) are negatively affected by these compounds. In fish histological alterations, similar to those found in this study, was seen in PCB exposed fish. Largemouth bass (*Micropterus salmoides*), from the Savannah River (South Carolina and Georgia, USA) contaminated by PCBs had hyperplasia of the gill epithelium and – chloride cells (Teh *et al*., 1997). The livers of fish exposed to PCBs had fatty change (fatlike vacuolation) in the livers (Teh *et al*., 1997; Grinwis *et al*., 2001). The fish from Parys had epithelial hyperplasia

(Table 8) and was the site with the highest PCB contamination (Table 7). One fish from Parys had fatlike vacuolation which might be attributed to PCB contamination.

PFOS is part of the PFC group. It is both lipophillic and hydrophilic, and it is ubiquitous in the environment (Hansen *et al*., 2002; Yeung *et al*., 2009; Post *et al*., 2012). PFCs were banned and restricted in 2009 (Stockholm Convention, 2011). Extensive studies of PFC effects have been undertaken on human health (Völkel *et al*., 2008; Butenhoff *et al*., 2009) and wildlife (Giesy & Kannan, 2001; Hoff *et al*., 2003; Hoff *et al*., 2005; Bossi *et al*., 2005; Du *et al*., 2009; Yeung *et al*., 2009). Hepatic lesions (including fatlike vacuolation) were seen in eel (*Anguilla anguilla*) and carp (*Cyprinus carpio*) with PFOS present in the livers (Hoff *et al*., 2005) and Standerton was the site with the highest PFOS accumulation and was where a fish sampled had both micro- and macrovesicluar steatosis foci (Figure 22 & 23). Another fish, from Standerton, had hyalinization in its liver (Figure 20). The appearance of hyaline droplets is caused by the disruption of protein synthesis in the liver (Van Dyk *et al*., 2007). PFOS has been found to decrease serum proteins in fish (Hoff *et al*., 2005) and is capable of liver damage (Hoff *et al*., 2003). It is therefore possible that PFOS could disrupt protein synthesis in the liver of fish, but further investigation is required.

Some metals are essential to sustain life but are toxic at high levels (Duffus, 2002, Dallas & Day, 2004). Metals form stable bonds with proteins creating active sites of enzymes or function as catalysts in redox reactions (Dallas & Day, 2004). Metallothioneins are proteins, induced by genes to use in metal detoxification. Metallothioneins bind to metals such as Cu, Zn, Cd, Hg and Ag protecting the organism against the metals until its excreted. However, high metal concentrations can overwhelm the metallothioneins in which case the unbound metals interact with the tissue and cause damage. This is the so-called “spill-over hypothesis” (Newman, 2010).

Chromium was detected in the sediment at all the sites (Table 3) and in high concentrations in the fish (relative to the other elements) (Figure 13). Chromium is one of the least toxic metals (Eisler, 1986; Dallas & Day, 2004). Experimental- and feral fish exposed to Cr, had this metal accumulated in their gills, liver and kidney (Eisler, 1986). In rainbow trout (*Oncorhynchus mykiss*), Cr inhibited liver enzymes and caused liver lesions (Eisler, 1986). Perfused gills due to epithelial hyperplasia also occured in this fish at different Cr concentrations (Eisler, 1986). The *Clarias gariepinus*, at all the sites, exhibited hyperplasia in their gills. Standerton had the most fish with this alteration (Table 9) and was the site that had the highest levels of Cr in the sediment (Table 3) and fish tissue (Figure 13).

Zinc is an essential micronutrient, forming the active site of various metalloenzymes, including DNA and RNA polymerases (Dallas & Day, 2004). In fish, Zn accumulates in the liver, gills and kidneys (Dallinger *et al*., 1987). Van Dyk *et al*. (2007) found fatty change and hyalinization of hepatocytes in the livers of *C. gariepinus* exposed to Zn. Fatlike vacuolation was also seen in the sturgeon (*Acipenser ruthenus*) from the Danube River basin that were exposed to heavy metals, especially Zn (Poleksic *et al*., 2010). Fatlike vacuolation was seen in one fish each from Boegoeberg, Parys and Standerton. Steatosis was seen at Standerton (micro- and macro vasicular steatosis) and Rooipoort (macro-vasicular steatosis). All these sites had Zn present in the sediment and fish tissue (Table 3 & Figure 15). Hyalinization was only found in one fish from Standerton (Figure 20). Standerton also had the second highest Zn levels in the sediment (Table 3), but the lowest levels in the fish tissue (Figure 15).

Silver is one of the most toxic, but least studied metals (Coleman & Cearley, 1974). Silver is toxic to fish in both acute and chronic exposures and it accumulates in the gills and livers (Coleman & Cearley, 1974; Hogstrand & Wood, 1996; Morgan & Wood, 2004). Coleman & Cearley (1974) undertook laboratory studies on the effects of Ag on largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*). Results from this study showed behavioural change due to Ag exposure. The bass experienced respiratory difficulty during the exposures. After death, the gills were red and non-mucous (Coleman & Cearley, 1974). The fish therefore did not suffocate from mucous covering the gills, but the surface area of the gills could have been reduced to such an extent that the oxygen demand of the fish under stress could not be met. Although these authors did not do histology, from what was described, gill alterations due to the Ag exposure are quite possible. An alteration that could have caused this is epithelial hyperplasia that could have led to lamellar fusion. Another gill alteration is telangeicstasia. Silver could have caused the pillar cells in the gills to rupture, causing pooling of blood (explaining the red colouring observed by Coleman & Cearly (1974), decreasing the effectiveness of oxygen exchange).

Silver has the ability to disrupt ion regulation in the gills. The Ag ions inhibit the exchange of Na and Cl ions leading to a stress response and fish tend to die from circulatory failure (Hogstrand & Wood, 1996). Ag also inhibits Na- and K ion ATPase (Hogstrand & Wood, 1996; Morgan & Wood, 2004), which according to Evans (1987), results in the increase of chloride cells. The levels of Ag in the fish tissue were notably higher than the other metals (Figure 13). Ag was also one of two metals that accumulated (Au accumulation not of concern due to low levels in fish) (Table 15). The alterations found in this study compared to what was found in literature corresponded with the histological results. Chloride cell- and epithelial hyperplasia was seen in fish from Boegoeberg (Table 9).

The detoxification function of the liver and the fact that the gills are in direct contact with the water could be the reason why these two organs had more alterations than the kidneys. Even though *C. gariepinus* had Ag and high levels of PFOS accumulated in the muscle tissue, the semi quantitative histopathological assessment results from all the mean organ indices were within class 1 and 2, indicative of normal tissue structure with slight and moderate alterations.

It is important to keep in mind that the alterations found in the target organs, were classified as slight alterations (Table 11) and that these organs appear to be in functional states. The possible causes of these alterations were the metals and POPs detected at the sites, but other factors could also have played a role in the pathology seen. The water quality parameters of each site, parasitic infections (Table 8), and other possible chemical contaminants not measured for could have been the reason these organs showed the alterations found.

5.4 Limited human health risk assessment

The consumption of contaminated fish can have serious health implications for humans (Du Preez *et al.*, 2002; Sidhy, 2003; Oliveira-Ribeiro *et al*., 2005; Miranda *et al*., 2008). A limited human health risk assessment was performed, predicting whether humans consuming the fish in this study would have a hazardous and/or cancerous risk by the pollutants found in the fish fillets. The results showed that the toxicants responsible for the highest health risk were Ag, Hg, As and Cr.

The POPs levels were very low in the fish and the human health risk assessment showed no carcinogenic or non-carcinogenic risk from these chemicals. The metals were assessed for non-carcinogenic risk, because there are no cancer slope factors available for these metals. The non-cancer risk is indicated by the hazard index (HI) (Heath *et al*., 2004). The results obtained were interpreted using a scale described by Lemly (1996) (cf. Results section 4.5) to determine the magnitude of the risk.

The scenarios used for this assessment were long (10 years) and short term (5 years) exposure of both children and adults (Table 12). The demographic group chosen for the risk assessment was subsistence fisherman, consuming fish as their main protein source. The fish intake rate (400 g per day) for this risk assessment was assumed to be constant and that of subsistence fisherman along the Orange-Vaal River.

Ag, Hg, As, and Cr showed moderate and high risk (Figure 25). A distinct pattern was seen in these metals' HI values. The pattern of the highest HI between the metals relative to the scenarios showed that the hazard (HI) was greatest for the 10 year exposure of children (Sc2)

then adults exposed for 10 year and children exposed for 5 year (Sc1&4), then the 5 year exposure of the adults (Sc3). (cf. Results section 4.5).

The HI for Ag and Hg were the highest at Boegoeberg. These high HI values for Ag and Hg coincided with the levels for these elements that were the highest in the fish of Boegoeberg. The highest As and Cr levels in the fish were from Standerton, and this site had the highest HI values. The reference dose (RfD) for each metal determines the HI. The reference doses are the maximum acceptable oral doses of toxic substances set up by the US EPA (US EPA, 2012). These reference doses are chemical specific. Mercury had a lower RfD than the other metals, calculating a higher HI (cf. Materials and Methods, section 3.7, equation 9), due to its high toxicity

As expected for both the children and the adults, the longer exposure term created a higher HI for all the elements. The higher HI for the children indicates that they are the more sensitive life stage and therefore extra care should be taken with the quality of their food.

The fact that the 10 year exposure period of the adults were similar to the 5 year exposure period of the children predicts that although adults might not show acute toxic reaction to the level of the metals in their fish food, they will be chronically exposed to the metals if they were to each as much fish, weigh as much and consume fish as long as and as frequently as were assumed in the scenario. Children would show earlier signs of toxicity, if the assumptions of the scenarios were to be fulfilled.

The ingestion of Ag (in its solid form), has no health effects in humans. It is because this form of Ag is biologically inert, and would pass through the body without being absorbed (Dartmouth, 2012) or metabolised and excreted (Faust, 1992). Insoluble Ag salts can be bio-transformed into soluble silver sulphide albuminates, which bind to complexes with amino- or carboxyl groups of proteins (WHO, 2006). The only health effect Ag found in literature is argyria. Argyria is a gray or blue-gray, permanent discolouration of the skin and mucous membranes that is not a toxic effect *per se*, but is considered cosmetically disfiguring (Faust, 1992 WHO, 2006; Panyala *et al* 2008; Dartmouth, 2012).

Mercury can enter the body in different forms and pathways (Health Canada, 2007). In contaminated fish, Hg is in its organic, methyl-mercury form. Mercury poisoning became a concern after the Minamata Bay (seafood contamination) and the Niigata (freshwater fish) epidemics (US EPA, 1997; Cope, 2004; Health Canada, 2007; Newman, 2010). Mercury has many severe effects on human health (cf. Literature study section 2.2.4). It is for this reason that the intake of contaminated food had been regulated by national and international guidelines

(US EPA, 1997; Health Canada, 2007) and the public was made aware of its dangers and how to avoid Hg contaminated foodstuffs (Sydney Health & Fertility, 2007; WHO, 2007).

In Kwa-Zulu Natal Oosthuizen & Ehlrich (2001) calculated hazardous risk for humans consuming fish (*Clarias gariepinus* and *Cyprinus carpio*) from rivers and dams polluted by a Hg processing plant. The HI's calculated at these sites were similar (HI 4-30) to that of this study. Similarly, catfish contaminated with Hg, from gold mining activities in Tanzania, posed hazardous risk for human consumption (Taylor *et al*., 2005). From the results found by these authors, the human health risk was calculated according to the parameters of scenario 2 (of this study). The results showed that the hazard risk at the mining impacted site was extremely high (HI 247). In Indonesia, (Castilhos *et al*., 2006) calculated a human health risk assessment on fish also contaminated by mining activities. The HI was relatively lower than this study's, with the highest Hg HI value of 9.9.

The high HI values of Ag and Hg in the present study are cause for concern. Silver might cause argyria in children eating the contaminated fish, but of greater concern is Hg. Mercury can have detrimental effects on the health of the subsistence fisherman and their families consuming the fish from the study sites (especially Boegoeberg and Parys) causing effects such as: Damage to the central nervous system (Dallas & Day, 2004; Patrick, 2006; Vinceti *et al*., 2010), kidneys (Drazniowsky *et al*., 1985; Nordberg *et al*., 2009), and liver (Barcelos *et al*., 2011). Mercury can cause retardation in the development of humans and have cancerous effects (Newman, 2010).

6. Conclusion

Elemental analysis of the sediment showed them to be enriched with heavy metals, polluting the Orange-Vaal system. Ag, Ni, Se, Hg, Cr, Mn, Cu, As and U were the elements contributing most to the sediment pollution. The enrichment factors indicated towards anthropogenic activities causing the pollution. Rooipoort was the worst affected by the pollution as shown by the sediment indices. The organic pollutants were not contributing to the pollution of the sediment.

Those elements that bio-accumulated in the fish were Ag and Au when compared to sediment levels of these metals. Not one of the metals for which international fish consumption guidelines could be found, exceeded those levels. In spite of the very low sediment levels of the organic pollutants, PFOS accumulated in almost all fish and fish from Parys had three groups of organic pollutants accumulated: PCBs, PBDEs and PFOS.

The accumulated levels of PFOS in the fish are of concern since these levels were greater than what had been reported in northern hemisphere studies. Very little is known about the levels of this relatively new POP in other South African systems.

Even though pollutants were detected in the muscle tissue of *Clarias gariepinus* they appear to be in functional health (normal tissue structure with slight and moderate alterations) according to the histological assessment. The POPs and metals detected at all the sites therefore had no severe effect on the histology of the fish. The histology of the Standerton fish pointed to stressors other than those analysed in this study.

Although the histological assessment indicated that the fish had no severe alterations from the pollutants, the human health risk assessment indicated to non-cancerous hazard from prolonged consumption of these fish, specifically for Hg and Ag and to a lesser extent Cr and As.

The results from this study clearly point out that the Orange-Vaal system is polluted. The pollution needs to be regularly monitored and efforts should be made to decrease it to preserve the ecosystem and the health of the people depending on it.

7. Recommendations

- A second survey where seasonal variation is accommodated will address the influence of high flow and low flow on pollutant levels in the sediment as well as how much run-off from the surroundings contributes to concentrations.
- More sites on the Orange River, specifically upstream of its confluence with the Vaal River and closer to its Lesotho origins, will give a better representation of that system.
- Other toxicants that influence aquatic biota should be researched, to identify unknown pollutants at each site e.g.: bio-toxins e.g. mycotoxins, other organic pollutants e.g. PAHs, current use agricultural pesticides and associated chemicals such as growth stimulants and fertilisers.
- Non-pollutant stressors such as flow regime, droughts and floods, new dams and roads, deteriorating infrastructure and other human activities that damage the riparian zone must be included.
- Sampling fish species (carp, tilapia, yellowfish), from different trophic levels, would deepen the understanding of the damage to an ecosystem caused by pollutants and stressors.
- Further investigation on the presence of PFOS in the Orange-Vaal River and other rivers in South-Africa is needed. It is important to determine the extent of this generally new pollutant's contamination in our country's waterbodies as its chemical behaviour is different from all other POPs.

8. References

ADU-KUMI S, KAWANO M, SHIKI Y, YEBOAH PO, CARBOO D, PWAMANG J, MORITA M & SUZUKI N. 2010. Organochlorine pesticides (OCPs), dioxin-like polychlorinated biphenyls (dl-PCBs), polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in edible fish from Lake Volta, Lake Bosumtwi and Weija Lake in Ghana. *Chemosphere* 81: 675-684.

AGIUS C & ROBERTS RJ. 2003. Melano-macrophage centres and their role in fish pathology. *Journal of Fish Diseases* 26(9): 499-509.

ALBAS A. 2001. Histologiehandleiding. Histologietegniek: Praktiese aanwysings. Department of Zoology, University of Stellenbosch 3-14 p.

AMEH EG & AKPAH FA. 2011. Heavy metal pollution indexing and multivariate statistical evaluation of hydrogeochemistry of River PovPov in Itakpe Iron-Ore mining area Kogi State Nigeria. *Pelagia Research Library Advances in Applied Science Research* 2(1): 33-46.

AMUNDSENAT PA, STALDVIK FJ, LUKINB AA, KASHULINB NA, POPOVA OA & RESHETNIKOV YS. 1997. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *The Science of the Total Environment* 201: 211-224.

ANGLO-AMERICAN. 2010. Kumba Iron Ore Ltd, Board Of Directors' Annual Report 2010. www.kumba.co.za/reports/kumba_ar2010/board/pdf/
comm_full.pdf Date of access 12 March 2012.

ANGLOGOLD-ASHANTI. 2006. Country Report: South Africa-Vaal River. www.anglogoldashanti.co.za/subwebs/informationforinvestors/reporttosociety06/files/Val_River.pdf Date of access: 21 October 2012.

ANGULO E. 1996. The Tomlinson Pollution Load Index applied to heavy metal, 'Mussel-Watch' data: a useful index to assess coastal pollution. *The Science of the Total Environment* 187: 19-56.

ANTHONY JA, ROBY DD & TURCO KR. 2000. Lipid content and energy density of forage fishes from the northern Gulf of Alaska. *Journal of Experimental Marine Biology and Ecology* 248: 53-78.

ANZECC, AUSTRALIAN & NEW ZEALAND ENVIRONMENT AND CONSERVATION COUNCIL. 2000. National Water Quality Management Strategy: Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Volume 2. www.mincos.gov.au/__data/assets/gfmwq-guidelines-vol2-8-4.pdf Date of access: 16 June 2011.

ATGIN RS, EL-AGHA O, ZARARSIZ A, KOCATAŞ A, PARLAK H & TUNCEL G. 2000. Investigation of the sediment pollution in Izmir Bay: trace elements. *Spectrochimica Acta Part B* 55: 1151-1164.

ATSDR, AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY. 2012. Toxicological profile for copper. www.atsdr.cdc.gov/toxprofiles/tp132-c2.pdf Date of access: 12 September 2012.

AYAS Z, EKMEKCI G, OZMEN M & YERLI SV. 2007. Histopathological changes in the livers and kidneys of fish in Sariyar Reservoir, Turkey. *Environmental Toxicology and Pharmacology* 23: 242-249.

BANTLE JA. 1995. FETAX: A developmental toxicity assay using frog embryos. *Fundamentals of Aquatic Toxicology. Effects, Environmental Fate, and Risk Assessment.* 2nd Edition, RAND GM, Ed. Taylor & Francis, Washington DC.

BARCELOS GRM, ANGELI JPF, SERPELONI JM, GROTTO D, ROCHA BA, BASTOS JK, KNASMÜLLER S & BARBOSA F. 2011. Quercentin protects human-derived liver cells against mercury-induced DNA-damage and alterations of the redox status. *Mutation Research* 726: 109-115.

BARNHOORN IEJ, BORNMAN MS, JANSE VAN RENSBURG C & BOUWMAN H. 2009. DDT residues in water, sediment, domestic and indigenous biota from a currently DDT-sprayed area. *Chemosphere* 77: 1236-1241.

BARNHOORN IEJ, VAN DYK JC, PIETERSE GM &BORNMAN MS. 2010. Intersex in

feral indigenous freshwater *Oreochromis mossambicus*, from various parts in the Luvuvhu River, Limpopo Province, South Africa. *Ecotoxicology and Environmental Safety* 73: 1537–1542.

BELICHEVA LA & SHAROVA JN. 2011. Assessment of Fish Health Status under Long-Term Water Pollution: Vygozero Reservoir, North-West Russia. *Proceedings of the 8th International Scientific and Practical Conference* 11: 368-373.

BERGLUND O, NYSTRÖM P & LARSSON P. 2005. Persistent organic pollutants in river food webs: influence of trophic position and degree of heterotrophy. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 2021-2032.

BERNET D, SCHMIDT H, MEIER W, BURKHARDT-HOLM P & WAHLI T. 1999. Histopathology in fish: proposal for protocol to assess aquatic pollution. *Journal of Fish Diseases* 22:25-34.

BERNET D, SCHMIDT-POSTHAUS H, WAHLI T & BURKHARDT-HOLM P. 2004. Evaluation of two monitoring approaches to assess effects of waste water disposal on histological alterations in fish. *Hydrobiologia* 524: 53-66.

BERTAZZI PA, CONSONNI D, BACHETTI S, RUBAGOTTI M, BACCARELLI A, ZOCCHETTI C & PESATORI AC. 2001. Health Effects of Dioxin Exposure: A 20-Year Mortality Study. *American Journal of Epidemiology* 153(11) 1031-1044.

BONEFELD-JORGENSEN EC. 2004. The human health effect programme in Greenland, a review. *Science of the Total Environment*. 331: 215–231.

BOORMAN GA, BOTTS S, BUNTON TE, FOURNIE JW, HARSHBARGER JC, HAWKINS WE, HINTON DE, JOKINEN MP, OKIHIRO MS & WOLFE MJ. 1997. Diagnostic criteria for degenerative, inflammatory, proliferative nonneoplastic and neoplastic liver lesions in medaka (*Oryzias latipes*): consensus of a National Toxicology Program Pathology Working Group. *Toxicological Pathology* 25: 202-210.

BORNMAN MS, VAN VUREN JHJ, BOUWMAN H, DE JAGER C, GENTHE B & IEJ BARNHOORN. 2007. Endocrine disruptive activity and the potential health risk in an urban nature reserve. Water Research Commission Report No. 1505/1/07. Pretoria.

BOSSI R, RIGET FR, DIETZ R, SONNE C, FAUSER P, DAM M & VORKAMP K. 2005. Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. *Environmental Pollution* 136: 323-329.

BOUWMAN, H. 2004. South Africa and the Stockholm convention on persistent organic pollutants. *South African Journal of Science* 100: 323–328.

BOUWMAN H, POLDER A, VENTER B & SKAARE JU. 2008. Organochlorine contaminants in cormorant, darter, egret, and ibis eggs from South Africa. *Chemosphere* 71: 227-241.

BRANCH S. 2004. Teratogenesis, Chapter 13. *Modern Textbook of Toxicology*, 3rd Edition. HODGSON E, Ed. John Wiley & Sons, Inc. Hoboken, New Jersey. p:557.

BRAND M, MAINA J, MANDER M &O'BRIEN G. 2009. Characterisation of the social and economic value of the use and associated conservation of the yellowfishes in the Vaal River. Water Research Commission Report No KV 226/09. Pretoria.

BRAUNE E & ROGERS KH. 1987. The Vaal River catchment: Problems and research needs. South African Scientific Programmes Report No: 143.

BRUSLÉ J & GONZÀLEZ G. 1996. The Structure and Function of Fish Liver. Chapter 6. *Fish Morphology: Horizon of New Research*, 1st Edition. MUNSHI JSD & DUTTA, Eds. Science Publishers, Inc. p. 434.

BUCAS K. 2006. Natural and anthropogenic influences on the water quality of the Orange River, South Africa. Unpublished M.Sc Thesis. University of Johannesburg. Aucklandpark. https://ujdigispace.uj.ac.za/handle/10210/
456?show=full Date of access: 12 May 2011.

BUNTON TE. 1996. Experimental Chemical Carcinogenesis in Fish. *Toxicological Pathology* 24(5): 603-618.

BUTENHOFF JL, EHRESMAN DJ, CHANG S, PARKER GA & STUMP DG. 2009. Gestational and lactational exposure of potassium perfluorooctanesul-fonate (K^+PFOS)

in rats: Development neurotoxicity. *Reproductive Toxicology* 27: 319-330.

CAMARGO MPM & MARTINEZ CBR. 2007. Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. *Neotropical Icthyology* 5 (3): 327-336.

CARPENTER DO. 2006. Polychlorinated biphenyls (PCBs): Routes of exposure and effects on human health. *Reviews on Environmental Health* 21(1): 1-23.

CARVALHO CEV, OVALLE ARC, REZENDE CE, MOLISANI MM, SALOMÃO MSMB &LACERDA LD. 1999. Seasonal variation of particulate heavy metals in the lower Paraíba do Sul River, R.J., Brazil. *Environmental Geology* 37(4): 297-302.

CASTILHOS ZC, RODRIGUES-FILHO S, RODRIGUES APC, VILLAS-BÔAS RC, SIEGEL S, VEIGA MM & BEINHOFF C. 2006. Mercury contamination in fish from gold mining areas in Indonesia and human health risk assessment. *Science of the Total Environment* 368: 320-325.

CHAKRAVARTY M& PATGIRI AD. 2009. Metal pollution assessment in sediments of the Dikrong River, N.E. India. *Journal of Human Ecology* 27(1): 63-67.

CHAPMAN PM. 2007. Heavy metal – Music not science. *Environmental Science and Technology* 41 (12): 6.

CHAPMAN PM. 2012. Heavy metal – cacophony, not symphony. *Integrated Environmental Assessment and Management* 8(2): 216.

CHEUNG KC, LEUNG HM, KONG KY & WONG MH. 2007. Residual levels of DDTs and PAHs in freshwater and marine fish from Hong Kong markets and their health risk assessment. *Chemosphere* 66: 460-468.

CHUTTER FM. 1963. Hydrobiological studies on the Vaal River in the Vereeniging Area, Part 1. *Hydrobiologia* 21;1-79.

CHUTTER FM. 1967. Hydrobiological studies on the Vaal River and some of its tributaries, including an introduction to the ecology of *Simulium* in its lower reaches. PhD (Zoology) Thesis, Rhodes University.

CHUTTER FM. 1969. The effects of silt and sand on the invertebrate fauna of streams and rivers. *Hydrobiologia* 34(1): 57-76.

COLEMAN RL & CEARLEY JE. 1974. Silver toxicity and accumulation in largemouth bass and bluegill. *Bulletin of Environmental Contamination and Toxicology* 12(1):53-61.

COPE WG. 2004. Exposure Classes, Toxicants in Air, Water, Soil, Domestic and Occupational Settings, Chapter 4. *Modern Textbook of Toxicology*, 3rd Edition. HODGSON E, Ed. John Wiley & Sons, Inc. Hoboken, New Jersey. p:557.

COSTA LG, GIORDANO G, TAGLIAFERRI S, CAGLEIRI A & MUTTI A. 2008. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potential adverse health effects. *Acta Biomed* 79: 172-183.

COSTOPOLOU D, VASSILIADOU I, PAPADOPOULOS A, MAKROPOLOUS V & LEONDIADIS L. 2006. Levels of dioxins, furans and PCBs in human serum and milk of people living in Greece. *Chemosphere* 65:1462–1469.

COULIBALY A, OUATTARA IN, KONÉ T, DOUBA VN, SNOEKS J, GOORÉ G & KOUMÉLAN EP. 2007 First results of floating cage culture of the African catfish *Heterobranchus longifilis* Valenciennes, 1840: Effect of stocking density on survival and growth rates. *Aquaculture* 263: 61-67.

CULLING CFA. 1974. Handbook of distopathological and histochemical techniques. 3rd ed. Butterworths 712p.

DAFF, DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES. 2011. Maize market value chain profile 2010-2011. http://www.daff.gov.za/docs/AMCP/MaizeMVCP2010-2011.pdf Date of access: 17 March 2012.

DALLAS H F & DAY JA. 2004. The effect of water quality variables on aquatic ecosystems: a review. Water Research Commission Report No. TT 224/04. Pretoria.

DALLINGER R, PROSI F, SEGNER H & BACK H. 1987. Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia* 73: 91-98.

DANSCHER G. 1981. Histochemical demonstration of heavy metals. *Histochemistry* 71: 1-16.

DARNERUD PO. 2003. Toxic effects of brominated flame retardants in man and in wildlife. *Environment International* 29: 841-853.

DARTMOUTH. 2012. The Fact on Silver: Dartmouth toxic metals superfund research program. http://www.dartmouth.edu/~toxmetal/toxic-metals/more-metals/silver-faq.html Date of access: 31 October 2012.

DI GULIO RT, HABIG C & GALLAGHER EP. 1993. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. *Aquatic Toxicology* 26: 1-22.

DIKIO ED. 2010. Water quality evaluation of Vaal River, Sharpeville and Bedworth Lakes in the Vaal region of South Africa. *Research Journal of Applied Sciences, Engineering and Technology* 2(6): 574-579.

DIN. 2000. DIN 38414 S24, Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung d Schlamm und Sedimente (Gruppe S) d Teil 24: Bestimmung von polychlorierten Dibenzo-dioxinen (PCDD) und polychlorierten Dibenzofuranen (PCDF).

DMITRUK U, PIAŠCIK M, TABORYSKA B & DOJLIDO J. 2008. Persistent organic pollutants (POPs) in bottom sediments of the Vistula River, Poland. *Clean* 36 (2): 222 – 229.

DRAZNIOWSKY M, PARKINSON IS, WARD MK, CHANNON SM & KERR DN. 1985. Raised serum nickel concentrations in chronic renal failure. *Proceedings of the European Dialysis and Transplant Association - European Renal Association. European Dialysis and Transplant Association - European Renal Association. Congress* 21: 241-246.

DU Y, SHI X, LIU C, YU K & ZHOU B. 2009. Chronic effects of water-borne PFOS exposure on growth, survival and hepatotoxicity in zebrafish: A partial life-cycle test.

Chemosphere 74: 723-729.

DUFFUS JH. 2002. "Heavy Metals" a Meaningless term? (IUPAC Technical Report). *Pure and Applied Chemistry* 74: 793-807.

DU PREEZ HH, HEATH RGM, SANDHAM LA & GENTHE B. 2002. Methodology for assessment of human health risks associated with the consumption of contaminated freshwater fish in South Africa. *Water SA* 29(1): 69-90.

DWA a, DEPARTMENT OF WATER AFFAIRS. 2004. Lower Orange ISP report. Development of ISPs for central region: lower Orange WMA. http://www.dwaf.gov.za/ Documents/Other/WMA/14/LowerOrangeISPJul04Chap2.pdf Date of access: 28 July 2011.

DWA b, DEPARTMENT OF WATER AFFAIRS. 2004. Integrated Strategic Perspective: Upper Orange Water Management Area. DWAF Report Number: P WMA 13/000/00/0304. Pretoria, South Africa. http://www.dwaf.gov.za/Documents/ Other/WMA/13/optimised/UPPER%20ORANGE%20REPORT.pdf Date of access: 18 February 2012.

DWA c, DEPARTMENT OF WATER AFFAIRS 2004. Integrated Strategic Perspective: Lower Orange Water Management Area. DWAF Report Number: P WMA 14/000/00/0304. Pretoria, South Africa. http://www.dwaf.gov.za/ Documents/Other/WMA/14/LowerOrangeISPJul04Intro.pdf Date of access: 18 February 2012.

DWA a, DEPARTMENT OF WATER AFFAIRS. 2010. Drought conditions persist (media release). http://www.dwaf.gov.za Date of access: 16 February 2010.

DWA b, DEPARTMENT OF WATER AFFAIRS. 2010. Water quality concepts. http://www.dwaf.gov.za Date of access: 16 February 2010.

DWA a, DEPARTMENT OF WATER AFFAIRS. 2011. Introduction to the Orange River basin. http://www.dwa.gov.za/orange/intro.aspx Date of access: 23 May 2011.

DWA b, DEPARTMENT OF WATER AFFAIRS. 2011. Sishen iron ore mine.

http://www.dwaf.gov.za/orange/Low_Orange/sishen.htm Date of access: 27 July 2011.

DWA a, DEPARTMENT OF WATER AFFAIRS. 2012. Upper Orange/Senqu. http://www.dwaf.gov.za/orange/up_orange/uporange.aspx Date of access: 25 July 2012.

DWA b, DEPARTMENT OF WATER AFFAIRS. 2012. The Middle Orange. http://www.dwaf.gov.za/orange/mid_orange/midorang.aspx Date of access: 25 July 2012.

DWA c, DEPARTMENT OF WATER AFFAIRS. 2012. The Lower Orange. http://www.dwaf.gov.za/orange/low_orange/default.aspx Date of access: 25 July 2012.

EARLE A, MALZBENDER D, TURTON A & MANZUNGU E. 2005. A preliminary basin profile of the Orange/Senqu River. AWIRU, University of Pretoria. http://www.anthonyturton.com/admin/my_documents/ my_files/Orange_Profile_4_Apl_2.pdf Date of access: 27 July 2011.

EISLER R. 1986. Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report no. 85 (1.6) 60p.

ENGINEERINGNEWS. 2012. SA chemicals and petrochemicals industry in focus. http://www.engineeringnews.co.za/article/a-snapshot-of-the-south-african-chemicals-and-petrochemicals-industry-2009-03- Date of access:9 March 2012.

ENGUIX GONZÁLEZ A, TERNEDO RODRÍGUEZ M, JIMÉNEZ SÁNCHEZ C, FERNÁNDEZ ESPINOSA CA & BARRAGÁN DE LA ROSA FJ. 2000. Assessment of metals in sediments in a tributary of Guadalquivir river (Spain). Heavy metal partitioning and relation between the water and sediment system. *Water, Air, and Soil Pollution* 121: 11–29.

ESKOM. 2012a. Matla power station. http://www.eskom.co.za/c/article/43/matla-power-station/ Date of access: 21 October 2012.

ESKOM. 2012b. Arnot power station. http://www.eskom.co.za/c/article/8/arnot-power-station/ Date of access: 21 October 2012.

ESKOM. 2012c. Duvha power station. http://www.eskom.co.za/c/article/21/duvha-power-station/ Date of access: 21 October 2012.

ESKOM. 2012d. Kendal power station. http://www.eskom.co.za/c/article/28/kendal-power-station/ Date of access: 21 October 2012.

ESKOM. 2012e. Camden power station . http://www.eskom.co.za/c/article/9/camden-power-station/ Date of access: 21 October 2012.

ESKOM. 2012f. Majuba power station. http://www.eskom.co.za/c/article/39/majuba-power-station/ Date of access: 21 October 2012.

ESKOM. 2012g. Tutuka power station. http://www.eskom.co.za/c/article/47/tutuka-power-station/ Date of access: 21 October 2012.

EU, EUROPIAN UNION. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. :eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri%3DOJ:L:2006:364:0005:0024:EN:PDF Date of access: 23 August 2011.

EVANS DH. 1987. The fish gill: Site of action and model for toxic effects of environmental pollutants. *Environmental Health Perspectives 71: 47-58.*

FAIREY R, LONG ER, ROBERTS, CA, ANDERSON BS, PHILLIPS, BM, HUNT JW, PUCKETT HR & WILSON CJ. 2001. An evaluation of methods for calculating mean sediment quality guideline quotients as indicators of contamination and acute toxicity to amphipods by chemical mixture. *Environmental Toxicology and Chemistry* 20: 2276–2286.

FAROMBI EO, ADELOWO OA & AJIMOKO YR. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus)* from Nigeria Ogun River. *International Journal of Environmental Research and Public Health* 4 (2): 158-162.

FAUST RA. 1992. Toxicity Summary for Silver. Oak Ridge Reservation Environmental Restoration Program. Date of access: 2 November 2012.

FRIDAY GP. 1998. Ecological Screening Values for Surface Water, Sediment, and Soil. :www.osti.gov/bridge/servlets/purl/4764-2uJvjR/webviewable/4764.pdf Date of access: 17 July 2011.

FROESE R & PAULY D. 2010. Fishbase: Species Summary. www.fishbase.org/summary/SpeciesSummary.php?id=5129. DOA 4 May 2012.

FROMBERG A, CLEEMANN M & CARLSON L. 1999. Review on persistent organic pollutants in the environment of Greenland and Faroe Islands**.** *Chemosphere* 38(13): 3075-3093.

GABE M. 1976. Histological techniques. 1st ed. Springer-Verlag: New York. 1106p.

GARRETT RH & GRISHAM CM. 2005. Biochemistry. 3rd ed. Belmont: Thomas Brooks/Cole. 1086 p.

GIESY JP & KANNAN, K. 2001. Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science and Technology* 35: 1339-1342.

GEERAERTS C & BELPAIRE C. 2010. The effects of contaminants in European eel: a review. *Ecotoxicology* 19: 239-266.

GERHARDT A. 2002. Bioindicator species and their use in biomonitoring. *Environmental Monitoring* 1 *www.eolss.net/Sample-Chapters/C09/E6-38A-01-07.pdf.* Date of access: 6 September 2012.

GERIĆ M, CERAJ-CERIĆ N, GAJSKI G, VASILIĆ Ž, CAPUDER Ž & GARAJ-VRHOVAC V. 2012. Cytogenetic status of human lymphocytes after exposure to low concentrations of p,p‘-DDT, and its metabolites (p,p’-DDE, and p,p‘-DDD) *in vitro*. *Chemosphere* 87: 1288-1294.

GHSI, GLOBAL HEALTH & SAFETY INNITAITIVE. 2009. Perfluorinated Compounds (PFCs) and Human Health Concerns. www.healthybuilding.nethealthcare2009-04-20PFCs_fact_sheet.pdf Date of access: 26 September 2012.

GLASER D & CONNOLLY JP. 2002. A model of *p,p*’-DDE and total PCB bioaccumulation in birds from the Southern California Bight. *Continental Shelf Research*

22: 1079-1100.

GOUIN K, MACKAY D, JONES T & MEIJER SN. 2004. Evidence for the “grasshopper” effect and fractionantion during long-range atmospheric transport of organic contaminants. *Environmental Pollution* 128: 139-148.

GOUROUNTI K, LYKERIDOU K, PROTOPPA E & LAZARIAS A. 2008. Mechanisms of actions and health effects of organochlorine substances: a review. *Health Science Journal* 2(2): 89-99.

GRINWIS GCM, VAN DEN BRANDHOF EJ, ENGELSMA MY, KUIPER RV, VAAL MA, VETHAAK AD, WESTER PW & VOS JG. 2001. Toxicity of PCB-126 in European flounder (*Platichtys flesus*) with emphasis on histopathology and cytochrome P4501A induction in several organ systems. *Archives of Toxicology* 75: 80-87.

GROENEWALD M. 2000. Bioaccumulation of metals and the general health of fish from the Vaal Dam and Vaal River Barrage. M.Sc (Zoology) Dissertation, Rand Afrikaans University.

GROMAN DB. 1986. Histology of the Striped Bass. *American Fisheries Society* Monograph 3. 116 p.

HAMILTON SJ. 2004. Review of selenium toxicity in aquatic food chain. *Science of the Total Environment* 326: 1-31.

HANCOCK FM. 1973, The ecology of the diatoms of the Klip River, Southern Transvaal. *Hydrobiologia* 42(2-3): 243-284.

HANSEN KJ, JOHNSON HO, ELDRIDGE JS, BUTENHOFF JL & DICK LA. 2002. Quantitative Characterization of Trace Levels of PFOS and PFOA in the Tennessee River. *Environmental Science and Technology* 36: 1681-1685.

HANSEN N, GUTTMAN E & LARSSON Å. 2006. The effect of different holding conditions for environmental monitoring with caged Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Environmental Monitoring* 9(2): 994-999.

HALSALL CJ. 2004. Investigating the occurrence of persistent organic pollutants

(POPs) in the arctic: their atmospheric behaviour and interaction with the seasonal snow pack. *Environmental Pollution* 128: 163–175.

HEALTH CANADA. 2007. Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption. Date of access: 2 November 2012.

HEATH RGM, DU PREEZ HH, GENTHE B & AVENANT-OLDEWAGE A. 2004. Freshwater fish and human health reference guide. Water Research Commission Report No. TT213/04. Pretoria.

HECHT T, UYS W & BRITZ PJ. 1988. The culture of sharptooth catfish *Clarias gariepinus* in southern Africa. South African National Scientific Programmes Report No 153. Pretoria.

HODGSON E. 2004. Introduction to Toxicology, Chapter 1. *Modern Textbook of Toxicology*, 3rd Edition. HODGSON E, Ed. John Wiley & Sons, Inc. Hoboken, New Jersey. p:557.

HODSON ME. 2004. Heavy Metals – geochemical bogey man? *Environmental Pollution* 129: 341-343.

HOFF PT, VAN DONGEN W, ESMANS EL, BLUST R & DE COEN WM. 2003. Evaluation of the toxicological effects of perfluorooctane sulfonic acid in the common carp (*Cyprinus carpio*). *Aquatic Toxicology* 62: 349-359.

HOFF PT, VAN CAMPENHOUT K, VAN DE VIJVER K, COVACI A, BERVOETS, L, MOENS L, HUYSKENS G, GOEMANS G, BELPAIRE C, BLUST R & DE COEN W. 2005. Perfluorooctane sulfonic acid and organo-halogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environmental Pollution* 137(2): 324-333.

HOGSTRAND C & WOOD M. 1998. Toward a better understanding of the bioavailability, physiology, and toxicity of silver in fish: Implications for water quality criteria. *Environmental Toxicology and Chemistry* 17(4): 547-561.

HINTON DE & LAUREN DJ. 1990. Integrative histopathological approaches to

detecting effects of environmental stressors on fishes. *American Fisheries Society Symposium* 8:51–66.

HPA a, HEALTH PROTECTION AGENCY. 2009. Nickel Toxicological overview. www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1236757324101 Date of access: 15 July 2012.

HPA b, HEALTH PROTECTION AGENCY. 2009. PFOS + PFOA Toxicological overview. www.health.state.mn.us/divs/eh/risk/guidance/gw/pfos.pdf Date of access: 13 July 2012.

HUMASON GL. 1979. Animal tissue techniques. 4th ed. W.H. Freeman and Company: San Francisco. 661p.

IRIS, INTEGRATED RISK INFORMATION SYSTEM. 2012. http://www.epa.gov/IRIS/backgrd.html Date of access: 14 August 2012.

ISO. 2004. Draft International Standard ISO/DIS 22032 2004: Water quality: determination of selected polybrominated diphenyl ethers in sediment and sewage sludge. Method using extraction and gas chromatography/mass spectrometry (ISO/TC 147/SC 2). International Organization for Standardization, Geneva.

ISTAS. 2012. RISCTOX: Toxic and hazardous substances database: Persistent Organic Pollutant (POP). http://www.istas.net/risctox/en/index.asp?idpagina=1185 Date of access: 10 August 2012.

ITIS. 2010 a. ITIS Report: Clarias gariepinus. http://www.itis.gov Date of access: 28 October 2010.

JAGOE CH, FAIVRE A & NEWMAN MC. 1996. Morphological and morphometric changes in the gills of mosquitofish (*Gambusia holbrooki*) after exposure to mercury (II). *Aquatic Toxicology* 34: 163-183.

JOMOVA K & VALKO M. 2011. Advances in metal induced oxidative stress and human disease. *Toxicology* 283: 65-87.

JONES KC & DE VOOGT P. 1999. Persistent organic pollutants (POPs): state of the

science. *Environmental Pollution* 100: 209-221.

KHANKI GR. 2007. Chemical contaminants in milk and public health concerns: a review. *International Journal of Dairy Science* 2(2): 104-115.

KIDD KA, BOOTSMA HA, HESSLEIN RH, MUIR DCG & HECKY RE. 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: Importance of trophic level and carbon source. *Environmental Science and Technology* 35: 14-20.

KOGEVINAS M. 2001. Human health effects of dioxins: cancer, reproductive and endocrine system effects. *Human Reproduction Update* 7(3) 331-339.

KRALIK M. 1999. A rapid procedure for environmental sampling and evaluation of polluted sediments. *Applied Geochemistry* 14: 807-816.

KUROSAKI K, NAKAMURA T, MUKAI T & ENDO T. 1995. Unusual findings in fatal case of poisoning with chromate compounds. *Forensic Science International* 75: 57-65.

LEMLY AD. 1996. Evaluation of the Hazard Quotient Method for the Risk Assessment of Selenium. *Ecotoxicology and Environmental Safety* 35: 156-162.

LENNTECH WATER TREATMENT SOLUTIONS. 2011. http://www.lenntech.com/processes/heavy/heavy-metals/heavy-metals.htm Date of access: 4 May 2011.

LETCHER RJ, BUSTNES JO, DIETZ R, JENSSEN BM, JØRGENSEN EH, SONNE C, VERREAULT J, VIJAYAN MM & GABRIELSEN GW. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment* 408:2995–3043.

LOUIZ I, BEN-ATTIA M& BEN-HASSIE OK. 2009. Gonadosomatic index and gonad histopathology of *Gobius niger* (Gobiidea, Teleost) from Bizerta lagoon (Tunisia): Evidence of reproduction disturbance. *Fisheries Research* 100: 266-273.

LUKIN A, SHAROVA J, BELICHEVA L & CAMUS L. 2011. Assessment of fish health status in the Pechora River: Effects of contamination. *Ecotoxicology and Environmental*

Safety 74: 355-365.

MALLATT J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences* 24: 630-648.

MARCHAND MJ, VAN DYK JC, PIETERSE GM, BARNHOORN IEJ & BORNMAN MS. 2008. Histopathological alterations in the liver of the sharptooth catfish *Clarias gariepinus* from polluted aquatic systems in South Africa. *Environmental Toxicology* 24: 133-147.

MARCHAND MJ. 2009. A histology-based fish health assessment to determine the health status and edibility of two indicator fish species from Roodeplaat Dam Unpublished M.Sc Thesis. University of Johannesburg. Aucklandpark. https://ujdigispace.uj.ac.za/handle/10210/3708 Date of access: 6 March 2012.

MARCHAND MJ, VAN DYK JC, BARNHOORN IEJ & WAGENAAR GM. 2012. Histopathological changes in two potential indicator fish species from a hyper-eutrophic freshwater ecosystem in South Africa: a baseline study. *African Journal of Aquatic Science* 37(1): 39-48.

MARTIN S & GRISWOLD W. 2009. Centre For Hazardous Substances Research: Human Health Effects of Heavy Metals. *Environmental Sciences and Technology Briefs for Citizens* 15: 1-6.

MARVIN C, GRAPENTINE L & PAINTER S. 2003. Application of a sediment quality index to the lower Laurentian Great Lakes. *Environmental Monitoring and Assessment* 91: 1-16.

MBEDI INFORMATION SERVICES. 2012a. Oil Refining in South Africa, An Overview. http://www.mbendi.com/indy/oilg/ogrf/af/sa/p0005.htm Date of access: 9 March 2012.

MBEDI INFORMATION SERVICES. 2012b. Silver mining in South Africa, An Overview. http://www.mbendi.com/indy/mining/silv/af/sa/index.htm Date of access: 19 October 2012.

McCARTHY T & RUBIDGE B. 2005. The Story of Earth and Life: a southern African

perspective on a 4.6-billion-year journey. 1st Edition. Cape Town: Struik Publishers. 333p.

McCARTHY TS & VENTER JS. 2006. Increasing pollution levels on the Witwatersrand recorded in the peat deposits of the Klip River wetland. *South African Journal of Science* 102: 27-34.

McDONALD TA. 2002. A Perspective on the potential health risks of PBDEs. *Chemosphere* 46: 745-755.

McHUGH KJ, SMIT NJ, VAN VUREN JHJ, VAN DYK JC, BERVOETS L, COVACI A & WEPENER V. 2011. A histology-based fish health assessment of the tigerfish, *Hydrocynus vittatus* from a DDT-affected area. *Physics and Chemistry of the Earth* 36: 895-904.

McKAY G. 2002. Dioxin characterisation, formation and minimisation during municipal solid waste (MSW) incineration: review. *Chemical Engineering Journal* 86: 343-368.

MENESES M, SCHUHMAVHER M & DOMINGO JL. 2004. Health risk assessment of emissions of dioxins and furans from a municipal waste incinerator: comparison with other emission sources. *Environment International* 30: 481-489.

MEYBECK M, HOROWITZ AJ & GROSBOIS C. 2004. The geochemistry of Seine River Basin particulate matter: distribution of an integrated metal pollution index. *Science of the Total Environment* 328; 219-236.

MILLER JR, HUDSON-EDWARDS KA, LECHLER PJ, PRESTON D & MACKLIN MG. 2004. Heavy metal contamination of water, soil and produce within riverine communities of the Rıo Pilcomayo basin, Bolivia. *Science of the Total Environment* 320:189-209.

MIRANDA AL, ROCHE H, RANDI MAF, MENEZES ML & OLIVEIRA RIBEIRIO CA. 2008. Bioaccumulation of chlorinated pesticides and PCBs in the tropical fresh water fish *Hoplias malabricus*: Histopathological, physiological and immunological findings. *Environmental International* 34:939-949.

MLAMBO SS, VAN VUREN JHJ, BARNHOORN IEJ & BORNMAN MS. 2009.

Histopathological changes in the reproductive system (ovaries and testes) of *Oreochromis mossambicus* following exposure to DDT. *Environmental Toxicology and Pharmacology* 28: 133–139.

MOHAMED FAS. 2009. Histopathological Studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. *World Journal of Fish and Marine Sciences* 1 (1): 29-39.

MOHIUDDIN KM, ZAKIR HM, OTOMO K, SHARMIN & SHIKAZONO N. 2010. Geochemical distribution of trace metal pollutants in water and sediments of downstream of an urban river. *International Journal of Environmental Science and Technology* 7(1): 17-28.

MORGAN TP & WOOD CM. 2004. Relationship between gill silver accumulation and acute silver toxicity in the freshwater rainbow trout: Support for the acute silver biotic ligand model. *Environmental Toxicology and Chemistry* 23(5): 1261-1267.

NAIK HK, MISHRA MK & ROA KARANMAN UM. 2009. Rheological characteristics of fly ash slurry at varying temperature environment with and an additive. *2009 World of Coal Ash (WOCA) Conference- May 4-7, 2009. Lexington, KY, USA.* http://www.flyash.info/ Date of access: 22 October 2012.

NASH TH & GRIES C. 1995. The use of lichens in atmospheric deposition studies with an emphasis on the arctic. *The Science of the Total Environment* 160/161: 729-736.

NEUMANN M, SCHULZ R, SCHÄFER, MÜLLER W, MANNHELLER W & LEISS M. 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. *Water Research* 36: 835-842.

NEWMAN MC. 2010. Fundamentals of Ecotoxicology. 3rd Edition. CRC Press: Boca Raton. 541 p.

NIEUWOUDT C, QUINN LP, PIETERS R, JORDAAN I, VISSER M, KYLIN H, BORGEN AR, GIESY JP & BOUWMAN H. 2009. Dioxin-like chemicals in soil and sediment from residential and industrial areas in central South Africa. *Chemosphere* 76: 774-783.

NIKALJE SB, MULEY DV & ANGADI SM. 2012. Histopathological changes in gills of a

freshwater major carp *Labeo rohita* after acute and chronic exposure to textile mill effluent (tme). *International Journal of Environmental Sciences* 3(1): 108-118.

NORDBERG GF, JIN T, WU X, LU J, CHEN, LEI L, HONG F & NORDBERG M. 2009. Prevalence of kidney dysfunction in humans- Relationship to cadmium dose, metallothionein, immunological and metabolic factors. *Biochimie* 91: 1282-1285.

NOREÑA-BARROSO E, SIMÁ-ÁLVAREZ, GOLD-BOUCHOT G & ZAPATA-PÉREZ O. 2004. Persistent organic pollutants and histological lesions in Mayan catfish *Ariopsis assimilis* from the Bay of Chetumal, Mexico. *Marine Pollution Bulletin* 48 :263–269.

NCB, NORTHERN CAPE BUSINESS. 2012. Economic sector: Agriculture & agri-processing. http://www.northerncapebusiness.co.za/pls/cms/ti_secout.secout_prov?p_sid=1&p_site_id=164. Date of access: 17 March 2012.

NCSU, NORTH CAROLINA STATE UNIVERSITY. 2011. North Carolina Water Quality Program, Water Sheds: Heavy metals. http://www.water.ncsu.edu/watershedss/info/hmetals.html Date of access: 21 April 2011.

NNAJI JC, UZAIRU A, HARRISON GFS & BALARABE ML. 2007. Evaluation of cadmium, chromium, copper, lead and zinc concentrations in the fish head/viscera of *Oreochromis niloticus* and *Synodontis schall* of River Galma, Zaria, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 6(10): 2420-2426.

OECD, Organisation for Economic Co-operation and Development. 2003. OECD Environmental Indicators: Development, Measurement and use. *www.oecd.org/dataoecd/7/47/24993546.pdf.* Date of access: 26 August 2012.

OETKEN M, STACHEL B, PFENNINGER M & OEHLMANN J. 2005. Impact of a flood disaster on sediment toxicity in a major river system- the Elbe flood 2002 as a case study. *Environmental Pollution* 134: 87-95.

OLIVEIRA RIBEIRO CA, VOLLAIRE Y, SANCHEZ-CHARDI A & ROCHE H. 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. *Aquatic Toxicology* 74: 53-69.

OOSTHUIZEN J & ELHRICH R. 2001. The impact of pollution from a mercury processing plant in Kwazulu-Natal, South Africa, on the health of fish-eating communities in the area: An environmental health risk assessment. *International Journal of Environmental Health Research* 11: 41-50.

ORASECOM. 2011. Orange-Senqu River Awareness Kit. http://www.orangesenqurak.org/people/environment+well+being/sustainable+livelihoods/subsistence+farming.aspx. Date of access: 16 March 2012.

OSHA, OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION. 2004. Part 1910: Occupational Safety and Health Standards. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10118 Date of access: 24 July 2012.

ÖZTÜRK M, ÖZÖZEN G, MINARECI O & MINARECI E. 2009. Determination of heavy metals in fish, water and sediments of Avsar dam lake in Turkey. *Iranian Journal of Environmental Science & Engineering* 6(2): 73-80.

PALANISAMY P, SASIKALA G, MALLIKARAJ D, BHUVANESHWARI N & NATARAJAN GM. 2011. Histopathological lesions in gill of air-breathing catfish *Mystus cavasius* exposed to of electroplating industrial effluent nickel. *International Journal of Applied Biology and Pharmaceutical Technology* 2(2): 150-155.

PANYALA NR, PEÑA-MÉNDEZ EM & HAVEL J. 2008. Silver or silver nanoparticles: a hazardous threat to the environment and human health? *Journal of Applied Biomedicine* 6: 117-129.

PARTICK L. 2006. Lead toxicity, a review of literature. Part I: Exposure, evaluation and treatment. *Alternative Medicine Review* 11(1): 2-22.

PIETERSE GM, MARCHAND MJ, VAN DYK JC & BARNHOORN IEJ. 2010. Histological alterations in the testes and ovaries of the Sharptooth catfish (Clarias gariepinus) from an urban nature reserve in South Africa. *Journal of Applied Ichthytology* 26: 789-793.

POLEKSIC V, LENHARDT M, JARIC I, DJORDJEVIC D, GACIC Z, CVIJANOVIC G & RASKOVIC B. 2010. Liver, gills, and skin histopathology and heavy metal content of

the Danube Sterlet (*Acipenser ruthenus* Linnaeus, 1758). *Environmental Toxicology and Chemistry* 29(3): 515-521.

PORĘBA R, GAĆ P, PORĘBA M & ANDRZEJAK R. 2011. Environmental and occupational exposure to lead as a potential risk factor for cardiovascular disease. *Environmental Toxicology and Pharmacology* 31: 267-277.

POST GB, COHN PD & COOPER KR. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature. *Environment Research* 116: 93-117.

PRAVEENA SM, AHMED A, RADOJEVIC M, ABDULLAH MH & ARIS AZ. 2007. Heavy metals in mangrove surface sediment of Mengkabong lagoon Sabah: multivariate and geo-accumulation index approach. *International Journal of Environmental Research* 2(2): 139-148.

RAILSBACK LB. 2008. An Earth Scientist's Periodic Table of the Elements and Their Ions. Department of Geology, University of Georgia. http://www.gly.uga.edu/railsback/PT.html Date of access: 20 October 2012.

RATTE HI. 1999. Bioaccumulation and Toxicity of Silver Compounds: a Review. *Environmental Toxicology and Chemistry* 18(1): 89-108.

RAURET G. 1997. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. *Talanta* 46: 449-455.

ROBERTS RJ. 1989. Fish Pathology. 2nd Edition. London: Baillière Tindall. 466p.

ROGAN SMUC N, VRHOVINK P, DOLENEC T, SERAFIMOVSKI T, TASEV G & DOLENEC M. 2009. Assessment of the heavy metal contamination in the surficial sediments of Lake Kalimanci (Macedonia): a preliminary study. *Materials and Geoenvironment* 56(4): 437-447.

ROGERS JM & KAVLOCK RJ. 2008. Development Toxicology, Chapter 10. *Casarett & Doull's Toxicology. The Basic Science of Poisons*, 7th Edition. Klaasen CD Ed. McGraw Hill, New York.

ROOILAND W. 2011. Mining and Minerals. http://economic.ncape.gov.za/index.php/option=com_content&view=article&id=141&Itemid=231 Date of access: 13 October 2012.

ROSS G. 2004. The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicology and Environmental Safety* 59: 275–291.

ROYCHOUDHURY AN AND STRAKE MF. 2006. Partitioning and mobility of trace metals in the Blesbokspruit: Impact Assessment of dewatering of mine waters in the East Rand South Africa. *Applied Geochemistry* 21:1044-1063.

RUIZ F. 2001. Trace metals in estuarine sediments from the south-western Spanish Coast. *Marine Pollution Bulletin* 42: 482–490.

SAHA SB, MITRA A, BHATTACHARYYA SB & CHUODHURY A. 2001. Status of sediment with special reference to heavy metal pollution of a brackish water tidal ecosystem in northern Sundarbans of West Bengal. *Tropical Ecology* 42(1): 127-132.

SCHANTZ SL. 1996. Developmental Neurotoxicity of PCBs in Humans: What Do We Know and Where Do We Go From Here? *Neurotoxicology and Teratology* 18(3): 217-227.

SCHMID P, KOHLER M, GUJER E, ZENNEGG M & LANFRANCHI M. 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. *Chemosphere* 67 S16-S21.

SCHWAIGER J, WANKE R, ADAM S, PAWERT M, HONNEN W & TRIEBSKORN R. 1997. The use of histopathological indicators to evaluate contaminant-related stress in fish. *Journal of Aquatic Ecosystem Stress and Recovery* 6: 73-86.

SEREDA BL & MEINHARDT HR. 2005. Contamination of the water environment in malaria endemic areas of KwaZulu-Natal, South Africa, by agricultural insecticides. *Environmental Contamination and Toxicology* 75: 530–537.

SHEPPARD SC, SHEPPARD MI, GALLERAND MO, SANIPELLIB. 2005. Derivation of ecotoxicity thresholds of uranium. *Journal of Environmental Radioactivity* 79: 55-83.

SIDHY KS. 2003. Heath benefits and potential risks related to consumption of fish or fish oil. *Regulatory Toxicology and Pharmacology* 38: 336-344.

SKELTON P. 2001. A complete guide to the freshwater fishes of Southern Africa. 2nd Edition. Cape Town: Struik Publishers. 395 p.

SPIES RB, STEGEMAN JJ, HINTON DE, WOODIN B, SMOLOWITZ R, OKIHIRO M & SHEA D. 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. *Aquatic Toxicology* 34: 195-219.

STRANDBERG B & HITES RA. 2001. Concentrations of organochlorine pesticides in winecorks. *Chemosphere* 44: 729-735.

SYDNEY HEALTH & FERTILITY. 2007. Mercury in Fish: Information Sheet. Date of access: 2 November 2012.

TAYLOR H, APPLETON JD, LISTER R, SMITH, CHITAMWEBA D, MKUMBO O, MACHIWA JF, TESHA AL & BEINHOFF. 2005. Environmental assessment of mercury contamination from the Rwamagasa artisanal gold mining centre, Geita District, Tanzania. *Science of the Total Environment* 343: 111-133.

TEH SJ, ADAMS SM & HINTON DE. 1997. Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquatic Toxicology* 37: 51-70.

THE STOCKHOLM CONVENTION ON PERSISTENT ORGANIC POLLUTANTS (POPs) 2011. http://chm.pops.int/Convention/The%20POPs/tabid/673/language/en-US/Default.aspx Date of access: 3 May 2011.

TOTTEN SM, MORAN SR & GROSS DL. 1969. Effect of Industrial Pollution on the Aquatic and Shore Angiosperm Flora in the Ottawa River, Allen and Putman Counties, Ohio. *The Ohio Journal of Science* 69 (4): 226-242.

TRAN TT, CHOWANADISAI W, LÖNNERDAL B, LE L, PARKER M, CHICZ-DEMET A & CRINELLA FM. 2002. Effects of neonatal dietary mangenese exposure on brain

dopamine levels and neurocognitive functions. *NeuroToxicity* 23: 645-651.

TSIPLAKOU E, ANAGNOSTOPOLOUS CJ, LAIPIS K, HAROUTOUNIAN SA & ZERVAS G. 2010. Pesticides residues in milks and feedstuff of farm animals drawn from Greece. *Chemosphere* 80: 504-512.

TURKEZ H, GEYIKOGLU, TATAR A, KELES MS & KAPLAN I. 2012. The effects of some boron compounds against heavy metal toxicity in human blood. *Experimental and Toxicologic Pathology* 64: 93-101.

UNEP. 2011. United Nations Environmental Programme, Chemicals: POPs. http://www.chem.unep.ch/POPs/default.htm Date of access: 3 May 2011.

UN FAO, UNITED NATIONS FOOD AND AGRICULTURE ORGANISATION. 2005. Crop and Food Supply Assessment Mission to Lesotho. ftp://ftp.fao.org/docrep/fao/008/J5513e/J5513e00.pdf Date of access: 16 March 2012.

UN FAO, UNITED NATIONS FOOD AND AGRICULTURE ORGANISATION. 2012. Fisheries and Aquaculture Department, Species fact sheet: *Clarias anguillaris*. http://www.fao.org/fishery/species/2197/en Date of access: 10 September 2012.

US DOI, UNITED STATES DEPARTMENT OF THE INTERIOR. 2009. United States Geological Survey, 2009, Minerals Yearbook: South Africa. minerals.usgs.gov/minerals/pubs/country/2009/myb3-2009-sf.pdf Date of access: 9 March 2012.

URBACH J. 2007. Inconclusive study on DDT has potentially mortal consequences. Human Health Policy. May 2007. http://www.healthpolicyunit.org/scripts/documents Date of access: 3 October 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROCTETION AGENCY. 1994. Method 1613 Tetra- through octa-chlorinated dioxins and furans by isotope Dilution HRGC/HRMS. http://www.epa.gov/region9/qa/pdfs/1613.pdf Date of access: 21 April 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROCTETION AGENCY. 1996.

Method 3050B Acid digestion of sediment, sludges, and soils. http://www.epa.gov/wastes/hazard/testmethods/sw846/pdfs/3050b.pdf Date of access 4 May 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROCTETION AGENCY. 1997. Mercury study report to congress, Volume VII: Characterization of human health and wildlife risks from mercury exposure in the United States. Date of access: 2 November 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROCTETION AGENCY. 2000a. Guidance for assessing contaminant data for use in fish advisories. Volume 1: Fish sampling and analysis. 3rd ed. water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume1_v1cover.pdf Date of access: 16 April 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2000b. Guidance for assessing chemical contaminant data for use in fish advisories, Volume 2: Risk assessment and fish consumption limits third edition. http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume2_v2cover.pdf Date of access: 19 June 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROCTETION AGENCY, 2008. Method 1668B Chlorinated biphenyl congeners in water, soil, sediment, biosolids, and tissue by HRGC/HRMS. water.epa.gov/scitech/methods/cwa/bioindicators/upload/2009_01_07_methods_method_1668.pdf DOA: 16 April 2012.

US EPA, UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2009. Persistent organic pollutants: A global issue, a global response. http://www.epa.gov/oia/toxics/pop.html Date of access: 9 May 2011.

US EPA, UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2012. http://www.epa.gov/iris/rfd.htm Date of access: 3 November 2012.

USERO J, GONZÁLEZ-REGALADO E & GRACIA I. 1996. Trace metals in the bivalve *Chamelea gallina* from the Atlantic coast of southern Spain. *Marine Pollution Bulletin*

32(3) 305-310.

VAN AARDT WJ & BOOYENS J. 2004. Water hardness and the effects of Cd on oxygen consumption, plasma chlorides and bioaccumulation in *Tilapia sparrmanii. Water SA* 30 (1): 57-64.

VAN AARDT WJ & ERDMANN R. 2004. Heavy metals (Cd, Pb, Cu, Zn) in mudfish and sediments from three hard-water dams of the Mooi River catchment, South Africa. *Water SA* 30(2): 211-218.

VAN AARDT WJ & HOUGH M. 2007. The effect of short-term Cu exposure on the oxygen consumption and Cu accumulation of mudfish (*Labeo capensis*) and the largemouth bass (*Micropteris salmoides*) in hard water. *Water SA* 33 (5): 701-708.

VAN DYK JC, PIETERSE GM & VAN VUREN JHJ. 2007. Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. *Ecotoxicology and Environmental Safety* 66: 432–440.

VAN DYK JC, MARCHAND MJ, SMIT NJ & PIETERSE GM. 2009a. A histology-based fish health assessment of four commercially and ecologically important species from the Okavango Delta panhandle, Botswana. *African Journal of Aquatic Science* 34(3): 273-282.

VAN DYK JC, MARCHAND MJ, PIETERSE GM, BARNHOORN IEJ & BORNMAN MS. 2009b. Histological changes in the gills of *Clarias gariepinus* (Teleostei: Clariidae) from a polluted South African urban aquatic system. *African Journal of Aquatic Science* 34(3): 283-291.

VAN DYK JC, COCHRANE MJ & WAGENAAR GM. 2011. Liver histopathology of the sharptooth catfish *Clarias gariepinus* as a biomarker of aquatic pollution. *Chemosphere* 87(4): 301-311.

VAN STEENDEREN RA, THERON SJ & HASSER AJ. 1987. The occurrence of organic micro-pollutants in the Vaal River between Grootdraai Dam and Parys. *Water SA* 13(4):209-214.

VAN WYK F. 2001. An integrated manual for the management, control and protection of the Vaal River Barrage Reservoir. Unpublished M.Sc Thesis. Rand Afrikaans University. Aucklandpark. https://ujdigispace.uj.ac.za/handle/10210/1981 Date of access: 9 May 2011.

VAROL M. 2011. Assessment of heavy metal contamination in sediments of the Tigris River (Turkey) using pollution indices and multivariate statistical techniques. *Journal of Hazardous Materials* 195: 355-364.

VELMURUGAN B, SELVANAYAGAM M, CENGIZ EI & UNLU E. 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environmental Toxicology and Pharmacology* 24: 286-291.

VINCETI M, WEI ET, MAGAGOLI C, BERGOMI C & VIVOLI G. 2001. Adverse health effects of selenium in humans. *Reviews of Environmental health* 16(4): 233-251.

VÖLKEL W, GENZEL-BOROVICZÉNY O, DEMMELMAIR H, GEBAUER C, KOLETZKO B, TWARDELLA D, RAAB U & FROMME H. 2008. Perfluorooctane sulphonate (PFOS) and Perfluorooctanoic acod (PFOA) in human breast milk: Results of a pilot study. *Internationa Journal of Hygiene and Environmental Health* 211: 440-446.

WAGNER A & BOMAN J. 2003. Biomonitoring of trace elements in muscle and liver tissue of freshwater fish. *Spectrochimicia Acta Part B* 58: 2215-2226.

WEPENER V, VAN DYK C, BERVOETS L, O'BRIEN G, COVACI A & CLOETE Y. 2011. An assessment of the influence of multiple stressors on the Vaal River, South Africa. *Physics and Chemistry of the Earth* 36: 949-962.

WHO, WORLD HEALTH ORGANIZATION. 2006. Elemental speciation in human health risk assessment, Environmental health Criteria 234. Date of access: 2 November 2012.

WHO, WORLD HEALTH ORGANIZATION. 2007. Exposure to mercury: a major public health concern. Date of access: 2 November 2012.

WHO, WORLD HEALTH ORGANIZATION. 2012. Mercury and health Fact sheet N361. http://www.who.int/mediacentre/factsheets/fs361/en/index.html Date of access: 12 September 2012.

WOLF JC & WOLFE M. 2005. A brief overview of non-neoplastic hepatic toxicity in Fish. *Toxicologic Pathology* 33: 75-85.

WRC Water Research Commission. 2009. Vaal River water quality: A matter of life... http://www.wrc.org.za/News/Pages/VaalRiverwaterqualityAmatteroflife.aspx Date of access: 7 May 2011.

YAMAKZAKI M, TANIZAKI Y & SHIMOKAWA T. Silver and other trace metals in a freshwater fish, *Carasius auratius langsdorfii*, from the Asakawa Riverin Tokyo, Japan. *Environmental Pollution* 94(2): 83-90.

YAO Y, MASUNAGA S, TAKADA H & NAKANISHI J. 2002. Identification of polychlorinated dibenzo-*p*-dioxin and coplanar polychlorinated biphenyl sources in Tokyo Bay, Japan. *Environmental Toxicology and Chemistry* 21(5): 991-998.

YEUNG LWY, YAMASHITA N, TANIYASU S, LAM PKS, SINHA RK, BOROLE DV & KANNAN K. 2009. A survey of perfluorinated compounds in surface water and biota including dolphins from the Ganges River and in other waterbodies in India. *Chemosphere* 76: 55-62.

ZIMMERLI S, BERNET D, BURKHARDT-HOLM P, SCHMIDT-POSTHAUS H, VONLANTHEN P, WAHLI T & SEGNER H. 2007. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. *Aquatic Sciences* 69: 11-25.

Table 16: Concentrations of POPs, TEF and TEQ value from sediment of the Orange-Vaal River system

	Sediment (ng/kg)					TEQ values (TEQng/kg)			
Compound	Boegoeberg	Rooipoort	Parys	Standerton	TEF values	Boegoeberg	Rooipoort	Parys	Standerton
2,3,7,8-TCDD	0.05	0.05	0.05	0.05	1	0.05	0.05	0.05	0.05
1,2,3,7,8-PeCDD	0.05	0.05	0.05	0.05	1	0.05	0.05	0.05	0.05
1,2,3,4,7,8-HxCDD	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
1,2,3,6,7,8-HxCDD	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
1,2,3,7,8,9-HxCDD	0.05	0.05	0.05	0.06	0.1	0.005	0.005	0.005	0.006
1,2,3,4,6,7,8-HpCDD	0.15	0.15	0.76	0.15	0.01	0.0015	0.0015	0.0076	0.0015
OCDD	0.7	0.5	5.4	0.7	0.0003	0.00021	0.00015	0.00162	0.00021
2,3,7,8-TCDF	0.05	0.05	0.07	0.10	0.1	0.005	0.005	0.007	0.010
1,2,3,7,8-PeCDF	0.05	0.05	0.05	0.05	0.03	0.002	0.002	0.002	0.002
2,3,4,7,8-PeCDF	0.05	0.05	0.05	0.05	0.3	0.015	0.015	0.015	0.015
1,2,3,4,7,8-HxCDF	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
1,2,3,6,7,8-HxCDF	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
1,2,3,7,8,9,-HxCDF	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
2,3,4,6,7,8-HxCDF	0.05	0.05	0.05	0.05	0.1	0.005	0.005	0.005	0.005
1,2,3,4,6,7,8-HpCDF	0.15	0.15	0.15	0.15	0.001	0.00015	0.00015	0.00015	0.00015
1,2,3,4,7,8,9-HpCDF	0.15	0.15	0.15	0.15	0.001	0.00015	0.00015	0.00015	0.00015
OCDF	0.5	0.5	0.5	0.5	0.0003	0.00015	0.00015	0.00015	0.00015
PCB 77	3	3	5	3	0.0001	0.0003	0.0003	0.0005	0.0003
PCB 81	0.1	0.1	0.3	0.1	0.0003	0.00003	0.00003	0.00009	0.00003
PCB 126	0.1	0.1	0.1	0.1	0.1	0.01	0.01	0.01	0.01
PCB 169	0.1	0.1	0.1	0.1	0.03	0.003	0.003	0.003	0.003
PCB 105	3	3	7	3	0.00003	0.00009	0.00009	0.00021	0.00009
PCB 114	1	1	1	1	0.00003	0.00003	0.00003	0.00003	0.00003
PCB 118	10	10	23	10	0.00003	0.00030	0.00030	0.00069	0.00030
PCB 123	1	1	1	1	0.00003	0.00003	0.00003	0.00003	0.00003
PCB156	1	1	3	1	0.00003	0.00003	0.00003	0.00009	0.00003
PCB157	1	1	1	1	0.00003	0.00003	0.00003	0.00003	0.00003
PCB 167	1	1	1	1	0.00003	0.00003	0.00003	0.00003	0.00003
PCB 189	1	1	1	1	0.00003	0.00003	0.00003	0.00003	0.00003
					Σ TEQs	0.17256	0.17250	0.18290	0.17856

Table 17: Geo-accumulation index for heavy metals in the sediment from sites in the Orange- and Vaal Rivers

	Igeo Bg	Igeo Rp	Igeo Ps	Igeo Sdn
Cr	-1.65	0.67	-0.58	0.74
Mn	-1.91	-0.08	-1.40	-0.12
Fe	-1.90	-1.05	-3.21	-1.19
Co	-0.84	0.20	-0.69	0.42
Ni	0.27	1.86	0.43	1.43
Cu	-0.09	0.76	-0.25	0.99
Zn	-1.32	-0.54	-1.26	-0.57
As	-0.06	1.06	-0.13	0.50
Se	3.73	6.53	6.43	6.56
Ag	5.06	6.81	5.06	5.41
Cd	5.62	3.56	2.87	2.50
Pt	-1.26	-0.32	-1.77	-0.98
Au	3.44	2.57	2.06	1.57
Hg	-1.93	-2.19	-3.29	-2.17
Pb	-2.46	-1.33	-2.77	-0.84
U	-3.27	2.76	-3.32	-1.77

Igeo scale	
0<	unpolluted
0-1	unpolluted
1-2	moderately to unpolluted
2-3	moderately polluted
3-4	moderately to highly polluted
4-5	highly polluted
>5	very highly polluted

Table 18: Metal concentrations in *Clarias gariepinus* from all sites

Boegoeberg

mg/kg	Mean	Min	Max	Stdev
Cr	0.211	0.16	0.27	±0.0317
Mn	0.056	0.046	0.064	±0.0054
Fe	1.67	1.4	2	±0.2263
Co	0.00392	0.0018	0.0091	±0.0024
Ni	0.0337	0.025	0.05	±0.0082
Cu	0.063	0.036	0.14	±0.342
Zn	0.657	0.52	0.92	±0.141
As	0.0503	0.044	0.061	±0.005
Se	0.1397	0.097	0.21	±0.039
Ag	4.65	3.7	5.7	±0.675
Cd	0.002301	0.00046	0.0077	±0.0024
Pt	0.01984	0.0032	0.069	±0.023
Au	0.2527	0.069	0.89	±0.259
Hg	0.0725	0.052	0.11	±0.016
Pb	0.01636	0.008	0.035	±0.0112
U	0.01913	0.0024	0.069	±0.0234

Rooipoort

mg/kg	Mean	Min	Max	Stdev
Cr	0.268	0.25	0.28	±0.009
Mn	0.0458	0.039	0.064	±0.007
Fe	1.38	1.1	1.7	±0.209
Co	0.00144	0.0012	0.0018	±0.0002
Ni	0.0253	0.022	0.031	±0.002
Cu	0.042	0.029	0.064	±0.0098
Zn	0.632	0.45	0.99	±0.166
As	0.0552	0.051	0.061	±0.003
Se	0.1119	0.085	0.16	±0.02
Ag	2.65	2.2	3.2	±0.29
Cd	0.000281	0.00023	0.00074	±0.0002
Pt	0.00207	0.0012	0.0039	±0.0008
Au	0.0273	0.023	0.034	±0.003
Hg	0.0411	0.032	0.058	±0.008
Pb	0.00608	0.0056	0.0067	±0.0003
U	0.000954	0.00048	0.0015	±0.0003

Parys

mg/kg	Mean	Min	Max	Stdev
Cr	0.252	0.23	0.27	±0.011
Mn	0.0471	0.036	0.057	±0.005
Fe	1.73	1.4	2.3	±0.27
Co	0.00192	0.0013	0.0027	±0.0004
Ni	0.0254	0.024	0.027	±0.0009
Cu	0.0449	0.032	0.065	±0.011
Zn	0.689	0.44	1.1	±0.17
As	0.049	0.044	0.054	±0.003
Se	0.161	0.12	0.2	±0.026
Ag	3.4	2.7	3.9	±0.385
Cd	0.000286	0.00023	0.00056	±0.0001
Pt	0.00245	0.0017	0.004	±0.0007
Au	0.0449	0.03	0.054	±0.0075
Hg	0.0464	0.04	0.054	±0.0058
Pb	0.00698	0.0056	0.0087	±0.001
U	0.001204	0.00081	0.0019	±0.0004

Standerton

mg/kg	Mean	Min	Max	Stdev
Cr	0.291	0.27	0.36	±0.031
Mn	0.0494	0.04	0.073	±0.001
Fe	1.61	1.1	2.3	±0.41
Co	0.00286	0.0021	0.004	±0.0006
Ni	0.0278	0.025	0.031	±0.0022
Cu	0.0487	0.031	0.12	±0.0265
Zn	0.552	0.33	0.93	±0.175
As	0.0596	0.051	0.071	±0.0077
Se	0.0771	0.035	0.13	±0.025
Ag	1.811	0.91	2.4	±0.601
Cd	0.000291	0.00023	0.00084	±0.0002
Pt	0.001385	0.00055	0.0026	±0.0007
Au	0.01818	0.0088	0.023	±0.006
Hg	0.0329	0.011	0.059	±0.015
Pb	0.00583	0.0022	0.011	±0.003
U	0.000654	0.00013	0.0012	±0.00034

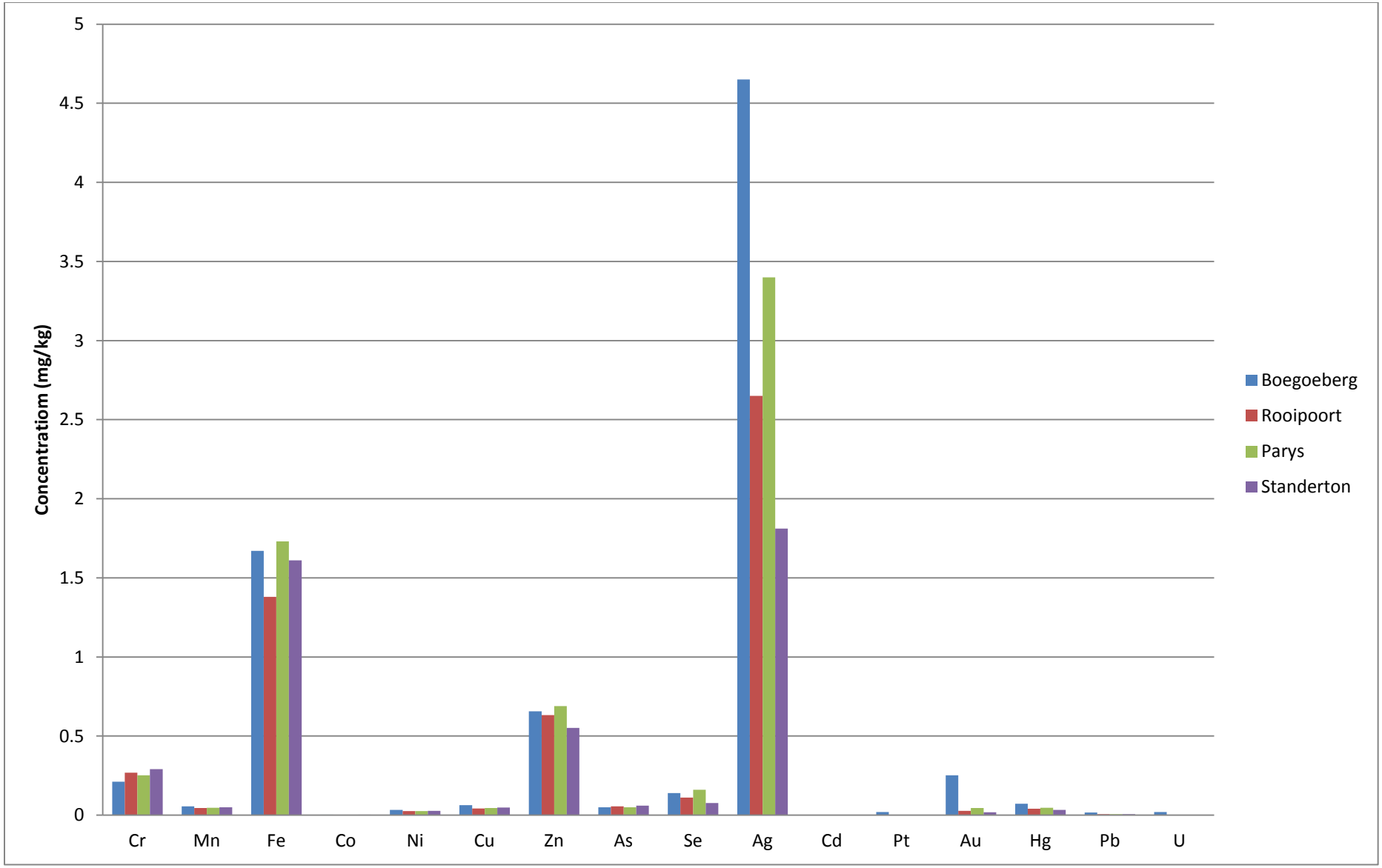

Figure 27: Metal concentrations in *Clarias gariepinus* from all sites

Table 19: Quality guidelines for metals in fish for human consumption as suggested by EU, 2006 , and Wagner & Boman (2003)

Metal	Cu	Zn	As	Se	Cd	Hg	Pb
mg/kg	10-100	40-100	0.1-5	0.3-2	5 E-04	0.5	0.5-10
Bg1	0.058	0.57	0.061	0.21	0.0077	0.11	0.035
Bg2	0.14	0.64	0.055	0.12	0.0045	0.07	0.033
Bg3	0.11	0.81	0.052	0.18	0.0042	0.078	0.029
Bg4	0.048	0.57	0.051	0.13	0.00074	0.062	0.011
Bg5	0.046	0.57	0.044	0.1	0.0011	0.078	0.01
Bg6	0.054	0.82	0.047	0.19	0.0013	0.064	0.0094
Bg7	0.04	0.52	0.049	0.11	0.0018	0.067	0.0098
Bg8	0.057	0.92	0.046	0.13	0.00056	0.058	0.0097
Bg9	0.036	0.52	0.051	0.097	0.00065	0.086	0.0087
Bg10	0.041	0.63	0.047	0.13	0.00046	0.052	0.008
Rp1	0.038	0.49	0.055	0.16	<4.6E-4	0.044	0.0063
Rp2	0.029	0.45	0.061	0.14	<4.6E-4	0.034	0.0056
Rp3	0.042	0.63	0.06	0.09	<4.6E-4	0.058	0.0065
Rp4	0.036	0.52	0.053	0.11	<4.6E-4	0.049	0.0056
Rp5	0.043	0.99	0.053	0.098	0.00074	0.045	0.006
Rp6	0.051	0.77	0.054	0.085	<4.6E-4	0.041	0.0056
Rp7	0.033	0.5	0.051	0.1	<4.6E-4	0.041	0.006
Rp8	0.041	0.59	0.054	0.12	<4.6E-4	0.035	0.0067
Rp9	0.043	0.62	0.056	0.096	<4.6E-4	0.032	0.0061
Rp10	0.064	0.76	0.055	0.12	<4.6E-4	0.032	0.0064
Ps1	0.032	0.44	0.049	0.2	<4.6E-4	0.053	0.0056
Ps2	0.042	0.62	0.046	0.15	<4.6E-4	0.053	0.0087
Ps3	0.06	0.7	0.048	0.16	0.00056	0.041	0.0078
Ps4	0.048	0.78	0.048	0.19	<4.6E-4	0.04	0.006
Ps5	0.046	0.63	0.049	0.16	<4.6E-4	0.054	0.008
Ps6	0.038	0.52	0.048	0.13	<4.6E-4	0.041	0.0069
Ps7	0.037	0.78	0.05	0.15	<4.6E-4	0.05	0.0072
Ps8	0.046	0.64	0.054	0.16	<4.6E-4	0.044	0.0074
Ps9	0.035	0.68	0.054	0.12	<4.6E-4	0.048	0.0064
Ps10	0.065	1.1	0.044	0.19	<4.6E-4	0.04	0.0058
Sdn1	0.038	0.55	0.052	0.088	<4.6E-4	0.034	0.0073
Sdn2	0.041	0.56	0.052	0.071	<4.6E-4	0.035	0.0056
Sdn3	0.062	0.93	0.051	0.098	<4.6E-4	0.039	0.006
Sdn4	0.048	0.54	0.057	0.082	<4.6E-4	0.046	0.011
Sdn5	0.039	0.6	0.053	0.066	<4.6E-4	0.038	0.0058
Sdn6	0.038	0.52	0.069	0.075	<4.6E-4	0.04	0.0062
Sdn7	0.12	0.71	0.059	0.067	0.00084	0.059	0.0092
Sdn8	0.035	0.35	0.071	0.13	<4.6E-4	0.011	0.0026
Sdn9	0.031	0.33	0.066	0.059	<4.6E-4	0.013	0.0024
Sdn10	0.035	0.43	0.066	0.035	<4.6E-4	0.014	0.0022