Polycyclic aromatic hydrocarbons (PAHs) in the aquatic ecosystems of Soweto/Lenasia

Report to the

WATER RESEARCH COMMISSION

by

Wihan Pheiffer[1], Rialet Pieters[1], Bettina Genthe[2], Laura Quinn[3], Henk Bouwman[1] & Nico Smit[1]

[1]North-West University

[2]Council for Scientific and Industrial Research

[3]National Metrology Institute of South Africa

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Water Research Commission

Private Bag X03

Gezina, 0031

orders@wrc.org.za or download from www.wrc.org.za

EXECUTIVE SUMMARY

BACKGROUND

Polycyclic aromatic hydrocarbons (PAHs) consist of fused benzene rings and the congeners have varying numbers of benzene rings, usually between two and six. They have a widespread distribution due to their formation by incomplete combustion of organic materials and are continuously released into the environment making them ever-present. The US EPA has earmarked 16 congeners that must be monitored and controlled because of their proven harmful effects on humans and wildlife. Anthropogenic activities largely increase the occurrence of these pollutants in the environment. A measurable amount of these PAHs are expected to find their way into aquatic ecosystems.

RATIONALE

In a previous study completed for the Water Research Commission (Project no K5/1561) on persistent organic pollutants in freshwater sites throughout the entire country, the PAHs had the highest levels of all of the organic pollutants analysed for. According to this study Soweto/Lenasia was particularly burdened with high PAH levels which was the main motivation for further, in-depth investigation into this area, focussing on the PAHs only.

OBJECTIVES AND AIMS

In the current study (K5/2242) the potential exposure of humans and wildlife to the 16 priority PAHs, was investigated. The sites were selected in the suburban areas of Moroka, Lenasia, Fleurhof, Eldorado Park, Orlando West, Orlando East, Nancefield and Dobsonville. The sites were named after these respective areas.

AIM 1

The main aim of this study was to determine the levels of the 16 priority PAHs in the Klip River that flows through the densely populated urban areas of Soweto and Lenasia.

AIM 2

In addition, the pollutant profile of the 16 parent PAHs in the sediments was investigated, by comparing site PAH composition percentages to determine origin of the pollution, i.e. pyrogenic vs petrogenic.

AIM 3

The final aim of this study was to determine the toxicity posed by the PAHs in the study area. This was done by assessing the sediments against sediment quality guidelines and quality indices. A very specific mechanism of toxicity: that mediated via the cellular receptor the aryl hydrocarbon receptor, was also investigated. The biochemical responses and overall health of the fish was also investigated. Finally, the potential risk to human health was gauged using a health assessment index.

METHODS

The levels of the PAHs in the sediment were determined by instrumental analysis in the sediment, fish tissue, and wetland bird eggs sampled within the study area. The PAHs were extracted using pressurised liquid extraction. The extracts was fractioned – to isolate the PAH containing fraction – using size exclusion. The extract was cleaned up with a silica/Florisil solid phase extraction (SPE) column. From the final extract, The PAHs were quantified with gas chromatography and time of flight mass spectrometry (GC-TOFMS).

The pollutant profile of the PAHs in the sediment was further extended into calculation of percentage congener contribution. Along with this, the percentage of low- and high molecular mass PAHs (LPAHs, HPAHs) and “carcinogenic” PAHs (CPAHs) were determined. The potential origins of PAHs measured were identified using diagnostic ratios.

The sediment toxicity was evaluated by comparing the levels to international sediment quality guidelines. The sediments were also assessed with sediment quality indices which describe sediment quality and ecological risk to benthic fauna. The investigation of the toxicity via the aryl hydrocarbon receptor was measured using the H4IIE-*luc* reporter gene bio-assay.

The effect on fish was explored by performing biomarker response assays. These included: acetylcholinesterase- and cytochrome P450 activity, malondialdehyde- and protein carbonyl content, as well as catalase and superoxide dismutase activity. Biomarkers reflect the biochemical responses to environmental stressors. Along with this, individual and community fish health was assessed using various indices: fish health assessment index and organo-somatic indices.

Finally, the potential health risk to the human population dependant on the water bodies in the study area was gauged by conducting a theoretical human health risk assessment.

RESULTS AND DISCUSSION

The chemical analysis of PAHs on the sediment, fish and bird eggs confirmed the presence of PAHs in the study area. The sediments showed significant levels of PAHs however analysis for PAHs in the biota produced little or no data – due to effective metabolism of the parent isomers.

The pollutant profile of the sediments indicated that the dominant sources of PAHs in the Soweto/Lenasia area are of pyrogenic origins, specifically from the burning of biomass and to a smaller extent, petroleum combustion, probably from vehicles in urban areas. The site of greatest concern was the site in the Moroka area. Other sites that were also of concern are Lenasia, Fleurhof, Eldorado Park and Orlando West as well as Orlando East. Moroka had the highest PAHs levels for both seasons and exceeded most of the sediment quality guidelines. The quality indices revealed the same results: Moroka scored the highest values for the sediment quality guideline index (SQG-I), indicating that this site poses a high ecological risk to benthic biota. Similarly, Fleurhof, Orlando West, Lenasia, and Eldorado Park showed a high probability of being toxic to benthic biota when considering the SQG-I. The sediment quality index (SQI) based on the chemical analysis data, indicate the quality of the sediments in terms of the PAH loads and according to this all the sites are of poor quality. In conjunction to these toxicity assessments the toxic equivalents (TEQs) of the study area (for both years) were all higher than the lower, interim sediment quality guideline (for the protection of fish) of Canada, except for Nancefield 2013. Moroka 2013 sediment exceeded also the higher probable effects level (PEL) of the Canadian guideline. The toxicity assessments identified Moroka's sediment as the site with the highest probable toxicity to both benthic organisms and fish.

In comparison to the TEQs calculated for the sediments, the bio-assay equivalents (BEQs) of the sites also showed that Moroka (2013) elicited the highest response in the H4IIE-*luc* bio-assay. However, the 2014 samples had Lenasia as the highest BEQ, far higher than Moroka, which was the second highest.

The biomarker results cannot be exclusively ascribed to the PAHs in the aquatic environment, because other compounds also present might have been – or contributed to – the cause of the responses observed in the biomarkers. Also, it was impossible to tell how much PAHs the individual fish were exposed to as they metabolised the PAHs and due to budget constraints the metabolites could not be quantified Even though it would be theoretically possible to relate the cytochrome P450 (CYP450) results to the TEQ and BEQ – which measures the same toxic mechanism of action – no clear relationship was seen.

The highest CYP450 response was from Orlando, and its sediment (Orlando East) had the second highest TEQ value for the season. The other biomarkers indicated that the fish were exposed to compounds that elicited selected responses, specifically the inhibition of acetylcholinesterase activity and the up-regulation of the catalase system.

The health assessment of the fish also corroborated that they most like were affected by xenobiotics. The condition factor showed that the fish were all in fair to good condition. The high prevalence of abnormalities in the livers (discolouration, deformation and fat deposition) seems to indicate to contaminant metabolism and the enlargement of the spleens in most fish – of which none had parasitic infections – supports the deduction that the health effects are from chemical contamination rather than natural factors (parasites, mechanical damage and malnutrition).

Possible health risk to humans consuming fish from the study area was investigated by conducting human health risk assessment by modelling risk from oral exposure. PAH levels in fish were extrapolated from levels found in the sediment. Benzo(a)pyrene and dibenz(a,h)anthracene were identified as the chemicals of concern even when they did not occur in high concentrations. The risk calculated at each site showed that there is no risk to humans living in the study area, contradictory to the previous WRC study in the area, on which this study is based.

The results obtained in this study indicate that there is a definite presence of PAHs in the Klip River of Soweto/Lenasia. The site that created the most concern based on the chemical analysis and toxicity assessments was Moroka followed by Lenasia and Eldorado Park. The biochemical responses and health assessment of the fish indicated that there are stressors present in the system – not necessarily PAHs – that activated the cytochrome P450s, inhibited neurotransmission enzymes, increased the anti-oxidation systems, and decreased the overall health. However, it seems that the humans in the area are not at risk of exposure to PAHs, at least not through ingestion of the fish from the area.

CONCLUSIONS

- The presence of PAHs in the sediments of Soweto/Lenasia was confirmed by the chemical analysis.
- The sources of these PAHs have been narrowed down to pyrogenic sources, mainly from biomass combustion. The ratios also identified petroleum combustion as a source of the HPAHs and this is most probably from vehicles as the study areas is situated in an urban area.

- The site of greatest concern is Moroka. Other sites that are of concern are Lenasia, Fleurhof, Eldorado Park and Orlando West as well as Orlando East. Even though Protea Glen had high CPAHs, according to the toxicological assessment of this site it ranked lower than the other sites. Moroka had the highest PAHs levels (ΣPAHs, ΣLPAHs, ΣHPAHs, and ΣCPAHs) of all the sites. It was also the site that ranked the highest in all the toxicological assessments – exceeding most guidelines, especially the PEL of the Canadian guidelines.
- For both the 2013 and 2014 seasons, Moroka had the highest SQG-I score, indicating that the site's sediment posed a high ecological risk to benthic biota. The SQI corresponds to the chemical analysis and guidelines – scoring the sediment quality of this site as poor in terms of PAH pollution. Lenasia and Eldorado Park also had high levels of PAHs. The 2014 sediments of these sites exceeded both sets of guidelines.
- The toxic equivalent quotients (TEQs) of samples from the study area (2013 & 2014) were all higher than the lower guideline (ISQG: for the protection of fish), except Nancefield 2013. Moroka 2013 sediments had the highest TEQ – that exceeded the higher guideline (PEL) – and in conjunction with the other toxicological tests indicates that this site posed a serious threat to biota, specifically benthic organisms and fish.
- Even though the chemical analysis of the fish and bird egg samples produced little to no quantifiable data in terms of the parent PAHs, there is evidence that there are PAHs present in the system – high sediment loads.
- The biomarker responses are difficult to appoint to specific exposure due to the lack of chemical data in the fish. Cytochrome P450 activity in the fish can be compared to the TEQ and BEQ of the sediment data, seeing that the same mode of action is used (Ah-receptor mediated responses). One would expect the cytochrome activity to correspond to the TEQ and BEQ, but contradicting responses were observed for Fleurhof (2013) as well as Nancefield (2013): the lowest CYP450 response was in fish from Fleurhof (2013), which in turn had the highest TEQ and BEQ results for the sediment for the same year. The second highest 2013 CYP450 response was from the fish from Nancefield but its sediment had the lowest TEQ and BEQ values. Some of this discrepancy could be attributed to the fact that fish were sampled from dams and sediment from streams feeding the dams, and although the sampling sites were in close proximity to each other this might explain the observed differences. This discrepancy was unexpected as we assumed that the transportation of the PAHs to sites close together would be the same. The expectation of having high CYP450 responses from coinciding high TEQ and BEQ levels in surrounding sediment was

met for Orlando: the highest CYP450 response was from Orlando, and its sediment (Orlando East) had the second highest TEQ value for the same year.

- The other biomarkers indicated that there were compounds present in the study area that elicited responses in the fish, specifically the inhibition of acetylcholinesterase activity and the up-regulation of the catalase system that could probably not be ascribed to PAH levels.

RECOMMENDATIONS FOR FUTURE RESEARCH

- The chemical analysis of the metabolised PAHs would complete the picture of what is happening to the parent PAHs after entering the animals' bodies. This would however, necessitate more funding because these analytical standards are expensive and not always readily available in South Africa. Each of the 16 parent PAHs has more than two metabolites that could be quantified chemically increasing the analytical load and associated expenses.
- The biomarker response results could not conclusively be attributed to the PAHs, and therefore a broad spectrum screening for a much larger variety of organic chemical pollutants is advised for this densely populated area of Gauteng. Chemical compounds that can be considered include: polychlorinated biphenyls, brominated flame retardants, organochlorine pesticides, plasticisers, pharmaceuticals and personal care products and perfluorinated compounds, just to name a few compound classes.
- The number of bio-assays can be broadened to include assays capable of detecting endocrine disruptive effects.
- Evaluation of fish species composition and numbers to further describe pollution effects in the system.
- Add a social component to the study in which the human population's physical interaction and dependence on the Klip River running through Soweto/Lenasia is quantified, i.e. using questionnaires and interviewing the citizens.
- Incorporating results from this study into management of this water catchment one must keep in mind that PAHs are mainly airborne. Therefore, a successful monitoring program of any water catchment for these compounds would require an integrated approach including air quality monitoring.

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Prof J van Vuren	University of Johannesburg (Reference committee)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
Acea	Acenaphthene
Acey	Acenaphthylene
AChE	Acetylcholine esterase
AHH	Aryl hydrocarbon hydroxylase
AhR	Aryl-hydrocarbon receptor
Ant	Anthracene
ARNT	Aryl hydrocarbon receptor nuclear translator
ASE	Accelerated solvent extraction
BaA	Benzo(a)anthrancene
BaP	Benzo(a)pyrene
BbF	Benzo(b)fluoranthene
BgP	Benzo(ghi)perylene
BkF	Benzo(k)fluoranthene
BSA	Bovine serum albumin
CAT	Catalase activity
CCME	Canadian Council of Ministers of the Environment
Chr	Chrysene
CPAH	Carcinogenic polycyclic aromatic hydrocarbon
CRM	Certified reference material
CYP450	Cytochrome P450
Db	Dobsonville
DBA	Dibenzo(ah)anthracene
DCM	Dichloromethane
ddH_2O	Double distilled water
dm	Dry mass
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulphoxide

DNPH	2,4-Dinitrophenylhydrazine
DRE	Dioxin response element
dSPE	Dispersive solid phase extraction
DTPA	Diethylene triamine penta-acetic acid
EC	Effective concentration
ECOD	7-ethoxycoumarin-O-deethylase
EDTA	Ethylene diamine tetra-acetic acid
ElD	Eldorado Park
ELISA	Enzyme linked immuno-sorbent assay
EROD	Ethoxyresorufin-O-deethylase
ETS	Electron transport system
FBS	Foetal bovine serum
Fl	Fleurhof
Fla	Fluoranthene
Flu	Fluorene
GCXGCMS-TOF	Tandem gas-chromatography mass spectrometry time of flight
GCMS-TOF	Gas-chromatography mass spectrometry time of flight
GHB	General homogenising buffer
GPC	Gel permeation chromatography
GSH	Glutathione
GST	Glutathion-S-transferase
HDPE	High density polyethylene
HPAH	High molecular mass polycyclic aromatic hydrocarbon
InP	Indeno(1,2,3-cd)pyrene
ISQG	Interim sediment quality guideline
LAR	Luciferase activating reagent
Le	Lenasia
LOD	Limit of detection
LOQ	Limit of quantification
LPAH	Low molecular mass polycyclic aromatic hydrocarbon

MDA	Malondialdehyde
MeOH	Methanol
Mo	Moroka
MTT	3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
Nap	Naphthalene
NIST	National Institute of Standards and Technology
NMISA	National Metrology Institute of South Africa
NWU	North-West University
OD	Optical density
OE	Orlando East
OW	Orlando West
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate buffered saline
PC	Protein carbonyls
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PEC	Probable effects concentration
PEL	Probable effects level
PG	Protea Glen
Phe	Phenanthrene
PLE	Pressurised liquid extraction
PMSF	Phenyl methanesulphonyl fluoride
POPs	Persistent Organic Pollutants
Pyr	Pyrene
QuEChERS	Quick, Easy, Cheap, Rugged and Safe
REP	Relative effective potency
RLU	Relative light units
ROS	Reactive oxygen species

SDS	Sodium dodecyl sulphate
SOD	Superoxide dismutase
SPE	Solid phase extraction
SQG-I	Sediment quality guideline index
SQI	Sediment quality index
TCA	Trichloroacetic acid
TCDD	Tetrachlorodibenzo-*p*-dioxin
TEC	Threshold effects concentration
TEF	Toxic equivalent factors
TEQ	Toxic equivalent quotient
TMP	1,1,3,3- Tetramethoxypropane
TOC	Total organic carbon
UDP-GT	Uridine 5-diphosphate-glucuronosyltransferase
US EPA	United States Environmental Protection Agency
v/v	Volume/volume
WWTP	Waste water treatment plant

1 INTRODUCTION AND OBJECTIVES

1.1 South Africa's water and pollution dilemma

South Africa is a water scarce country and is ranked as the 30th driest country in the world. Even though water scarcity is a global challenge, sub-Saharan Africa, especially southern Africa is hardest hit (Cessford et al., 2005; DWA, 2014). Brand et al. (2009) states that South Africa over-utilized its water resources because of the attitude that these resources are inexhaustible. It is believed that many parts of South Africa has reached or are approaching the point where viable freshwater resources are fully utilised (Cessford et al., 2005; DWA, 2014) placing our ecosystems under immense pressure (Dallas & Day, 2004). Anthropological influences like pollution, misuse and poor management of water resources created environmental problems such as poor water quality and the diminishing of ecosystem health (Brand et al., 2009).

The quantity of our water resources are already under pressure and the decrease in quality escalates the problem. According to the South African National Water Act (Act 36 of 1998) we need to implement monitoring programs to assess aquatic ecosystem health. This, if implemented correctly and efficiently, along with resource management, will promote and support the improvement of aquatic ecosystems.

As a consequence of the above many studies have reported on water quality of South African and the effect pollution has on the aquatic environment. These studies focussed on industrial and agricultural pollutants (Ansara-Ross et al., 2012; Du Preez et al., 2005; Schulz & Peall, 2001) and heavy metals (Jooste et al., 2014; Kotze et al., 1999; Van Aardt & Erdmann, 2004). However, there is a paucity in the knowledge of organic pollutants of industrial origins in South African systems, and lately some studies have been conducted to fill this knowledge gap (Barnhoorn et al., 2010; 2015; Nieuwoudt et al., 2009; 2011; Quinn et al., 2009).

1.2 Polycyclic aromatic hydrocarbons (PAHs) and the aquatic environment

Polycyclic aromatic hydrocarbons are a group of organic pollutants composed of carbon and hydrogen atoms arranged in 2 or more fused aromatic rings (Sims & Overcash, 1983; Kehle, 2009). Of the numerous PAHs that exist, the US EPA has identified 16 priority PAH congeners (US EPA, 2008) based on their toxicity, carcinogenicity and mutagenicity (Karlsson & Viklander 2008; Myers et al., 1994; Vethaak et al., 1996). These priority PAHs are: naphthalene, acenaphthene, acenaphthylene, anthracene, phenanthrene, fluorene, fluoranthene, pyrene, benzo(g,h,i)perylene, indeno[1,2,3-cd]pyrene, benzo(a)anthracene,

chrysene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, and dibenz(a,h)anthracene. Their widespread occurrence is due to their formation processes (Kehle, 2009), high volume releases (Zhang & Tao, 2009) and slow degradation (Ahrens & Dupree, 2010), which allows them to remain in the environment at high concentrations. Anthropogenic activities largely increase the occurrence of these pollutants in the environment. An estimated 520 000 tons of the 16 priority PAHs were released in Africa alone during 2004, contributing to 18.8% of global emissions (Zhang & Tao, 2009).

PAHs have their origin from both pyrogenic and petrogenic sources. Pyrogenic PAHs are created by the incomplete combustion of organic material such as fossil fuels, wood and industrial waste (Angerer et al., 1997; Lui et al., 2009) while petrogenic PAHs are released from crude oils and refined petroleum products, such as diesel, kerosene, petrol and industrial oil products (Yunker et al., 2002, Lui et al., 2009). The PAHs are distributed into the neighbouring environments by means of long range atmospheric transport and consequent deposition of the pollutants. Waste water discharge, urban runoff and oil spills/leakages directly pass into the aquatic environment (Chen & Chen, 2011; Pies et al., 2008; Van Metre et al., 2000). Once in the aquatic environment the PAHs adhere to the sediment (Lui et al., 2009), due to their high hydrophobicity and strong affiliation to organic matter. This is how sediment becomes a sink for these pollutants (Chen & Chen, 2011). When bottom feeders and sediment dwelling organisms ingest PAHs they enter the food chain and may have negative effects on the biota as well as bio accumulate within the higher trophic levels (Lu et al., 2012; Wang et al., 2010).

1.3 Scope and aims of the study

The direct PAH emissions to soil, water and sediment is not known, and there is little data for South African freshwaters (Das et al., 2008; Moja et al., 2013; Nekhavhambe et al., 2014; Nieuwoudt et al., 2011; Quinn et al., 2009; Roos et al., 2012). We therefore know that PAHs are present in the South African environment, specifically in the section of the Vaal River catchment running through the Vaal Triangle (Moja et al., 2013; Nieuwoudt et al., 2011). The total concentration of PAHs in the former study ranged between 44 and 39 000 ng/g, dry mass (dm) and the concentration of carcinogenic PAHs ranged between 19 and 19 000 ng/g, dm (Nieuwoudt et al., 2011). The concentrations of native congeners in the water ranged between 23.5 and 110.8 µg/ℓ (Moja et al., 2013). Pyrogenic (burning) processes were the most likely sources, with minimal petrogenic (derived from fuels and oils) contributions. PAH levels were in the same range as levels reported from other countries.

In the study completed for the Water Research Commission (Project no K5/1561) on POPs in freshwater sites throughout the entire country, the PAHs had the highest levels of all of the organic pollutants analysed for. One of the sites with the highest PAH levels, was in Soweto/Lenasia with 5 408 ng/g (Roos et al., 2012). The cumulative probability of developing cancer resulting from exposure to benzo(a)pyrene at this site as a result of exposure to fish contaminated with benzo(a)pyrene was calculated to be between 0.181 and 0.859 in 1 000. [This can be rounded off to 2 in 10 000 and 9 in 10 000]. This is much higher than what is considered as an acceptable risk (approximately 6 in 10 000 versus the acceptable risk of 1 in 100 000 of the WHO (2001)].

The findings of Roos et al. (2012) led to the need to investigate the Soweto/Lenasia area in more detail as it showed to be experiencing high PAH exposures and therefore lead to this study (WRC K5/2422). The main aim of this was to determine the levels of the 16 priority PAHs in the Klip River that flows through the densely populated urban areas of Soweto and Lenasia where high levels were previously found. This aim was achieved by the following objectives:

Measure concentrations of 16 parent PAHs in sediment at 9 sites over a two year period.

Measure concentrations of 16 parent PAHs in fish tissue at 4 sites over a two year period.

Measure concentrations of 16 parent PAHs in wetland bird eggs over a two year period.

In addition to the main aim, we also wanted to investigate the pollutant profile of the 16 parent PAHs in the sediments, by:

comparing site PAH composition percentages by grouping congeners with the same number of cyclic rings to investigate similar pollution profiles between sites.

calculation of diagnostic ratios to determine origin of the pollution, i.e. pyrogenic vs petrogenic.

The final aim of this study was to determine the toxicity posed by the PAHs in the study area. In order to accomplish this, the aim was broken down into the following objectives:

Assessing sediment toxicity to benthic organisms, by comparing levels to international sediment quality guidelines and calculating sediment quality indices.

Investigating a very specific form of toxicity: that of aryl hydrocarbon receptor mediated toxicity, in sediment using the H4IIE-*luc* reporter gene bio-assay.

Investigating biochemical responses of the fish to the environmental stressors by performing biomarker response assays.

Investigating individual and community fish health by applying health indices for fish

Gauging potential risk to human health by conducting a theoretical human health risk assessment.

2 LITERATURE REVIEW

2.1 The Klip River Catchment

The Klip River catchment is a sub-catchment of the Upper Vaal River Water Management Area (WMA) (DWAF 2004). It is situated in South Africa's most densely populated province Gauteng, draining the Witwatersrand region, the southern part of Johannesburg, one of the most developed urban areas in Africa (Kotze, 2002, DWAF 2009). The Klip River is the largest tributary of the Vaal River, and together these rivers supply the largest portion of the surface flow of the WMA, downstream of the Vaal Dam (DWAF, 2004). It flows mainly southwards where it joins the Vaal River near Vereeniging.

For the sake of convenience the Klip River catchment was divided into regions based on the Klip River's tributaries and their position within the catchment. The Klip River originates in the south of Roodepoort, northwest of Soweto (Figure 1). The river flows south and then turns east along the south of Soweto (Howie & Otto, 1996) (referred to as Region 1 for this study, Figure 1). Here the Klip Spruit joins the Klip River. The Klip Spruit originates north of Soweto, and flows south through the centre of Soweto (referred to as Region 2, Figure 1). The Klip River receives water from three waste water treatment plants (WWTPs), Olifantsvlei, Bushkoppies and Goudkoppies, that are situated in this area (Figure 1). The river continues to flow past the south of Johannesburg towards the east, where the Riet Spruit flows into the Klip River (Region 3, Figure 1) and continues towards the Vaal confluence (Region 4, Figure 1) near Vereeniging (Howie & Otto, 1996; Kotze, 2002).

Figure 1: Klip River catchment showing the Klip River and its tributaries from origin to confluence with the Vaal River

Domestic users of the river mainly include rural settlements along the Klip River and its tributaries. The water utility, Rand Water, supplies potable water from the river to various municipalities within the catchment (Howie & Otto, 1996; Kotze, 2002). Industrial use of the water (Region 1 & 2) is restricted to the middle reaches of the catchment. Main water users are processing industries, such as product packaging, roofing and cladding material producers, three waste water treatment plants and mines (Kotze, 2002). Water for industrial use is also supplied by Rand Water. Mining (gold, base metals and industrial minerals) is the

most important activity in the upper catchment of the Klip River (DWA, 2012). Agricultural activities such as livestock watering and crop irrigation also use water from the catchment (Kotze, 2002).

Because the Klip River flows through the Witwatersrand region it is considered as one of South Africa's most polluted rivers (McCarthy et al., 2006; 2007). The mining activities and WWTPs in the catchment are the primary sources of point pollution while diffuse pollution mainly consist of informal settlements and old mine slime dams/waste dumps (Kotze, 2002). A summary of the potential pollution in the Klip River was compiled by Kotze (2002) (Table 1).

Table 1: The potential sources of point- and diffuse pollution in the Klip River (adapted from Kotze, 2002).

	Point source pollution		Diffuse pollution	
Klip River upstream from Klip Spruit confluence (Region 1)	Mining activity:	Durban Deep Roodepoort Mine (mine water pumping ceased in 1998)	Mining activity:	Slime dams Rock dumps Old mine waste sites
			Informal settlements:	Kagiso, Durban Deep Roodepoort Mine, Protea Glen, Doornkop West, Soweto, and Moroka
			Municipal:	Leaking sewage systems in informal settlements, mainly Soweto
			Industrial:	Chamdor industrial area
			Waste sites:	Closed solid waste site at Dobsonville
Klip Spruit (Region 2)	Power generation:	Orlando Power Station (ceased operation in 1998, plant collapsed in 2014)	Mining activity:	Slime dams (Central Gold Recovery), Rock dumps, Old mine waste sites
			Informal settlements:	Diepkloof, Power Park, Orlando East, and Pimville
			Municipal:	Leaking sewage systems in Soweto and surrounding suburbs
			Industrial:	Main Reef Road, Industria, Newtown and Selby areas
			Waste sites:	Marie Louise and Robinson Deep solid waste sites (active) and the Meredale solid waste site (closed)
Klip River between Klip Spruit and Riet Spruit confluence (Region 3)	Municipal:	Goudkoppies, Olifantsvlei and Bushkoppies WWTPs	Informal settlements:	Lenasia, Eldorado Park, Eikenhof
			Municipal:	Leaking sewage systems in Eldorado Park
			Industrial:	Nancefield and Olifantsvlei
			Waste sites:	Goudkoppies solid waste site
			Other:	Agricultural run-off
Riet Spruit tributary and Klip River to Vaal River confluence (Region 4)	Mining activity:	ERPM gold mine Glen Douglas dolomite mine	Mining activity:	Slime dams (Central Gold Recovery & Ergo Mine), Rock dumps, Old mine waste sites
	Municipal:	Rondebult, Dekema, Vlakplaats and Meyerton WWTPs	Informal settlements:	Central Johannesburg along Main Reef Road in Germiston, Katorus, kwa-Thema and Zonkizizwe
			Municipal:	Leaking sewers in Katorus area
			Industrial:	Village Deep, Alrode and Boksburg, Daleside, Meyerton and Iscor. Old Springfield Colliery
			Waste sites:	Henley-on-Klip, Walkerville & Waldrift solid waste sites (active) and Meyindustria solid waste site (closed)
			Other:	Agricultural run-off

2.2 Polycyclic aromatic hydrocarbons

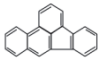
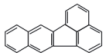
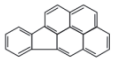
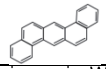
Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consisting of only fused aromatic rings, without functional groups or heteroatoms and are referred to as parent PAHs (Angerer et al., 1997; Sims & Overcash, 1983; Stogiannidis & Laane, 2015). These rings are arranged in clustered, angular or linear formations (Nadal et al., 2004). They are omnipresent in the environment and according to Neff et al. (2005) are major contributors to

detrimental effects in aquatic systems. There are 660 parent PAHs listed and from these US EPA has identified 16 priority PAHs for regulation and the need for priority monitoring of environmental quality because of the harm they can do to human and environmental health (Achten & Hofmann, 2009; Zhang & Tao, 2009). The priority PAHs are naphthalene, acenaphthene, acenaphthylene, anthracene, phenanthrene, fluorene, fluoranthrene, pyrene, benzo(g,h,i)perylene, indeno[1,2,3-cd]pyrene, benzo(a)anthracene, chrysene, benzo(a)-pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and dibenz(a,h)anthracene (US EPA, 2008) (Table 2).

2.2.1 Physical and chemical characteristics of polycyclic aromatic hydrocarbons

As these compounds are part of a very large group, they often differ from one another in physical and chemical characteristics based on their molecular mass and the number of their aromatic rings (CCME, 2008). The PAHs all have a high molecular mass with low volatility and the group is classified as semi-volatile (Ollivon et al., 1999) (Table 2) .They have a lipophilic nature with a high affinity for organic matter (Brenner et al., 2002; Morillo et al., 2007) rather than dissolving in water (Bertilsson & Widefalk, 2002). The group of congeners with 2 or 3 rings are referred to as low molecular mass PAHs (LPAHs) (relative to other PAHs), and the high molecular mass PAHs (HPAHs) are the 4-6 ring congeners (Table 2). The LPAHs tend to be more water soluble, but as the number of rings and molecular mass increase the hydrophilicity and mobility decreases (Iqbal et al., 2008). The HPAHs are more hydrophobic and lipophilic as well as have increased boiling- and melting points (Haritash & Kaushik, 2009). The high rate of release of these compounds (Boström et al., 2002; Haritash & Kaushik, 2009; Maliszewska-Kordybach et al., 2009) (Table 2) and these discussed attributes allow them to resist degradation in the environment (as indicated by the log K_{ow} values). Although the PAHs are not classified as persistent organic pollutants, their high volumes of release (Zhang & Tao, 2009) and large variety of sources (Maliszewska-Kordybach et al., 2009) allow them to become widespread at high concentrations, and because they degrade slowly under natural conditions – even more slowly in anoxic and low light conditions (Ahrens & Dupree, 2010) – PAHs are referred to by some researchers as pseudo-persistent.

Table 2: Physical and chemical characteristics of the 16 priority PAHs (Table adapted from Neff et al., 2005; Lee & Vu, 2010; Stogiannidis & Laane, 2015)

PAH	Structure	Abbreviation	No of rings	Molecular mass	Water solubility (mg/ℓ)	Vapour pressure (Pa)	Boiling point (°C)	$LogK_{ow}$	$LogK_{oc}$	Carcino-genicity
Napthalene		Nap	2	128.17	31	11	218	3.37	3.29	NC
Acenapthylene		Acey	3	152.2	3.9	9.0×10^{-1}	280	4.1	3.16	NC
Acenapthelene		Acea	3	152.22	3..9	3×10^{-2}	279	3.9	3.94	NC
Fluorene		Fl	3	166.2	1.9	9×10^{-2}	295	4.18	4.13	NC
Phenanthrene		Phe	3	178.2	1.1	2×10^{-2}	340	4.57	4.49	NC
Anthracene		Ant	3	178.2	0.05	1×10^{-3}	342	4.54	4.45	NC
Pyrene		Pyr	4	202.3	0.13	6×10^{-4}	393	5.18	4.83	NC
Fluoranthrene		Flu	4	202.26	0.26	1.2×10^{-3}	375	5.22	4.99	WC
Chrysene		Chr	4	228.28	0.002	1.4×10^{-6}	448	5.86	5.61	C
Benzo(a) anthracene		BaA	4	228.29	0.009	2.8×10^{-5}	400	5.6	5.57	C
Benzo(b) fluoranthene		BbF	5	252.3	0.0014	6.7×10^{-5}	481	5.8	6.16	C
Benzo(k) fluoranthene		BkF	5	252.3	0.0007	5.2×10^{-8}	480	6	6.18	–
Benzo(a)pyrene		BaP	5	252.3	0.003	7×10^{-7}	496	6	6	SC
Benzo(g,h,i) perylene		BgP	6	276.34	0.00026	1.4×10^{-8}	550	7.1	6.39	NC
Indeno[1,2,3-cd] pyrene		InP	6	276.3	0.00019	1.3×10^{-8}	536	6.6	6.6	C
Dibenz(a,h) anthrancene		DBA	6	278.35	0.0005	3.7×10^{-8}	524	6.5	6.59	C

NC = Non-carcinogenic; C = Carcinogenic; WC = Weak Carcinogenic; SC = Strong Carcinogenic; – = No information available

2.2.2 Sources of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons have both man-made and natural sources (Stogiannidis & Laane, 2015), but the release of PAHs from anthropogenic activities is one of the most important environmental pollution sources (Van Metre et al., 2000).

The widespread occurrence of PAHs is largely due to their formation and release in all processes of incomplete combustion of organic materials or high pressure processes (Kehle, 2009): production of cokes and carbon, coal power plants, petroleum processing, furnaces, fireplaces, gas and oil burners, and automobile sources and petroleum products (Angerer et al., 1997; Maliszewska-Kordybach, 1999; Yunker et al., 2002). Anthropogenic PAHs originate from two distinct processes namely pyrogenic- and petrogenic sources.

Pyrogenic PAHs are formed during the combustion of biomass (coal and petroleum, wood, and grass, and industrial waste) (Chen & Chen, 2011; He et al., 2014; Lui et al., 2009) in oxygen depleted and high temperature conditions (Saber et al., 2006). The HPAHs (4-6 ring PAHs) dominate the pyrogenic PAHs (Chen & Chen, 2011; Neff et al., 2005; Stogiannidis & Laane, 2015). Industrial processes such as power stations (Donahue et al., 2006; Li et al., 2014), coal mines (Pies et al., 2007), smelters (Næs & Oug, 1997; Booth & Gribben, 2005) and industrial waste removal (Domeño & Nerín, 2003) are major sources of pyrogenic PAHs. The most abundant PAHs formed through pyrogenesis are fluoranthrene, pyrene and to a lesser degree phenanthrene (Page et al., 1999). Carcinogenic PAHs (CPAHs) (benzo(a)pyrene, benzo(a)anthracene and benzo(b)fluoranthene) with pyrogenic origins are mainly released by motor vehicles (Dickhut et al., 2000; Van Metre at al 2000; Yadav et al., 2010). Whereas petrogenic sources predominantly consist of 2 and 3 ring PAHs (LPAHs) (Chen & Chen, 2011; Neff et al., 2005; Stogiannidis & Laane, 2015). Petrogenic PAHs are defined as the congeners that originate from petroleum products, including crude oil, petrol and diesel fuels, lubricants and their derivatives (Angerer et al., 1997; Maliszewska-Kordybach, 1999; Yunker et al., 2002; Saber et al., 2006). The PAH profile of different types of petroleum products vary depending on their production process, (Stout et al., 2001) for example, fuel with a lighter mass (jet fuel) contain more LPAHs than the heavier fuels, due to the distillation temperatures and the PAHs' boiling points and vapour pressures (Table 2). Apart from spillage and runoff, a major source of petrogenic PAHs is incomplete combustion of fuels. A significant amount of fuel is not ignited during pyrolytic processes (vehicles and combustion engines) (Bucheli et al., 2004; Van Metre et al., 2000)

2.2.3 Environmental fate of PAHs

When PAHs are released into the environment they, find their way into the aquatic environment and bind to the sediments (cf. physico-chemical properties) (Nadal et al., 2004). Here they are subjected to various degradation processes: chemical, photochemical and biological that may result in volatilisation, dissolution, and emulsification (Brenner et al., 2002; Page et al., 1996; Warren et al., 2003). Once the degradation processes are active, the physico-chemical properties of the congeners are changed (Kochany & Maguire, 1994; Page et al., 1996). Biological degradation of PAHs seems to be the main pathway of breakdown in sediments and soils (Lu et al., 2012; Wilson & Jones, 1993).

2.2.4 Toxicity of PAHs

PAHs exposure is a concern to organisms and humans as they are known to be mutagenic and carcinogenic (NTP, 2005). Studies of where humans are exposed to high levels of PAHs occupationally (industries) or where the products are themselves PAH sources (petroleum- and tar industries) have found that the PAHs accumulate via different exposure pathways and that it may have detrimental effects (Cirla et al., 2007; McClean et al., 2007; Väänänen et al., 2005). These effects are of developmental and reproductive nature, cytotoxicity (i.e. erythrocyte damage), DNA mutation and other health effects (Safe et al., 2010; US EPA, 2011; Zhang & Tao, 2009). Apart from the afore mention effects the PAHs are also known carcinogens (Myers et al., 1994; Qiao et al., 2006; Savinov et al., 2003) and according to Elmore & Boorman (2013) PAHs, such as benzo(a)pyrene and dibenz(ah)anthracene, are classified as genotoxic carcinogens – chemicals capable of producing cancer by directly altering cellular genetic material. The International Agency for Research on Cancer (IARC) has classified the carcinogenic PAHs (Table 2) into carcinogenic groupings: benzo(a)anthracene, benzo(a)pyrene and dibenz(ah)anthracene are classified as Group 2A (probably carcinogenic to humans) carcinogens, while benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene and indeno(1,2,3-cd)perylene are part of Group 2B (possibly carcinogenic to humans) carcinogens (OEHHA, 2001).

2.3 Biotic and abiotic matrices used for environmental studies

It is necessary to assess the concentrations of pollutants found in the environment as it is one measure of gauging the quality of the condition of the ecosystem and it helps to assess the potential toxicity in 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; Nieuwoudt et al., 2009; Varol, 2011) and biota (Forsberg et al., 2011; Miranda et al., 2008; Stentiford et

al., 2003). Pollutants in aquatic ecosystems generally exist in low levels in water (Öztürk et al., 2009) and mainly accumulate in the sediment (Öztürk et al., 2009; Praveena et al., 2008) and accumulate to higher levels into the food chain (Adams et al., 1993; Kidd et al., 2001).

2.3.1 Sediment as abiotic matrix for environmental studies

Sediment has a long residency in the aquatic systems and therefore it is an ideal matrix to assess for pollutants (Saha et al., 2001; Varol, 2011). Due to their variable physical and chemical properties, sediments are important sources for organic and inorganic pollutants (Praveena et al., 2008). During favourable conditions, they play a functional role in the mobilization of contaminants in aquatic systems (Öztürk et al., 2009). In riverine communities, the human population is both directly and indirectly exposed to sediment and its pollutants (Miller et al., 2004). Sediment is therefore an ideal environmental matrix to be included in a study of aquatic pollution (Praveena et al., 2008).

2.3.2 Fish used as biotic matrix for environmental studies

Fish is an ideal biotic matrix of aquatic studies, as they are represented in various trophic levels in food chains (Kidd et al., 2001). Biota acts as bio-indicators – which mean that groups or individual organisms can be 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 (Kidd et al., 2001). Fish have been used in numerous studies investigating organic pollutants (McHugh et al., 2011; Vives & Grimalt, 2002; Weber & Goerke, 2003; Wepener et al., 2011). The sharptooth catfish (*Clarias gariepinus*) is an opportunistic bottom-feeder, are omnivorous, and have also been found to be intentional detritus feeders, but are also known as formidable predators (Skelton, 2001). The sharptooth catfish is a hardy and resilient fish, surviving harsh conditions (Skelton, 2001).

The catfish was chosen as an indicator species because of its abundance in South Africa, their hardiness and because they are an apex predator. Their position on the food chain and their preference for bottom-dwelling in the aquatic systems make them ideal for studying exposure to pollutants, as well as the bio-accumulation and bio-magnification of organic chemical pollutants. These fish are also a valued food source, allowing for investigation in possible transfer of pollutants to humans.

2.3.3 Use of bird eggs as biotic matrix for environmental studies

Birds are also popular biotic matrices for environmental studies and have been used in various studies investigating organic pollution (Barnhoorn et al., 2009; Custer et al.,2001;

Khan et al., 2014; Pereira et al., 2009; Quinn et al., 2013;). Birds represent a different trophic level than fish, and because organic pollutants bio-accumulate and bio-magnify within food webs (Antoniadou et al., 2007; Herbert et al., 2011; Zhou et al., 2007), they can show the trophic transfer between different biotic matrices (fish to birds). The use of bird eggs specifically is considered to be a better matrix than the adult organism itself. Eggs are easy to handle and can be collected relatively fast and non-invasively (Medvedev & Markova, 1995). The eggs are representative of the female parent – as contaminants is transferred from the parent bird to her lipophilic eggs (Van den Steen et al., 2006; Verreault et al., 2006) – reflecting the pollutant body burden of that female parent (Braune, 2007). Wetland birds were chosen as bio-indicators for this study because they are exposed to pollutants from their feeding regimes, their direct habitat selection (aquatic systems) and breeding behaviours. Various types of wetland birds occur in the study area. The only heronry identified was in the study area was at Lenasia. Individual target species were not identified a priori and the species present at the heronry were sampled.

2.4 Sediment toxicity assessment

The assessment of a system's pollution status is achieved by means of various methods, indices, and guidelines. Indices are sets of aggregated and measured parameters or indicators (OECD, 2003), that are used to compare results indiscriminately between one another. Quality guidelines are sets of values that act as goals for environmental quality. These quality guidelines often have values that are specific for the purpose of the guideline – i.e. aimed at specific compounds and end points (protection of ecosystems, -benthic organisms, -aquatic life amongst others.) (MacDonald et al., 2000; Swartz, 1999).

The ecological toxicity risk that sediments may pose can be calculated with the sediment quality guideline index (SQG-I) (Fairey et al., 2001). The quality of the sediment can also be calculated in terms of the target compound or a mixture of compounds by using the sediment quality index (SQI) (Marvin et al., 2003). These tools can then be used to compare toxicity and quality of different sites in a study area in order to determine a status (of wellness) for that ecosystem.

The xenobiotics present in the sediment, have the potential to activate specific pathways in organisms, and through these pathways be toxic to the organism. The activation of the aryl-hydrocarbon receptor (AhR) – which is a ligand dependant transcription factor – regulates the expression of cytochrome P450 genes, specifically CYP1A1 (Aarts et al*.,* 1995). This enzyme is responsible for the metabolism of the activating xenobiotic. More detail on the mechanism of the transcription of the CYP450s and the AhR will be discussed later in this

report (cf. Biomarkers as biomonitoring tools & Relevance of bio-assays). The toxicity of AhR-ligands can be expressed as toxic equivalency factors (TEF), relative to the most toxic AhR-ligand, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Van den Berg et al., 2006). Using the TEF values and measured concentrations of the AhR-ligand compounds, the toxic equivalent quotient (TEQ) can be calculated as follows:

$$TEQ = \Sigma(Ci \times TEFi)$$

In turn, these TEQs can be compared to guidelines.

2.5 PAH source diagnostic ratios

Possible sources of PAHs can be identified by using diagnostic ratios. These ratios use the concentrations of specific congeners in a sample relative to one another. LPAH congeners with 2 and 3 rings are mostly released by petrogenic sources, while pyrogenic sources are dominated by 4-6 ring congeners (HPAHs). Thus, the ratio between the LPAHs and HPAHs can be used to identify pyrogenic or petrogenic sources (Scolo et al., 2000). The ratio between anthracene and phenanthrene (Ant/Ant+Phe) can also distinguish between pyrogenic and petrogenic sources (Chen & Chen, 2011; Pies et al., 2008). The nature of the pyrogenic source, i.e. identifying whether the combustion fuel was petroleum or biomass (grass, coal, wood), can further be classified using these congener ratios: Fla/Fla+Pyr (Lui et al., 2009; Yunker et al., 2002), BaA/BaA+Chr (Raza et al., 2013; Yunker et al., 2002) and InP/InP+BgP (Maliszewska-Kordybach et al., 2009; Yunker et al., 2002).

2.6 Biological indicators of environmental health and quality

An indicator is a parameter or a parameter derived value which provides information about, and describes the state of an area or an environment (OECD, 2003). In the case of this study, indicators state the overall health of the aquatic system of the greater Soweto/Lenasia area.

2.6.1 Fish health assessment index (FHAI) and gross body indices as indicators

The condition of an ecosystem is often reflected by the health of the organisms in the system. In the case of aquatic systems, fish are regarded as the representative species because of their position in the food web (Adams et al., 1993). The fish health assessment index (FHAI) and gross body indices have been used to evaluate the condition (overall health) of fish in a system (Heath et al., 2004).

Adams and co-authors (1993) describe the FHAI as a rapid and inexpensive quantitative index. It was developed as a field necropsy method, where the results provide a health profile of the fish, based on abnormalities observed in the tissue and organs of individuals sampled from a population (Adams et al., 1993; Goede & Barton, 1990). The index variables (cf. Materials and methods 2.4.2) are assigned numerical scores based on the degree of severity of damage that might have been caused by environmental stressors (Adams et al., 1993; Heath et al., 2004). The FHAI allows for statistical comparisons between data sets (Adams et al., 1993). Gross body indices such as the Fulton's condition factor (Cf), gonado-somatic index (GSI), hepato-somatic index (HSI), and spleen somatic index (SSI), are used to describe the state of physiological systems.

The Cf shows the volumetric relationship between the body mass and the total length of the fish. It expresses the condition (well-being, relative robustness or fatness) in numerical terms (Mortuza & Rohman, 2006). The GSI expresses the gonad size relative to the body size to describe sexual maturity or growth. It is used as a popular, simple and instantaneous measure of reproductive effort of a fish (Fouche et al., 2010). It also indicates irregularities, such as enlargements or tumours caused by contaminants (Stentiford et al., 2003). Fish livers are regarded as the main site of storage, bio-transformation and excretion of pollutants (Hinton & Laurén, 1990; Velmurugan et al., 2007) as well as a storage facility of energy reserves in the form of glycogen (Miranda et al., 2008). The HSI is the relationship between the liver mass and body mass and indicates the energy reserves of the fish or the effects of xenobiotics on the liver. The spleen is a lymphatic organ that plays a role in antigen degradation and antibody production. The SSI is the relationship between the spleen mass and body mass, and is used as an indication of immuno-responses (Rohlenová et al., 2011).

2.6.2 Biomarkers as indicators of environmental quality

The most common usage of the term 'bio-marker' is the measurement of the interaction between biological systems and environmental hazards (WHO, 1993). A more detailed definition is that biomarkers are the changes in the response on biochemical-, physiological- or morphological level, which can be related to the presence of xenobiotic chemicals (Bernet et al., 1999; Nikalje et al., 2012). Biomarkers are therefore regarded as indicators of environmental quality.

A biomarker 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 of biological organization (Newman, 2010) (Figure 2).

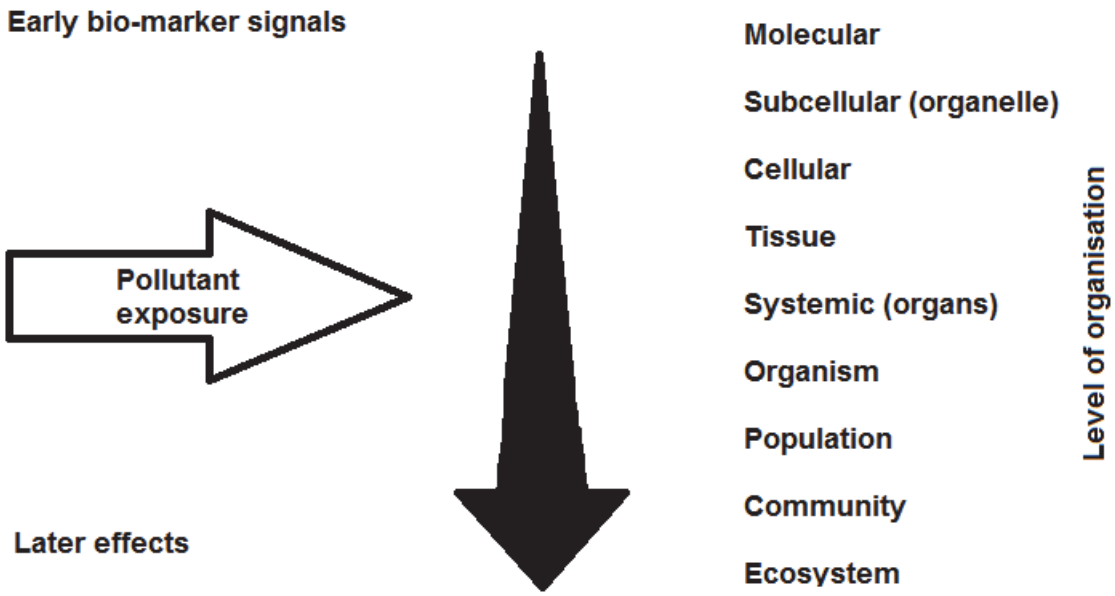


Figure 2: Levels of biological organisation and the order of response to pollutant stress [modified from Van der Oost et al. (2011)]

There are different classes of biomarkers, of which only two are used in this project: Biomarkers of (1) exposure and (2) effect. Biomarkers of exposure measure the product of the interaction of exogenous substances or their metabolites, and xenobiotics with target molecules or -cells within the body (US EPA, 2014; Van der Oost et al., 2003). The bio-markers of exposure used in this project are acetylcholine esterase (AChE), and cytochrome P450 activity (CYP450).

According to Van der Oost et al. (2003), biomarkers of effect are the measurable biochemical, physiological and other alterations within tissues and body fluids of an organism that are recognisable due to possible compromised health or disease. In this study cellular energy allocation (CEA) as well as bio-markers indicating oxidative stress were used. Biomarkers that show oxidative stress responses are catalase activity (CAT), superoxide dismutase (SOD), protein carbonyl formation (PC), and lipid peroxidation indicated by malondialdehyde content (MDA).

Acetylcholine esterase (AChE) is an enzymatic ester that hydrolyses the neurotransmitter acetylcholine. This deactivates acetylcholine and prevents constant nerve firing (Solé et al., 2006, 2010). These enzymes play a crucial role in the signal transmission in animals,

controlling functions such as movement, respiration, hormonal function and reproduction (Solé et al., 2010). AChE is found in the brains of fish, but is also found in large quantities in the liver (Van der Oost et al., 2003).

SODs are enzymes that form the first tier defence of the cellular antioxidant system. These enzymes catalyse the dismutation of superoxides (O_2^-) into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defence in nearly all cells exposed to oxygen and reactive oxygen species (ROS) (Pandey et al., 2003).

Catalase (CAT) is an enzyme belonging to the cellular antioxidant system and counteracts the toxicity of peroxide (Lionetto et al., 2003). CAT is produced in response to the increase of ROS and is also the second tier defence antioxidant system, hydrolysing the hydrogen peroxide formed by SOD (Pandey et al., 2003).

Lipid peroxidation is quantified by malondialdehyde content. MDA is formed when lipid membranes degrade when oxidised (Solé et al., 2006, 2010). Lipid peroxidation is an important reaction of cellular damage, as it can affect the cellular antioxidant system (Ferreira et al., 2007). MDA content is used to indicate if lipid damage has occurred in an organism due to oxidative stress and its levels indicate the severity of lipid peroxidation in an organism.

The oxidation of amino acid residues results in the formation of PCs. If the protein carbonyls increase they can cause damage to cellular systems and tissue and once PCs are formed they cannot be reversed (Parvez & Raisuddin, 2005). PCs decrease enzymatic functions and can cause delayed protein regeneration (Ferreira et al., 2007).

The cytochrome P450s (CYP450) are a superfamily of haeme-containing enzymes that are widely diverse with regards to substrate specificity and catalytic activity (Guengerich, 2008). The P450 enzymes are generally regarded as the enzymes which are the first defence against exogenous compounds (Liska, 1998). When an organism is exposed to a toxicant, the CYP450 enzymes are expressed (Ellero et al., 2010). This expression is the endpoint of the aryl-hydrocarbon receptor (AhR) mediated response (Hilscherova et al., 2000; Denison et al., 2004). The AhRs are located inside the cytoplasm of the cells. When AhR activating agents, such as PAHs, enter the cells, they bind onto the AhR complex. Upon binding, the AhR is transported into the nucleus, where it attaches onto a specific DNA sequence (called the dioxin response element, DRE), which consequently results in the transcription of the genes, such as the CYP450s (Aarts et al*.,* 1995; Denison et al., 2004; Whyte et al., 2004).

The inhibition and activation of the P450s of animals can be used as a biomarker of exposure as it reacts to the presence of toxicants.

2.7 Relevance of bio-assays

Chemicals introduced into the environment occur as complex mixtures. These complex mixtures interact with one another and the environment (Hecker & Giesy, 2011). Measuring the levels of these chemicals within an environmental sample is important to determine the level of pollution in that sample (Hilscherova et al., 2001). However, compounds can only be analysed if applicable analytical methods and standards exist (Garrison et al., 1996). The instrumental analysis of an environmental sample therefore does not take into account the interactions and synergy of the mixture and provide limited information on their potential biological effects (Hilscherova et al., 2000; Vanderperren et al., 2004). Bio-assays provide the estimations of the biological effects (Behnisch et al., 2002; Hilscherova et al., 2000; Koh et al., 2004) the substances have on living cells and tissues. Various types of bio-assays exist that investigate different endpoints. Bioassays were developed to answer the need for rapid and relatively inexpensive methods that detect and estimate relative potencies of complex mixtures (Baston & Denison, 2011) and quantifiably analyse the responses in a biological manner (Behnisch et al., 2002). These bio-assays can be performed in laboratories without using environmental test organisms. One of the many types of bio-assays includes the *in vitro* cell bio-assays. *In vitro* cell bio-assays offer a rapid and sensitive solution to the limitations of instrumental analysis.

In vitro cell bioassays are used to assess different modes of toxicity as endpoints of chemical groups such as genotoxic compounds, endocrine disrupting chemicals (EDCs), and aryl-hydrocarbon (Ah) ligands. The DNA-repair-deficient chicken DT40 B-lymphocyte cell line for example is used to screen and characterise genotoxicity of compounds (Ji et al., 2009). Similarly, the Ames test assesses genotoxic effects like point- and frame shift mutations using the *Salmonella* TA98 and TA100 strains respectively (Mortelmans & Zeiger, 2000). The effect of EDCs can be measured with various cell lines, focusing on different sections of the endocrine system. The H295R cell line measures endocrine disrupting activity by modulation of the steroidogenesis pathway (Hecker et al., 2006). Oestrogen activity is quantified using the MVLN oestrogen receptor-mediated luciferase reporter gene bio-assay (Demirpence et al., 1993). The androgenic chemical effects are measured similarly by means of the MDA-kb2, androgen receptor-mediated luciferase reporter gene bio-assay (Wilson et al., 2002). The Ah ligand mediated toxic responses can be quantified by measuring ethoxyresorufin-O-deethylase (EROD) activity (CYP1A1 activity) using the RTL-W1 cell line (Lee et al, 1993). The H4IIE-*luc* cell line also measures the CYP1A1 activity as

endpoint but quantifies the activity with a receptor-mediated luciferase reporter gene bio-assay (Sanderson et al., 1996).

Polycyclic aromatic hydrocarbons are known carcinogens and have adverse effects of human and wildlife health (Balch et al., 1995; Larsson et al., 2012; Spink et al., 2008). Some PAHs have their toxic effect by acting through the AhR, and a number of PAHs may also interfere with the oestrogen receptor (ER)-mediated signalling (Machala et al., 2001).

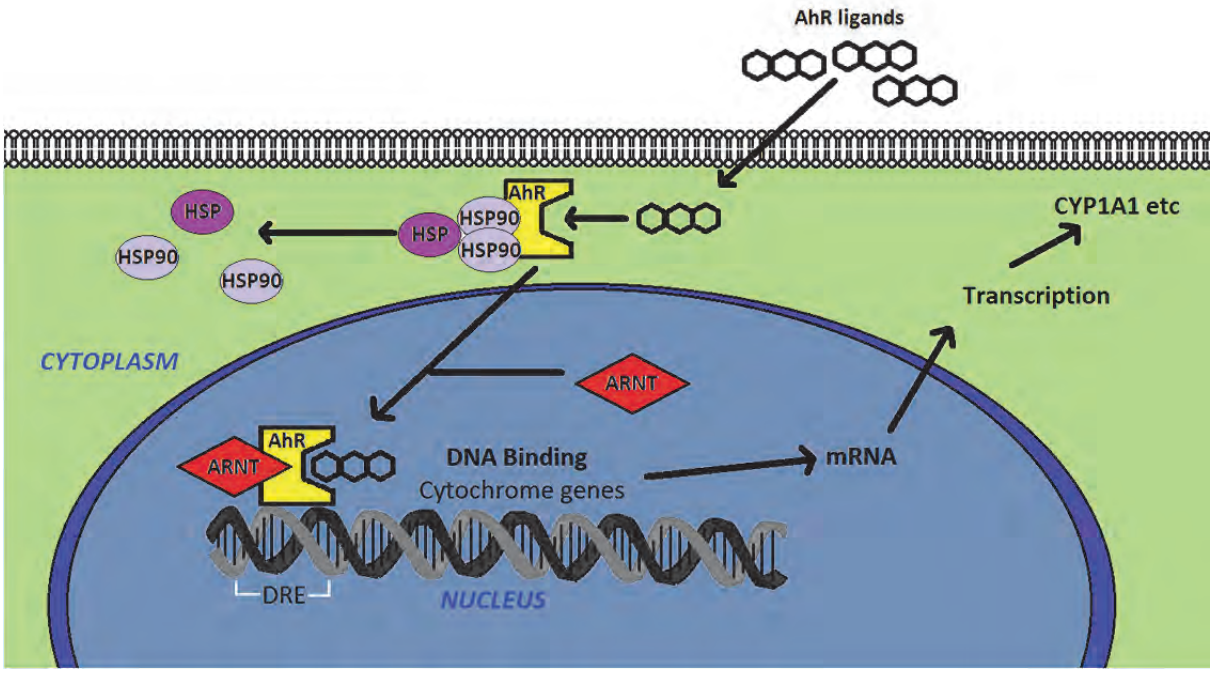


Figure 3: The mechanism of Ah-receptor mediated response in cells (Hilscherova et al., 2000). ARNT = aryl-hydrocarbon receptor nuclear translator, HSP = heat shock protein, DRE = Dioxin response element

The parent PAHs that bind to the Ah-receptor are: benzo(a)anthracene, chrysene, benzo(b+f)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)perylene and dibenz(ah)anthracene (Villeneuve et al., 1999). The AhR-ligands enter the cytoplasm of cells and bind to the AhR complexes – unbound AhRs are complexed with heat shock proteins (HSP) (Figure 3). Upon binding, the heat shock proteins dissociate which activates the complex (Hilscherova et al., 2000). The activated complex is translocated into the nucleus, where it rapidly forms a heterodimeric nuclear complex (Safe & Wormke, 2003) with the aryl hydrocarbon receptor nuclear translator (ARNT) protein (Hilscherova et al., 2000; Safe & Wormke, 2003) (Figure 3). The dimer-complex binds onto the dioxin response element, DRE – a specific DNA sequence in the CYP1A1 promoter (Denison et al., 2004; Hilscherova et al., 2000; Safe & Wormke, 2003;). Attachment to the DRE leads to the transcription of the adjacent responsive genes (Hilscherova et al., 2000), resulting in the up-regulation or induction of proteins responsible for detoxification (Baird et al., 2005) (Figure 3).

Cytochrome enzymes metabolise the PAHs by addition of an oxygen atom and in most cases this oxygen is reduced to a hydroxyl group (Tuvikene, 1995); further metabolism can result in epoxide-metabolites. The PAH-epoxide-metabolites are capable of binding to DNA during this stage of detoxification (Baird et al., 2005) causing mutagenesis. The reactive metabolites are conjugated by several enzymes: glutathion-S-transferase (GST), uridine 5-diphosphate-glucuronosyltransferase (UDP-GT) and glutathione (GSH) (Tuvikene, 1995; Baird et al., 2005). These enzymes complete biotransformation phase II: reducing the toxicity of the compound and making it easier to excrete.

The activation of the AhR has been seen to exhibit anti-oestrogenic cross-talk with the oestrogen receptor (Chen et al., 2001) blocking the oestrogen receptor (ER) (Safe, 2003). This cross-talk mechanism between the AhR-ER is complex, but involves the inhibition of oestrogen-responsive genes by DRE structures that bind to the AhR complex and so disrupt the oestrogen action through multiple mechanisms (Navas & Segner, 2000; Safe et al., 2003). The ability of PAHs to bind to DNA is therefore not the only mode of carcinogenesis (Baird et al., 2005).

Dioxin-like toxicity (AhR mediated toxicity) of PAHs was specifically investigated for this project at the proposed sediment sampling sites in Soweto/Lenasia.

The AhR mediated responses of PAHs can be quantified with the H4IIE-*luc* reporter gene bio-assay. The H4IIE-*luc* bio-assay results represent the total amount of bio-activity due to AhR-ligands present in the environmental sample as a result of gene activation. The H4IIE-*luc* reporter gene bio-assay consists of rat hepatoma cells that are stably transfected with a firefly luciferase reporter gene. The bio-assay indirectly measures cytochrome P450 induction – as mentioned above – which is an endpoint in the AhR mediated response (Denison et al., 2004; Hilscherova et al., 2000). The luciferase gene was inserted downstream of the cytochrome genes and the DRE in the H4IIE-*luc* cells. In the presence of luciferin (substrate for luciferase), light is produced (Figure 4). The amount of light that is released is directly proportional to the amount of AhR agonists present in the sample (Hilscherova et al., 2000).

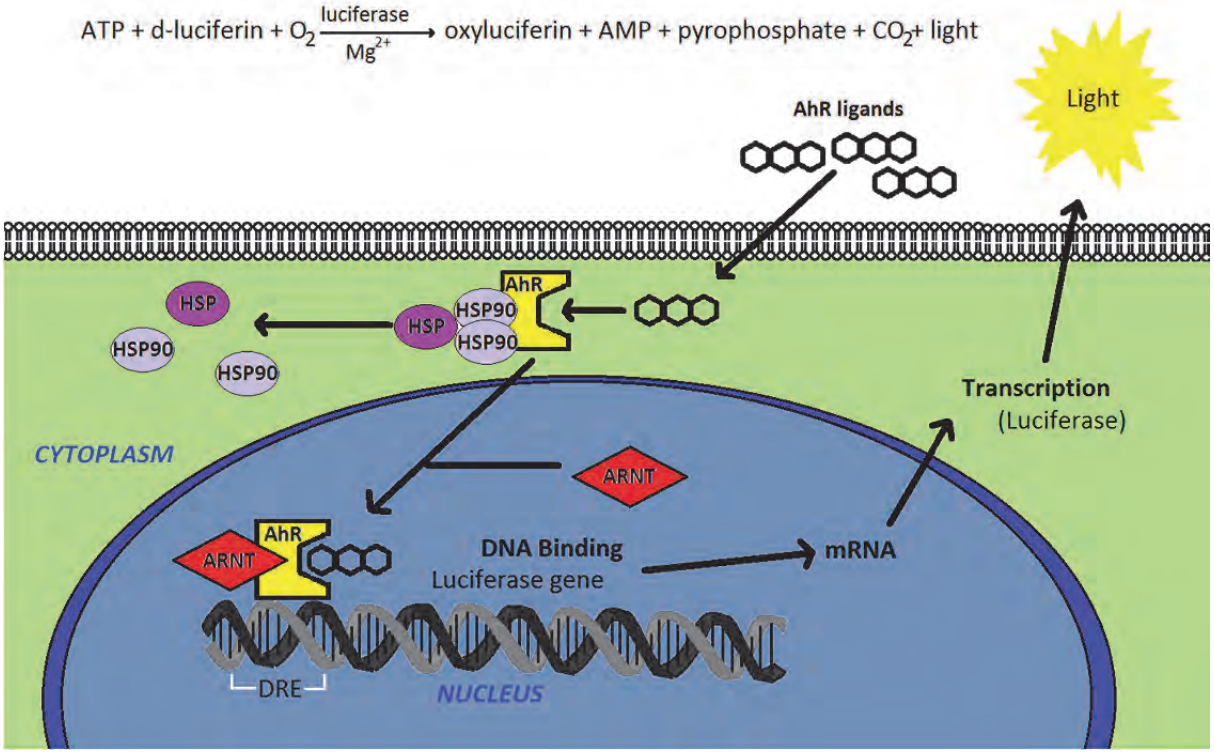


Figure 4: The mechanism of Ah-receptor mediated luciferase reporter gene response of the H4IIE-l*uc* bioassay (Hilscherova et al., 2000)

The toxicity of the sample can be quantified in terms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). The basis for this quantification is based on an assumption that the investigated sample is a diluted form of a reference compound, or a mixture of chemicals behaving like the reference compound, 2,3,7,8-TCDD, which is the most toxic congener of the Ah-receptor binding compounds (Yoo et al., 2006). The results are given as relative potency values (REP).

The results obtained from using this reporter gene bio-assay are: 1) establishing whether there are AhR agonists present in a sample and 2) quantify the toxicity of that sample relative to TCDD. The chemical data obtained from instrumental analysis identify the possible AhR agonists and in what concentrations they occur. The bio-assay and chemical analysis complement each other: the relative toxicity quotient can be calculated with the chemical data and compared to the biological toxicity equivalent. These equivalents can be used to assess the risk the compounds pose to humans and the environment (Yao et al., 2002) and can be compared to guidelines, such as international sediment quality guidelines.

3 MATERIALS AND METHODS

3.1 Site selection

The sampling area was in the Klip River catchment, focusing on that area of the catchment that encompasses greater Soweto. The sampling sites chosen were selected based on their position within the catchment. The sites are representative of the drainage area, as they are situated on the upper-, middle-, and lower stretch of the Klip River and its main tributary, the Klip Spruit, which flows through the urban areas of Soweto and Lenasia. Sediment was sampled from the 9 sites within suburbs. The sites were named after their suburbs: Protea Glen, Lenasia, Fleurhof, Moroka, Eldorado Park, Orlando West, Orlando East, Nancefield and Dobsonville (Figure 5 & 6). Fish were sampled from 4 dams: Lenasia, Fleurhof, Orlando and Nancefield, each of which drains into the Klip River (Table 3). After the collapse of the Orlando power station in late 2013, a massive fish kill was reported. No fish were sampled during the scheduled sampling event and evidence of the fish kill was confirmed by numerous fish carcasses. The Nancefield weir (Nc) (Figure 5) was initially selected to represent the farthest downstream area – however after the first sampling session was unsuccessful an alternative site within the area had to be identified. The closest to the original site where fishing was successful was in the Bushkoppies WWTP at the last of the maturation ponds, from where water flows into the Klip River and should be close to the environmental condition (Figure 5 & Table 3).

Potential egg sampling sites were scouted on foot and after no success, aerial reconnaissance was done to locate breeding colonies. After several aerial scouting trips, the only breeding colony within the study area was located in the Lenasia wetland, adjacent to the fish sampling site.

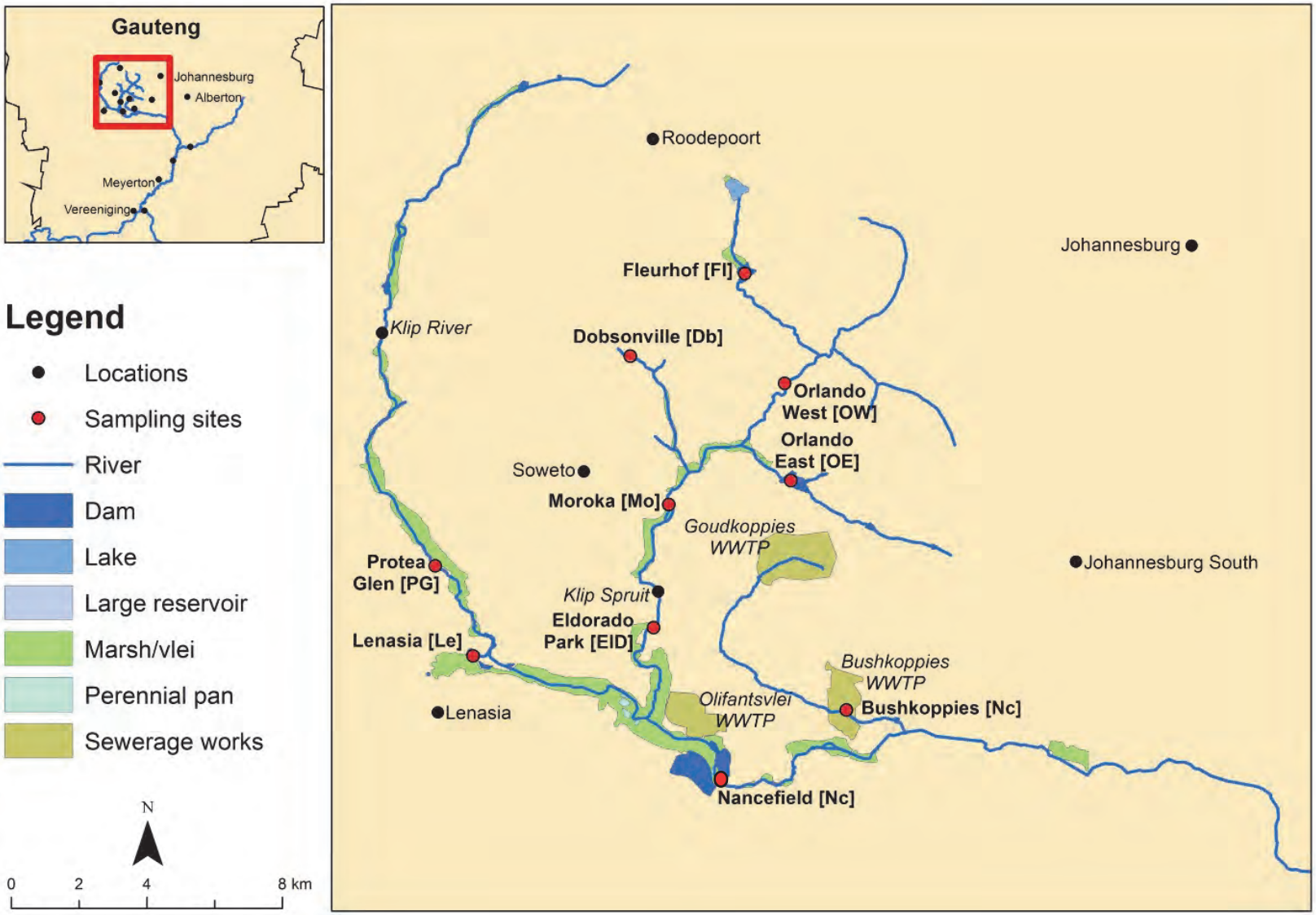


Figure 5: Sampling sites in the greater Soweto area

Table 3: Selected sites in the Greater Soweto/Lenasia co-ordinates, matrices sampled, and physical characteristics (2013 in grey and 2014 in white)

Sites	Site codes	Matrices sampled	River/dam characteristics	Flow rate (m/s)	Sediment grain size (%)							%TOC
					4000 µm	2000 µm	500 µm	212 µm	106 µm	53 µm	<53 µm	
Protea Glen 26°15'31.68"S 27°48'45.5"E	PG	Sediment	Deep pool, large rocks. Wetland reeds and riparian shrubs and trees	1	16.4	8.4	20	24	14.6	8.7	6.7	3.12
					7.7	12.9	27.9	22.3	13.1	10.2	6.1	2.05
Lenasia 26°18'8.33"S 27°50'10.8" E	Le	Sediment, fish and bird eggs	Large dam, forms part of a wetland system. Dam has water grass and weeds	N/A	32.6	4.6	9.1	27.9	15.8	5.6	2.6	1.41
					29	12.7	20.1	24.9	10.1	3.9	2	1.84
Fleurhof 26°12'03.49"S 27°54'31.87"E	Fl	Sediment and fish	Large dam, weed covered bottom, shore lined with reeds	N/A	0	0.4	18.8	36.5	21.5	13.4	7.8	0.42
					3.2	6.7	35.5	37.6	11.3	3.5	1.6	1.47
Dobsonville 26°13'22.89"S 27°52'40.35" E	Db	Sediment	Small dam draining into small stream.	N/A	0.6	0.5	17.7	53	15.8	6	4.9	1.28
					0	0.2	12.2	44.7	14.9	9.8	17.1	1.5
Orlando West 26°13'36.83"S 27°55'26.57"E	OW	Sediment	Relatively fast glides and riffles, followed by deeper glides with large boulders. Riparian zone covered in thick grassland	2.2	10.4	14.4	50.9	8.4	3.9	3.4	7	0.8
					0	0.3	0.7	14.8	11.8	31.1	38.5	1.07
Orlando East 26°15'21.63"S 27°55'18.97"E	OE	Sediment and fish	Old power plant reservoir, open areas along the shore line (barren or grass patches), reed beds	N/A	1.2	4.1	28.3	36	17.2	7.4	4.2	0.53
					0.3	8.5	38.4	28.1	10	8.9	5.1	0.71
Moroka 26°15'44.71"S 27°53'17.29"E	Mo	Sediment	Deep fast flowing pools, sandy banks lined with grass and reeds	3.19	0.5	1.6	37.9	46.3	13.5	1.9	0.5	2.07
					0	1.5	10.5	45.8	29.2	8.7	3.7	0.75
Eldorado Park 26°17'24.27"S 27°53'08.60"E	ElD	Sediment	Deep slow stretches linked with faster runs, banks dominated by grass and shrubs	2.68	8.6	10	22.1	23.5	15.3	13.1	7.6	2.24
					0	3.6	26.3	36.3	15.8	10.5	6.5	1.36
Nancefield 26°19'59.43"S 27°54'11.28"E	Nc	Sediment	Steady flowing, narrow and deep stretch downstream from weir. Rocky banks lined with reeds, veld grass and trees	1.5	2.9	6.9	21.2	27.3	21	13.7	6.9	0.87
					0	6.4	38.7	19.2	14.3	12.9	7.7	0.93
26°19'03.13" S 27°56'08.68" E	Nc	Fish	Last ponds of the Bushkoppies WWTP before flowing into the Klip River. Rocky shores lined with trees and veld grass	N/A								

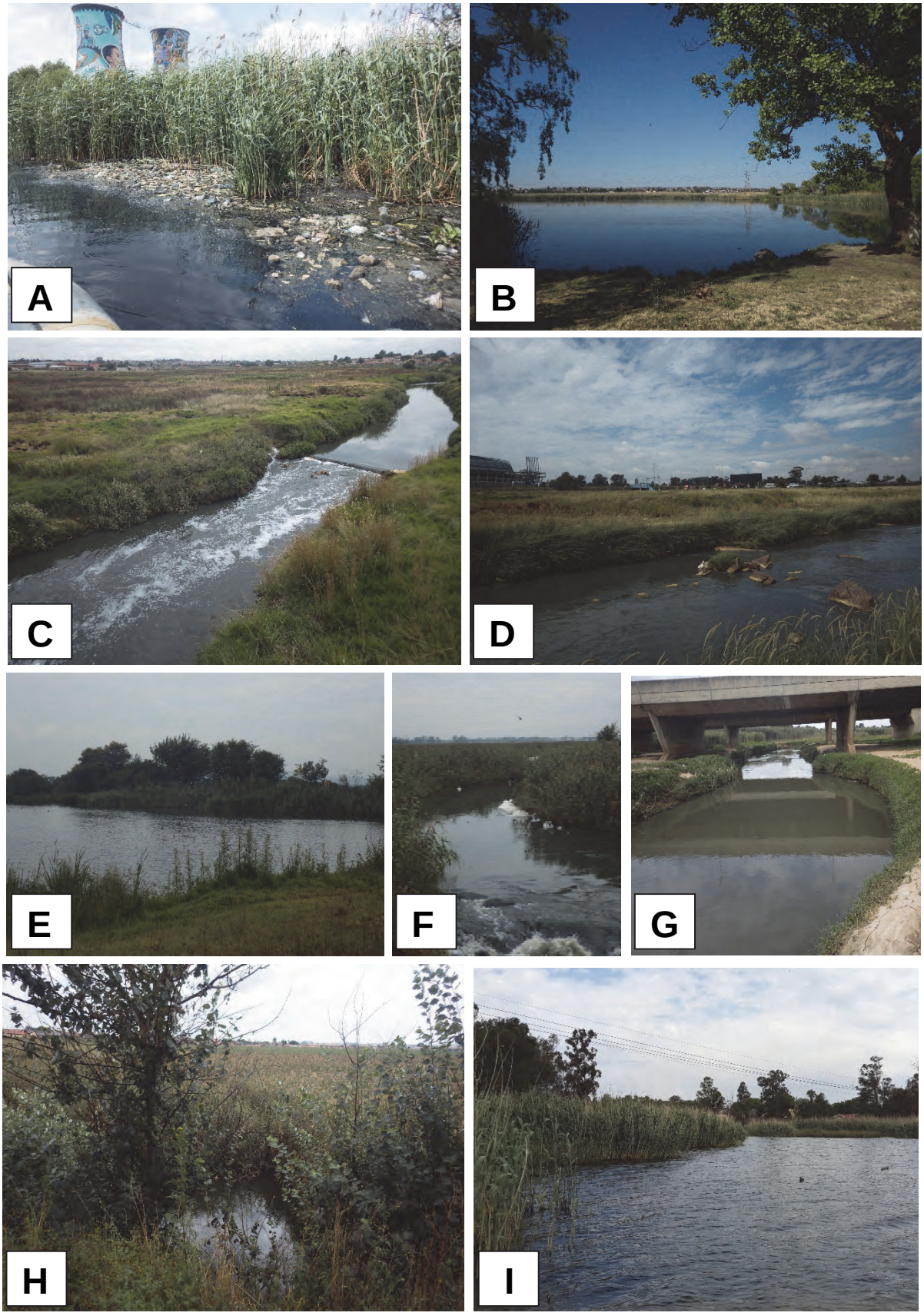


Figure 6: Sampling sites in the Soweto/Lenasia area: A) Orlando East B) Lenasia C) Eldorado Park D) Orlando West E) Bushkoppies WWTP F) Nancefield G) Moroka H) Protea Glen I) Fleurhof

3.2 Sampling

3.2.1 Sediment sampling

Surface sediment samples were collected at the nine sites. Composite samples (triplicate) were collected in a 5 m range, mixed thoroughly before storing it in high density polyethylene (HDPE) bottles, pre-cleaned (rinsed thrice with first acetone and then hexane) according to US EPA method 1613 (US EPA, 1994a). The samples were protected against microbial- and UV degradation by transporting at 4°C and storing at -20°C in the laboratory. The samples were air dried in the absence of light, ground and sieved (0.5 mm mesh size) to obtain homogenous samples (Kralik, 1999).

To determine the physical characteristics of the sediments, total organic carbon and grain size was determined. The loss-on-ignition method was used to determine the total organic carbon (Schumacher, 2002). The different sediment grain sizes was partitioned using a series of sieves (4 000-, 2 000-, 500-, 212-, 106-, 53 µm) (ISO, 2002).

3.2.2 Fish sampling

Sharptooth catfish (*Clarias gariepinus*) were sampled during the peak low flow season for two consecutive years, October 2013 and 2014 from the Lenasia, Fleurhof, Orlando and Nancefield (Bushkoppies) sites. They were sampled using gill- (118- and 150 mm) and fyke nets, and rod and line. The fish were euthanized by severing the spinal cord before the Fish Health Assessment Index (FHAI) and gross body indices were applied.

Samples were taken for chemical analysis and bio-marker purposes. Muscle fillet samples from the left ventral muscle behind the head plate, were collected for PAH analysis and liver and muscle tissue collected for bio-markers. Samples for PAH analysis were stored in pre-cleaned foil (washed with acetone and hexane), according to the EPA Method 1668B (US EPA, 2008). Muscle samples for chemical analysis were transported at 4ºC and stored at -20ºC. Biomarker samples were submerged in Hendrikson's buffer (40 mM tris-HCl, 10 nM b-mercapto-ethanol, 1 mM 0.04% bovine serum albumin [BSA], 1 nM EDTA) and transported in liquid nitrogen and the samples were stored at -80ºC.

The control group fish were caught from the Vaal River basin. These fish were depurated at the Water Research Group aquarium at the NWU Potchefstroom Campus for 6 months. They were kept in standard aquarium water that was replaced every 2 weeks and fed standard aquaculture feed. They were processed in the same manner as the fish sampled from the study site.

3.2.3 Wetland bird egg sampling

The wetland birds from which eggs could be collected were the black headed heron (*Ardea melanocephala)*, cattle egret (*Bubulcus ibis*), glossy ibis (*Plegadis falcinellus*), and the sacred ibis (*Threskiornis aethiopicus*). These birds' eggs were collected from the Lenasia wetland. After several aerial surveys, this was the only heronry found within the study area.

The wetland bird eggs were sampled from the nest in the heronries (accessed by boat). The largest egg was collected from each nest and marked with a pencil. The eggs were wrapped in pre-cleaned aluminium foil (US EPA, 2008) and also marked on the foil. The eggs were transported at 4°C and stored at -20°C in the laboratory.

3.3 Chemical extraction procedure

3.3.1 Sediment extraction

Approximately 5 g sediment was extracted for chemical analysis and 30 g for the bio-assay. The samples for both the chemical analysis and the bio-assay were extracted following the same extraction method except for one major difference: extracts destined for bio-assay analysis is not spiked with any standard as these would also elicit a response from the cells indistinguishable from that of the AhR ligands extracted from the sediment. Sediments were extracted with the accelerated solvent extraction (ASE) US EPA Method 3545 (US EPA, 1996) using the Dionex ASE 100® instrument. The extraction temperature was 100°C and the solvent used was 3:1 dichloromethane (DCM):hexane(v/v). Parameters set on the instrument are listed in Table 4.

Table 4: Extraction parameters for the accelerated solvent extraction method

Parameter	Setting
Pressure	11 721 kPa
Static time	5 minutes
Static cycles	2
N_2 purge	90 seconds
Flush volume	60%

The extract was evaporated under a gentle flow of nitrogen gas at 33°C and reconstituted in 2 mℓ DCM by means of solvent exchange. The sample was sent through gel permeation chromatograph columns (GPC) to collect the fraction containing PAHs in the extract as well as remove sulphur from the sample. A size-exclusion standard (corn oil, phthalate, perylene, methoxychlor and sulphur) (all standards from Sigma-Aldrich) was run first to calibrate the GPC and to determine the retention times of the standard compounds (US EPA, 1994b). A PAH standard (benzo(g,h,i)perylene and fluorene mixture) (Sigma-Aldrich) was also run through the GPC to determine the retention time of the target compounds. The PAH fraction

was collected (fraction collection started before the first PAH [benzo(g,h,i)perylene] passed the detector and stopped after the last PAH [fluorene] passed the detector) approximately 15.6-20.6 minutes. The PAH fraction was evaporated under nitrogen gas at 33°C and reconstituted in 10 mℓ hexane. Solid phase extraction was performed using a Supelco 12 mℓ 2g/2g LC-Si/Florisil® cartridge under gravitation. The cartridge was conditioned with 10 mℓ hexane. The conditioning solvent was discarded as waste. As soon as the conditioning solvent passed the matrix, but before it ran dry, the 10 mℓ sample was added and collected in a Turbo-Vap flask positioned under the SPE. Once the sample passed through the matrix, 24 mℓ DCM:hexane (1:1, v/v) was added, 6 mℓ at a time, ensuring the surface of the matrix never ran dry. The SPE was eluted with 8 mℓ DCM. The cartridge was left until there were no more drops leaving the SPE. The bio-assay samples were evaporated under nitrogen gas at 33°C and reconstituted in 1 mℓ hexane. The chemical analysis samples were reconstituted in 1 mℓ toluene. All samples were stored at -80°C.

These samples were sent for analysis to the National Metrology Institute of South Africa (NMISA). The concentrations of PAHs present in the sediments were determined with a gas-chromatography mass spectrometry time-of-flight (GCMS-TOF) instrumentation.

3.3.2 Biota chemical extraction

The fish and wetland bird eggs were extracted using a liquid-liquid extraction, coupled with dispersive solid phase extraction (dSPE) known as QuEChERS (**Qu**ick, **E**asy, **Che**ap, **R**ugged and **S**afe). This method was developed as a rapid screening extraction method after the Deep Water Horizon oil spill (Yeudakimau et al., 2012). The advantage of using this method for fish extraction is that it was developed for high lipid matrices, such as fish (Forsberg et al., 2011) and mussel (Madureira et al., 2014).

Fish fillet extraction

Homogenised fish samples were weighed off (2 g) and placed into the quecher extraction tubes (Agilent Technologies). Deuterated PAH standards were spiked at different concentrations for quality control and mixed by vortex. The samples were suspended in 5 mℓ double distilled water (ddH_2O), after which 10 mℓ extraction solvent, acetone:hexane (1:1,v/v) was added. Liquid-liquid extraction was performed by vigorously shaking the samples for 2 hours. The extraction salts (Agilent Technologies) were added to the samples and mixed by vortex, followed by a centrifuging step for 5 minutes at 181 g (5°C). The supernatants were decanted into Turbo-Vap flasks and were evaporated under nitrogen gas at 33°C to 1 mℓ. The flasks were rinsed with extraction solvent and the extract transferred

into the 1.5 mℓ dSPE tube (Agilent Technologies). The dSPE sorbent and extracts were mixed by vortex, and centrifuged for 5 minutes at 181 g (5°C). The supernatants were transferred into gas chromatography (GC) vials and evaporated under nitrogen gas at 33°C and reconstituted in 1 mℓ toluene.

Wetland bird egg extraction

The extraction of the bird eggs followed the same extraction process as the fish samples using the quecher method. An additional GPC clean-up step was included to remove excess fats. On the last step of the quecher method, the supernatant of the dSPE was evaporated under nitrogen gas at 33°C and solvent exchanged into 2 mℓ DCM, and from there followed the same protocol as the sediment extraction. Upon the last step of the GPC clean-up, the extract was evaporated under nitrogen gas at 33°C and solvent exchanged into 300 µℓ toluene.

3.3.3 Chemical analysis

The extracted samples were sent for analysis to the National Metrology Institute of South Africa (NMISA). The sediment, fish and egg samples were analysed for the 16 priority PAHs: naphthalene [Nap] acenaphthylene [Acey], acenaphthene [Acea], fluorene [Fl], phenanthrene [Phe], anthracene [Ant], fluoranthene [Fla], pyrene [Pyr], benzo(a)anthrancene [BaA], chrysene [Chr], benzo(b)fluoranthene [BbF], benzo(k)fluoranthene [BkF], benzo(a)pyrene [BaP], indeno(1,2,3-cd)pyrene [InP], benzo(ghi)perylene [BgP] and dibenzo(ah)anthracene [DBA]. The majority of the analyses was performed using a LECO gas chromatograph coupled to a time of flight mass spectrometer (GC-TOFMS) using an application specific column for PAH separation (Rxi-PAH). The GCxGCTOFMS (Rxi®-5SilMS and Rxi®-17SilMS columns) method was also employed for more sensitivity to detect the smaller molecular mass target compounds.

The limit of detection (LOD) and limit of quantification (LOQ) was calculated using linear regression analysis (Miller & Miller, 2000) with matrix matched standards, where S_a was defined as the intercept. The LOD was defined as three times the standard deviation of $S_{Y/X}$ and the LOQ was defined as ten times the standard deviation of $S_{Y/X.}$

3.4 Sample processing

3.4.1 H4IIE-*luc* tissue culture

Maintenance of H4IIE-*luc* cell culture

During routine maintenance of the cell culture, aseptic conditions were followed.

The H4IIE-*luc* cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich) with L-glutamine and 10% glucose, and without phenol red and sodium bicarbonate. The DMEM is supplemented with 10% foetal bovine serum (FBS) (Sigma-Aldrich) and 0.04 M sodium bicarbonate. The cells were kept in tissue culture dishes in an incubator at 37°C in humidified air (5% CO_2:95% air). The cells were rinsed with phosphate buffered saline (PBS) (Sigma-Aldrich) when media was changed and passaged using 1.5 mℓ trypsin (Highveld Biological) (Aarts et al., 1995).

H4IIE-*luc* reporter gene bio-assay

The method of the luminescence bio-assay is a modified version of that described by Tillet et al. (1991). The interior 60 wells of flat bottom 96 well microtiter plates were seeded with the 250 µℓ H4IIE-*luc* cells at a density of 80 000 cells/mℓ. The external 36 wells were filled with 250 µℓ phosphate buffered saline (PBS), to create a homogenous micro environment across each cell containing well. The plates were incubated at 37°C in humidified air (5% CO_2:95% air) for 24 hours before dosing. A series of three times diluted sample were dosed in triplicate at a volume of 2.5 µℓ per well, to generate a dose-response curve (Whyte et al*.,* 2004). Along with the environmental samples, controls were also dosed at 2.5 µℓ/well: a series of four times diluted 2,3,7,8-TCDD [160, 40, 10, 2.5, 0.625, 0.157 nM] (positive control); a 3 well solvent control (hexane); and a blank control, containing only cells and culture media (Hilscherova et al*.,* 2003). After 72 hr incubation the cells were microscopically inspected for viability and confluency. The culture media was removed and the cells were washed with Mg^{2+} and Ca^{2+} supplemented PBS. The ion supplemented PBS ensures that there is an excess of magnesium and calcium ions, which might be limiting factors during the light forming reaction of the bio-assay (Hilscherova et al*.,* 2000). The cell membranes were lysed using a lysis buffer for mammalian cell cultures (Sigma-Aldrich). The plates were frozen at -80°C for 30 minutes, to ensure that the cells have ruptured. The luminescence was recorded in a luminometer (Berthold multi-mode micro plate reader, model-LB941). The plate reader automatically injected 100 µℓ luciferase activating reagent (LAR) [20 mM tricine, 1.07 mM $Mg(CO_3)_2Mg(OH)_2{\cdot}5H_2O$, 2.67 mM $MgSO_4{\cdot}7H_2O$, 0.1 mM EDTA-disodium salt, 33.3 mM dithiothreitol, 270 µm coenzyme A, 530 µM ATP and 470 µM beetle luciferin (all

from Sigma-Aldrich)] (Villeneuve et al., 1999). The digestion of luciferin by luciferase produces light, measured in relative light units (RLUs).

Dose-response curves were prepared for the samples as well as the positive control by plotting the logarithm of the concentration (in the case of the control) or logarithm of the volume (in the case of the sample) on the x-axis and the %TCDDmax on the y-axis. The %TCDDmax was calculated by expressing the luminescence of each sample dilution as a percentage of the maximum luminescence generated by the positive control (2,3,7,8-TCDD) (Sanderson & Giesy, 1998). The relative effects potencies (REP) for the samples were calculated by dividing the effects concentration (EC20, EC50, EC80) of the positive control by the EC20-80 of the sample (Finney, 1971; Nieuwoudt et al., 2009) (The unit of these REPs is mass TCDD-equivalents/volume extract). Reporting all three REPs is necessary as it cannot be assumed that the complete mixture of the environmental samples will respond the same as TCDD (Villeneuve et al., 2000). The REP values were back calculated to represent the TCDD-eq in terms of the mass sediment extracted (Koh et al., 2005). The TCDD-eq calculated from bio-assay results are commonly known as bio-assay equivalents (BEQ) (Nieuwoudt et al., 2009).

The limit of quantification (LOQ) for the H4IIE-*luc* bio-assay was calculated by determining the mean EC_0 for the TCDD response curves. A 95% confidence interval was added to the average, for a ngTCDD/g value (Thomsen et al., 2003; Nieuwoudt et al., 2009).

MTT viability assay

A viability test was performed parallel to the luminescence bio-assay and dosed with the same series of samples and controls as in the bio- assay. The 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) viability assay was used to prevent false negative results in the luminescence bio-assay, where low or below LOD responses in the H4IIE-*luc* bio-assay might not necessarily be due to the absence of AhR agonists, but rather from cell toxicity. The mechanism of the MTT assay is that yellow MTT solution is metabolised by the living cells to form blue formazan crystals. The viability of the cells can be determined by spectrophotometrically quantifying the amount of formazan produced (Vistica et al., 1991).

The MTT plates were seeded, dosed, and incubated in the same manner as the bio-assay plates. On the fifth day, the MTT plates were rinsed with PBS, but did not receive lysis buffer. The negative control cells were killed with 100 µℓ MeOH before all the wells received 100 µℓ MTT solution and incubated for 30 min at 37°C, and air with 5% CO_2. The cells were inspected, to determine if the formazan crystals had formed. The excess MTT solution was

discarded and the formed crystals dissolved with 200 µℓ dimethyl sulphoxide (DMSO) (Sigma-Aldrich). After a waiting period of 15 minutes at room temperature, the optical density (OD) at 560 nm was measured (Berthold multi-mode micro plate reader, model-LB941) (Vistica et al*.,* 1991).

Viability was calculated by expressing the OD of the wells that received samples were expressed as a percentage of the OD from the control wells, representing 100% viable cells. A percentage below 80% indicated that the sample was cytotoxic that could have affected the H4IIE-*luc* bio-assay.

3.4.2 Fish health assessment index and gross body indices

The method followed was a modified version as described by Heath et al. (2004) and the scoring system proposed by Adams et al. (1993). The condition of various morphological characteristics was assessed using a score sheet. This score sheet has detailed descriptions for each condition that may occur, each with a corresponding field code and a corresponding numeric value. Fish health assessment started with the euthanasia of the fish by severing the spinal cord. The euthanized fish was measured and weighed and the values recorded. The external characteristics which are the eyes, skin, fins, gills, opercula and number of external parasites were evaluated and scored according to the score sheet. The fish were dissected for the internal evaluation. The internal characteristics which are the liver, spleen, bile and internal parasites were evaluated. The gonads, liver and spleen were dissected and weighed for their respective indices.

The FHAI score, the standard deviation and coefficient of variance were calculated, according to Adams et al. (1993). These values were used to compare population health within- and between sites, as well as over different sampling seasons. The mass and length recorded for the FHAI were used to calculate the Cf, by using the formula described by Nash et al*.* (2006):

$$\mathrm{K} = 100 \times \frac{\mathrm{W}}{\mathrm{L}^3}$$

where K is Fulton's Cf, W is mass in gram, and L is total length in centimetre. The GSI, HSI and SSI were calculated by dividing the index respective organ mass by the gutted body mass and multiplying by 100 (Rohlenová et al., 2011). The gross body indices thus show the percentage mass these organs contribute to the body mass.

2.4.3 Biomarker response assays

The mass of the tissue samples were noted and the appropriate buffer added. The first batch (labelled A) was for CAT, SOD, PC and CYP450 activity. A mass of 0.1 g liver tissue was measured and added to 1 mℓ general homogenising buffer (GHB) [0.1 M potassium phosphate buffer, 1.15% KCl, 1 nM EDTA, 0.1 nM phenyl methane sulphonyl fluoride (PMSF), 20% glycerol]. Batch B was for AChE and MDA, which was 0.05 g liver in 250 µℓ tris-sucrose buffer [25 mM tris-HCl, 250 nM sucrose]. From these batches aliquots were taken for each respected bio-marker. Protein content of each sample was determined using the Bradford (1976) method (read at 590 nm), using bovine serum albumin (BSA) as standard. Protein content is determined because the bio-markers are expressed as activity per milligram protein.

All reagents used in the biomarker analysis were acquired from Sigma-Aldrich, unless otherwise stated.

Acetylcholinesterase

The procedure of determining AChE activity was adapted from Ellman et al. (1961). The following were added to 24 wells of a 96 well microtitre plate: 210 µℓ potassium phosphate buffer (PPB) [0.09M, ph 7.4, K_2HPO_4 and KH_2PO_4], 10 µℓ s-acetylthiocholine iodide and 10 µℓ Ellmans's reagent (2,2'-dinitro-5,5' dithio-dibenzoic acid). Only 7 samples were analysed at a time to ensure accurate readings. After a 5 minute incubation at 37°C, 5 µℓ sample and a blank (GHB) was added into the wells in triplicate. The microtitre plate was tapped gently to ensure mixing. The reaction was read kinetically at 405 nm for 6 minutes at 1 minute intervals.

Catalase activity

Catalase activity was measured using the methodology from Cohen et al. (1970). Ten millilitres of the sample supernatant (batch A; centrifuged at 10 000 g for 10 minutes at 4°C) was added to the 96 well plate in triplicate, with 10 µℓ GHB as blank. Only 10 samples were analysed at a time. Hydrogen peroxide (H_2O_2) (93 µℓ) was added to each well and mixed gently by tapping. The plates were incubated at room temperature for 3 minutes. The reaction was halted by adding 19 µℓ sulphuric acid. Potassium permanganate ($KMNO_4$) (130 µℓ) was added immediately and OD read at 490 nm. The CAT assay is light sensitive and was performed in the dark.

Superoxide dismutase

The SOD method was adapted from Greenwald (1989). Each sample (4 µℓ) was added to the microtitre plate in triplicate. 245 µℓ DTPA/Tris buffer [50 nM tris-HCl, 0.1 M diethylene triamine penta-acetic acid (DTPA)] was added to each sample. The reaction was started by adding 4 µℓ pyrogallol and read kinetically at 560 nm for 5 minutes (11 intervals). This assay was also done in the dark as pyrogallol is light sensitive.

Lipid peroxidation (Malondialdehyde content)

The methodology from Ohkawa et al. (1979) as modified by Üner et al. (2005) to determine MDA content was followed. The homogenate of the second batch (B) was centrifuged at 10 000 g (4°C) for 10 minutes. A new set of sample tubes were used in this bio-marker assay. Twenty five microlitres homogenate supernatant was added to the new tubes along with 50 µℓ sodium dodecyl sulphate (SDS), 375 µℓ acetic acid, 375 µℓ thiobarbituric acid, and 175 µℓ ultra-pure water. The tubes were incubated for 30 minutes at 95°C in a water bath. After incubation, the samples were allowed to cool down to room temperature. 250 µℓ ultra-pure water and 1 250 µℓ butanol:pyridine solution (15:1) was added, vortexed and centrifuged at 2 700 g for 10 minutes at room temperature. The supernatant (245 µℓ) was read at 540 nm. This was done in triplicate. A 1,1,3,3-tetramethoxypropane (TMP) series was used as a standard and Tris/sucrose buffer as a blank. Both these were prepared the same as the samples.

Protein carbonyl

PC content was assayed as described by Levine et al. (1990) and modified by Floor & Wetzel (1998) (Parvez & Raisuddin, 2005). Post-mitochondrial supernatant of the batch homogenate (500 µℓ) was placed in a new sample tube set. An equal amount of 2,4-dinitrophenylhydrazine (DNPH) was added and allowed to react for an hour. The proteins were precipitated with 500 µℓ 6% trichloro acetic acid (TCA). The solution was centrifuged at 10 000 g for 3 minute. The TCA (supernatant) was discarded carefully and the protein pellet washed three times with absolute ethanol. After centrifuging at 10 000 g for ten minutes, 400 µℓ guanidine hydrochloride (to dissolve proteins) was added. The sample incubated for 15 minutes (37°C) and centrifuge again for 5 minutes (16 000 g). Absorbance was read at 390 nm in triplicate.

Cytochrome P450 activity

The cytochrome P450 activity was determined by using the DetectX P450 demethylating fluorescent activity enzyme linked immuno-sorbent assay (ELISA) kit from Arbor Assays (K011-F1). The samples were diluted with the assay buffer. The reaction was started with reconstituted nicotinamide adenine dinucleotide phosphate (NADPH) solution and incubated (37°C, 30 minute intervals). The reaction was stopped with the stop solution (acetic acid) and the formaldehyde detection reagent was added. The fluorescence of the samples was read at 510 nm with an excitation at a wavelength of 450 nm.

Statistical analysis of results

Correlation between the sum of the PAHs and the sediment grain size, as well as the total organic carbon, was done using Pearson's correlation calculation. Biomarker- and fish health assessment results were tested for normality using the D'Agostino & Pearson omnibus normality test and the Shapiro-Wilk normality test. One-way analysis of variance (ANOVA) was performed if the data was distributed normally and Tukey's Multiple Comparison test as a post-test. For non-parametric data, the Kruskal-Wallis test was performed with Dunn's Multiple Comparison Test as post-test. A $p<0.05$ was considered significant. Statistical analysis was done on Graphpad Prism version 5.

3.4.3 Sediment toxicity assessment

PAH sediment quality guidelines

Sediment quality guidelines chosen specifically for PAH contamination are the guidelines set-up by MacDonald and co-authors (2000) and the Canadian sediment quality guidelines from the Canadian Council of Ministers of the Environment (CCME) (2001), as South Africa does not have any sediment quality guidelines. The guidelines described by MacDonald et al. (2003) are consensus based sediment quality guidelines calculated on the PAH's toxicity to sediment dwelling organisms. The authors suggested these guideline values based on a three step evaluation. The first step was the matching of biological effects and sediment chemistry from published research. Secondly, each of these sediment samples' measured compounds was compared to its corresponding existing guidelines, predicting its toxicity. Finally, accuracy of prediction was evaluated by determining if the sediments were toxic to one or more aquatic organisms, using standardised toxicity tests (MacDonald et al., 2000). Toxicity was designated at levels where responses were significantly different from a reference or control. Non-toxicity was designated at levels where sediments did not cause a significant response in at least one test endpoint. The threshold effects concentrations (TEC;

lower values) were set at values where 75% of the sediments were correctly predicted as non-toxic. Similarly, the probable effects concentration (PEC; higher value) was set where 75% of the sediment samples were correctly predicted to be toxic. Thus, the values set out by the guidelines are the TEC, where concentrations below this value is expected not to have harmful effects to sediment dwelling organisms, and the PEC where concentrations above the value are expected to have harmful effects to benthic organisms at a more frequent interval. Guideline values for only Nap, Fl, Phe, Ant, Fla, Pyr, BaA, Chr, BaP, DBA and ΣPAHs (by addition) are available (expressed as µg/kg dm) (MacDonald et al., 2000).

The Canadian quality guidelines were implemented to protect, sustain and enhance the environment. They were created using environmental and human health risk protocols, thus they are used to protect human and animal health (CCME, 2012). In the case of the Canadian sediment quality guidelines, an interim sediment quality guideline (ISQG; lower value) and a probable effects level (PEL; higher value) for PAHs was created, to evaluate the degree to which adverse effects could occur from exposure to PAH containing sediment and so as a useful ecotoxicological assessment tool (CCME, 1999; 2012). This set of guidelines was derived on a similar principle as followed by MacDonald et al. (2000). These guidelines where derived by combination of both a modification of the National Status and Trends Program (NTSP) approach, as well as the spiked-sediment toxicity test (SSTT). The modified NSTP approach associates filed collected sediment concentrations of each compound measured, with any adverse biological effects observed (compiled in the Biological Effects Database for Sediments, BEDS). The SSTT approach is an independent evaluation of information from toxicity tests completed with spiked-sediments. This approach estimates the concentration of a chemical where adverse effects are not expected. The CCME has guidelines for Nap, Acey, Acea, Fl, Phe, Ant, Fla, Pyr, BaA, Chr, BaP and DBA (expressed as µg/kg 1%TOC).

Sediment indices

The sediment quality guideline index (SQG-I) is used to determine the ecological risk the sediment pose to benthic organisms (Fairey et al., 2011). The SQG-I incorporates the more protective guideline (lower) values and the measured concentrations of the target compounds to calculate the index value.

$$\text{SQG-I} = \frac{\Sigma_{i=1}^{n} C_{PAH}(\text{Sample})/C_{PAH}(\text{Threshold})}{n}$$

The SQG-I is the arithmetic mean of how many times the measured concentration ($C_{HM(Sample)}$) of individual PAHs at a specific site were higher than the lower guideline levels ($C_{HM(Threshold)}$) (Fairey et al., 2001).

In addition to the SQG-I, the quality of the sediment can be calculated in terms of the PAH contamination. The sediment quality index (SQI) as described by Marvin et al. (2003), incorporates the percentage of PAHs per site that did not meet the lower guidelines and their magnitude of exceedance.

$$\mathrm{SQI} = 100 - \frac{\sqrt{\mathrm{F}_1^2 + \mathrm{F}_3^2}}{\sqrt{2}}$$

The calculation of the index takes into account two elements, namely the scope (F_1) and amplitude (F_3). The scope is the percentage of variables that did not meet the guidelines

$$\mathrm{F}_1 = \left(\frac{\text{number of failed variables}}{\text{total variables}}\right) \mathrm{x}\ 100$$

Amplitude is the magnitude by which the failed variables exceed the guidelines.

$$\mathrm{F}_3 = \left(\frac{\mathrm{mdnc}}{0.001\mathrm{mdnc} + 0.01}\right)$$

Where:

mdnc = Mean degree of non-compliance

$$\mathrm{mdnc} = \sum_{\mathrm{i}=1}^{\mathrm{p}} \text{non-compliance}_\mathrm{i}$$

$$\text{non-compliance}_\mathrm{i} = \left(\frac{\text{failed test value}_\mathrm{i}}{\text{guideline}_\mathrm{i}}\right)$$

Failed test value = amount of samples not meeting guidelines
i = Individual guideline
p = Total amount of guidelines used

Toxic equivalent quotient calculation

The TEQ is calculated by the following equation:

$$\mathrm{TEQ} = \sum(\mathrm{C_i} \times \mathrm{TEF_i})$$

where the instrumentally determined concentration (C_i) is multiplied to the respective TEF_i and the sum total calculated. The Ah-receptor only binds to specific compounds-halogenated aromatic hydrocarbons such as the polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs), as well as PAHs. Villeneuve and co-authors (2002) derived TEF values for the PAHs that actively bind to the AhR using the H4IIE-*luc* bioassay. International guidelines available for TEQs are available for assessment (South

Africa does not have TEQ guidelines). The Canadian interim sediment quality guidelines (ISQG) for dioxin-like compounds were used in the assessment. This guideline was specifically created for the protection of fish from dioxin-like compounds (CCME, 2001), and seeing that PAHs use the same mechanism of action, it was used to evaluate PAH toxicity.

3.4.4 PAH source identification and compositions

The diagnostic ratios used to identify the possible sources of the PAHs (Cf. PAH diagnostic ratios) are listed in Table 5. The respected sources are determined by the ranges of each of the ratios.

Table 5: Diagnostic ratios for source identification of PAHs

	$\frac{\Sigma LPAHs}{\Sigma HPAHs}$	$\frac{Ant}{(Ant + Phe)}$	$\frac{Fla}{(Fla + Pyr)}$	$\frac{BaA}{(BaA + Chr)}$	$\frac{InP}{(InP + BgP)}$
Petrogenic	>1	<0.1	<0.4	<0.2	<0.2
Pyrogenic	<1	>0.1			
Pyrogenic: petroleum combustion			0.4-0.5	0.2-0.35	0.2-0.5
Pyrogenic: biomass combustion			>0.5	>0.35	>0.5

3.5 Human health risk assessment

Health risk assessments of chemical concentrations can provide the data to estimate the probability of health effects in exposed humans. 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 methodology used to asses this potential human health risk was that described by the US EPA (1987, 1992) and the WHO (2010). The health risk assessment consists of a 4 step process including hazard identification, dose-response assessment, exposure assessment, and lastly risk characterisation.

Hazard identification assesses the likelihood that exposure to chemical under specific exposure conditions will pose a threat to human health (US EPA, 2000; Heath et al., 2004). Information like physico-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 (Heath et al., 2004; IRIS, 2012; US EPA, 2000)

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 (Heath et al., 2004; Newman, 2004; US EPA, 2000).

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

Hazard quotient (HQ)

The hazard quotient was calculated for agents that cause non-cancer toxic effects, where the expected exposure to the agent is compared to an exposure that is assumed not to be associated with toxic effects.

For oral or dermal exposures the Average Daily Dose (ADD) was compared to a Reference Dose (RfD):

$$\mathrm{HQ} = \frac{\text{Average Daiy Dose}}{\text{Reference Dose}}$$

Any HQ less than 1 is considered to be safe for a lifetime exposure.

Cancer risks (CR)

For chemicals that may cause cancer if ingested, risk is calculated as a function of Oral Slope Factor and ADD and can be calculated by:

$$\mathrm{CR} = e^{-(\text{Oral Slope Factor} \times \text{Lifetime Average Daily Dose})}$$

Approach

The approach used to conduct the human health risk assessment for PAHs in environmental samples made use of the “reasonable maximum” concentrations detected in sample sites as a worst case scenario to determine what risks (if any) might be expected. This was carried out as a screening risk assessment. Where no PAH compounds were detected, the level of detection was used to determine what level of risk would exist if each type of sample were found to contain PAHs

The PAH concentration found in sediments was used to calculate the expected dose in fish and possible health risks were calculated for humans if fish from these areas is consumed. As no detectable parent PAHs were found in either collected fish or in eggs, a hypothetical risk assessment was performed using the limit of detection

The 95^{th} percentile for each PAH measured was calculated to represent the “reasonable maximum” concentration and used to calculate possible health risks for humans if fish from these areas is consumed. In addition to sediment samples, wild bird eggs and fish were analysed, but did not have detectable or quantifiable concentrations of PAHs over the study period). The limit of detection for each sample type (medium) was used to provide an indication of possible human health risks that may need to be taken into consideration.

The available reference doses and cancer slope factors used in the risk calculations are provided in Table 6.

Table 6: Chemical toxicity and carcinogenicity data used in the risk assessment

Chemical	CAS #	Chronic oral reference dose (mg/kg-day)	Chronic oral reference dose reference	Oral slope factor $(mg/kg\text{-}day)^{-1}$	Oral slope factor reference
Acenaphthene	000083-32-9	0.06	IRIS	N/A	
Acenaphthylene	000208-96-8	N/A		N/A	
Anthracene	000120-12-7	0.3	IRIS	N/A	
Benzo[a]pyrene	000050-32-8	N/A		7.3	IRIS
Benzo[b]fluoranthene	000205-99-2	N/A		0.73	Surrogate. WHO/TEF
Benzo[g,h,i]perylene	000191-24-2	N/A		N/A	
Benzo[k]fluoranthene	000207-08-9	N/A		0.073	Surrogate. WHO/TEF
Chrysene	000218-01-9			0.0073 or 0.12	IRIS and California EPA
Dibenz[a,h]anthracene	000053-70-3	N/A		7.3	Surrogate. WHO/TEF
Fluoranthene	000206-44-0	0.04	IRIS	N/A	
Fluorene	000086-73-7	0.04	IRIS	N/A	
Fluorine (Soluble Fluoride)	007782-41-4	0.06	IRIS	N/A	
Indeno[1,2,3-cd]pyrene	000193-39-5	N/A		7.3	Surrogate. WHO/TEF
Naphthalene	000091-20-3	0.02	IRIS	N/A	
Phenanthrene	000085-01-8	N/A		N/A	
Pyrene	000129-00-0	0.03	IRIS	N/A	

IRIS = Integrated Risk Information System; WHO = World Health organisation; TEF = Toxic equivalency factors

Cross-media transfer equations used to generate exposure estimates

The formulae used to generate the exposure concentrations based on sediment concentrations was that described by the US EPA (1990) for sediment to fish concentrations.

$$\mathrm{C(w)} = \frac{\mathrm{C\ (sd)}}{\mathrm{Koc} \times \mathrm{OC} \times \mathrm{DN}}$$

$$\mathrm{BCF} = (0.79 \times \log(\mathrm{Kow})) - 0.40$$

$$\mathrm{C(f)} = \mathrm{BCF} \times \left(\frac{\mathrm{fat}}{3}\right) \times \mathrm{C(w)}$$

Where:

- C(f) = Concentration in fish
- C(w) = Concentration in water
- C(sd) = Concentration in sediment
- DN = Sediment density (relative to water density of 1.0 kg/ℓ) (1.90)
- OC = Organic carbon fraction of sediment (4.00%)
- Koc = Octanol-carbon partition coefficient of the compound
- Kow = Octanol-water coefficient of the compound
- BCF = Bioconcentration factor

The dose estimates in this assessment, as well as the risk estimates derived from them, refer only to the specific exposure parameters that have been described in Table 7.

Table 7: Exposure parameters used to generate exposure estimates

Exposure parameter	Value
Events per year	350
Kg per event	0.054
Body mass	70 kg
Exposure duration	30 years

The average daily dose was calculated taking into account the concentration of the chemicals in sediment, for a 70 kg adult, assuming an intake of 0.054 kg fish on a daily basis (equivalent to 378 g per week). Risks are presented using 95th percentile concentrations of chemicals detected in the sediment, calculated to represent concentrations expected in fish. The 95th percentile represents the “reasonable maximum” risk.

4 RESULTS AND DISCUSSION

4.1 Chemical analysis results

The chemical analysis was done by the National Metrology Institute of South Africa (NMISA). The 16 USEPA priority PAHs analysed for where: naphthalene [Nap], acenaphthylene [Acey], acenaphthene [Acea], fluorene [Fl], phenanthrene [Phe], anthracene [Ant], fluoranthene [Fla], pyrene [Pyr], benzo(a)anthrancene [BaA], chrysene [Chr], benzo(b)fluoranthene [BbF], benzo(k)fluoranthene [BkF] benzo(a)pyrene [BaP], indeno (1,2,3-cd)pyrene [InP], benzo(ghi)perylene [BgP] and dibenzo(ah)anthracene [DBA]. They analysed all three matrices: sediment, fish fillet and bird eggs and only for the parent compounds of the already listed PAHs, i.e. no metabolites.

4.1.1 Sediment chemical analysis results

The detailed chemical analytical results of both 2013 and 2014 sediment samples are shown in Table 8. Samples that showed weak recoveries was subsequently reanalysed using a more sensitive GCxGC-MS-TOF. The samples that were below the limit of detection (LOD) and limit of quantification (LOQ) are reported as half LODs and half LOQs respectively. There was no correlation between the PAH concentrations and either total organic carbon content or the sediment grain size (Data not shown).

The summary of the results is reported in Table 8, which includes:

- individual PAH concentrations
- total PAHs (ΣPAHs),
- low molecular mass PAHs (ΣLPAH:Nap, Acey, Acea, Fl, Phe & Ant)
- high molecular mass PAHs (ΣHPAH: Fla, Pyr, BaA, Chr, BbF, BkF, BaP, InP, BgP & DBA)
- carcinogenic PAHs (ΣCPAH: BaA, Chr, BbF, BkF, BaP, InP & DBA).

In 2013 Moroka had the greatest ΣPAH of 3 684 µg/kg, followed by Protea Glen, 1 077 µg/kg. The lowest ΣPAH was at Nancefield and Dobsonville, 274 µg/kg; 275 µg/kg respectively (Table 8).

Table 8: Concentrations (µg/kg) of the PAHs in the sediment from the nine sites in the greater Soweto/Lenasia area for 2013 and 2014.

2013	Nap	Acey	Acea	Flu	Phe	Ant	Fla	Pyr	BaA	Chr	BbF	BkF	BaP	InP	DBA	BgP	ΣPAH	ΣLPAH	ΣHPAH	ΣCPAH
Protea Glen	119.8	2.8	77.4	31.1	112.7	22.2	156.3	118.4	69.0	79.2	83.2	40.3	14.0	47.0	23.0	80.7	1077.2	366.0	711.2	355.8
Lenasia	125.4	2.8	82.8	37.1	56.0	17.9	27.5	30.6	12.9	13.8	13.3	11.6	14.0	3.2	23.0	15.0	487.0	322.0	164.9	91.8
Fleurhof	70.1	0.8	22.9	12.8	99.0	22.7	169.0	128.6	79.0	41.1	45.3	29.0	14.0	26.6	23.0	34.3	818.1	228.3	589.8	257.9
Moroka	62.4	6.4	22.9	46.8	326.5	59.4	900.3	590.1	369.6	214.9	335.5	181.5	30.7	184.9	76.7	275.6	3 684.2	524.4	3 159.8	1 393.8
Eldorado Park	105.7	2.8	63.9	15.6	79.3	15.4	124.2	94.2	56.1	39.7	59.6	42.2	14.0	19.3	23.0	50.6	805.5	282.7	522.8	253.8
Orlando West	34.7	2.8	6.9	15.3	126.3	22.9	233.1	176.6	95.8	58.4	70.5	44.9	14.0	43.4	23.0	56.9	1025.6	208.8	816.7	350.1
Orlando East	144.2	7.1	100.8	52.5	138.5	27.8	148.1	119.9	55.3	36.0	42.5	27.3	17.3	31.8	23.0	45.4	1017.5	470.9	546.6	233.2
Nancefield	90.4	0.8	49.6	11.7	32.5	8.0	12.0	9.2	5.2	6.2	3.5	3.9	14.0	3.2	23.0	1.1	274.3	193.0	81.3	59.0
Dobsonville	59.5	0.8	22.9	5.4	29.8	7.0	24.5	9.2	14.3	14.1	16.6	9.5	14.0	10.6	23.0	14.5	275.7	125.4	150.4	102.2
2014	**Nap**	**Acey**	**Acea**	**Flu**	**Phe**	**Ant**	**Fla**	**Pyr**	**BaA**	**Chr**	**BbF**	**BkF**	**BaP**	**InP**	**DBA**	**BgP**	**∑PAH**	**∑LPAH**	**∑HPAH**	**∑CPAH**
Protea Glen	113.1	44.1	22.9	38.7	77.8	15.4	99.7	74.0	42.9	37.3	51.7	24.5	14.0	30.0	23.0	48.7	757.8	312.0	445.7	223.4
Lenasia	240.9	100.8	195.8	280.0	184.0	36.9	239.2	212.1	140.9	89.7	53.4	29.1	14.0	94.8	23.0	155.1	2089.7	1038.3	1051.4	445.0
Fleurhof	160.0	48.8	113.2	39.6	78.9	16.8	85.5	95.5	39.5	25.1	48.9	30.4	14.0	34.0	23.0	49.7	902.8	457.3	445.5	214.9
Moroka	512.7	421.2	470.1	328.3	385.2	92.4	1 086.3	786.6	373.3	241.9	60.0	33.2	44.6	184.9	76.7	272.2	5 369.5	2 209.9	3 159.6	1 014.5
Eldorado Park	255.8	92.0	210.4	52.4	207.5	54.0	479.4	358.7	202.4	127.3	53.8	32.2	28.4	96.1	23.0	139.7	2 412.9	872.0	1 540.9	563.1
Orlando West	122.8	116.9	76.1	119.5	57.6	19.1	103.3	80.5	47.2	30.3	53.8	33.2	14.0	21.2	23.0	28.3	946.7	511.9	434.8	222.8
Orlando East	94.3	0.8	22.9	13.8	42.6	10.3	37.4	30.6	14.6	13.8	50.8	31.1	14.0	9.2	23.0	12.0	421.3	184.8	236.5	156.6
Nancefield	80.6	0.8	22.9	16.5	105.7	23.2	105.3	81.9	46.9	32.0	43.9	32.1	14.0	20.1	23.0	27.5	676.3	249.7	426.6	211.9
Dobsonville	32.3	0.8	6.9	18.0	57.1	12.1	67.4	30.6	28.4	25.0	53.1	28.2	14.0	16.6	23.0	22.2	435.5	127.2	308.4	188.2
½ LOD	1.61	0.85	6.86	5.41	3.59	11.26	1.00	9.17	9.42	1.88	1.05	0.93	4.21	0.97	23.02	1.13				
½ LOQ	5.35	2.82	22.86	18.03	11.95	37.54	3.32	30.57	31.41	6.24	3.49	3.09	14.03	3.22	76.71	3.76				

Moroka also had the highest ΣPAH for the 2014 season, 5 369.5 µg/kg, which is the highest concentration of all seasons. Eldorado Park and Lenasia also had high ΣPAH concentrations (2 412 µg/kg and 2 089 µg/kg, respectively) (Table 8). The lowest value was at Orlando East (421 µg/kg), followed by Dobsonville (435 µg/kg) (Table 8). The study by Roos et al. (2011) had comparable results for the total PAHs. The highest levels of total PAHs from that study was 5 408 µg/kg, measured at a site between the Eldorado Park and Nancefield sites of this current study. Overall, the levels measured by Roos and co-authors (2011) were higher than measured during the 2013 and 2014 seasons (Table 9). In another study by Quinn et al. (2009), these authors investigated organic pollutants in the central part of South Africa, which include our study site. The mean ΣPAHs was lower the current study's levels (Table 9), however the range between minimum- and maximum concentrations was bigger (Quinn et al., 2009). In a study on PAH distribution from sources, Okedeyi et al. (2013) investigated PAHs in soils at coal-fired power stations and they found high levels of PAHs on site (Table 9), which declined over distance away from the source. The Lethabo- and Rooiwal (or Kelvin) power stations are 60- and 30 km away from this project's sampling sites – close enough for air transported PAHs. Internationally, the levels and ranges of PAHs in Europe are higher than in South Africa, but those from China are comparable (Table 9).

Table 9: Concentration of ΣPAHs from literature

Location	n	ΣPAHs (µg/kg)		Reference
		Mean	Range	
Klip River-Soweto/Lenasia, Gauteng, RSA	18	801	274 -2090	this study
Klip River-Soweto/Lenasia, Gauteng, RSA	12	1 362	215 -5408	Roos et al., 2011
Multiple rivers-Vaal Triangle, Free State & Gauteng, RSA	9	641	44 -2799	Quinn et al., 2009
Klip River-Vereeniging, Gauteng, RSA	1	580		Nieuwoudt et al., 2011
Riet Spruit-Vanderbijlpark, Gauteng, RSA	1	2 800		Nieuwoudt et al., 2011
Lethabo Power station, Free State, RSA *	1	18 750		Okedeyi et al., 2013
Matla Power station, Mpumalanga, RSA *	1	20 650		Okedeyi et al., 2013
Rooiwal Power station, Gauteng, RSA *	1	14 440		Okedeyi et al., 2013
Morava- & Drevnice Rivers, Czech Republic	13	18 620	3 500 -61 700	Hilscherova et al.,2001
Yellow River, China	14	1 414	464 -2 035	Xu et al., 2007
Yangtze Delta, China	11	1 556	133 -2 981	Chen et al., 2004

* Samples from power stations are soil samples

Moroka had the highest values for ΣLPAH, ΣHPAH, and ΣCPAH (Table 8) for both the 2013 and 2014 seasons. A temporal change was noted in the Moroka results between the two sampling events. There was a considerable increase in the ΣLPAH concentration at the site, increasing from 524 µg/kg in 2013 to 2 209 µg/kg in 2014 (Table 8).

Both Eldorado Park and Lenasia also had high concentrations of low- and high molecular PAHs for the 2014 season, where Eldorado Park had predominantly HPAHs (1 540 µg/kg, Table 8).

As mentioned above, Moroka had the highest concentrations for all the representative levels, including the sum of the carcinogenic PAHs. The second highest ΣCPAH for 2013 was Protea Glen (355 µg/kg) (Table 8) and the lowest Nancefield, 59 µg/kg (Table 8). The least of the ΣCPAH for 2014 was from Dobsonville (188 µg/kg) (Table 8). The only results obtained from 2013 that had a higher CPAHs level of Roos et al. (2011) was for Moroka. The 2014 samples that were comparable to the Roos et al. (2011) results were Lenasia, Moroka and Eldorado Park.

4.1.2 Fish tissue chemical analysis results

No PAHs (in native form) were detected in the fish samples. The reason for this is the ability of vertebrates to bio-transform xenobiotics (Newman, 2010). Xenobiotics are biotransformed via 2 pathways. Firstly, if a xenobiotic is hydrophilic, it goes through the phase I pathway of biotransformation. This is where a polar conjugate is introduced by means of oxidatiive, reductive and/or hydrolytic processes (Newman, 2010; Tuvikene, 1995). Xenobiotics that are already water soluble are directly passed to the phase II pathway. This pathway involves the conjugation of xenobiotics or their phase I metabolites. The conjugates that are added to the compounds include acetate, amino acids, glutathione, glucuronic acid, methyl groups and sulphate, to name a few (Newman, 2010; Tuvikene, 1995). Once conjugation has taken place the xenobiotic is water soluble and can be excreted from the organism (Newman, 2010; Tuvikene, 1995). The main PAH metabolism pathway in fish involve cytochrome P450 (CYP450), monooxygenases, epoxide hydrolase and conjugating enzymes (Tuvikene, 1995). The metabolism of PAHs is well studied and the collective process is described in detail by Tuvikene (1995): The subfamily of the CYP450 genes that are activated in fish by PAHs is the CYP1A family. After the PAH has bound to the Ah-receptor (Cf.Introduction: Biomarkers and Relevance of bio-assays), the P450s are induced. Specific forms of P4501A1 are induced after exposure to PAHs: aryl hydrocarbon hydroxylase (AHH), ethoxyresorufin-O-deethylase (EROD) and 7-ethoxycoumarin-O-deethylase (ECOD). The release of these enzymes results in the addition of an oxygen atom and in most cases this

oxygen is reduced to a hydroxyl group by monooxygenases. After these reactions the metabolites are conjugated by several enzymes-glutathion-S-transferase (GST), uridine 5-diphosphate-glucuronosyltransferase (UDP-GT) and glutathione (GSH). These enzymes complete biotransformation phase II: reducing the toxicity of the compound and making it easier to excrete

For this project only the parent PAHs were analysed and not their metabolites as well due to financial constraints. The “no PAHs detection” isn’t necessarily indicative of no PAHs present in the biota, merely proving the ability of the animals to metabolise PAHs.

4.1.3 Wetland bird egg chemical analysis results

The samples that were below the limit of detection (LOD) and limit of quantification (LOQ) are reported as half LODs and half LOQs respectively.

The chemical analysis results for the wetland bird eggs were similar to that of the fish results. Most of the PAHs were metabolised and only Nap, Phe and to a lesser extent Acea were quantifiable (Table 10). The presence of naphthalene and phenanthrene can be attributed to their resistance to metabolism and affinity to accumulate in fish (Liang et al., 2007), which form the main part of piscivorous birds’ diets. The presence of these PAHs in the egg samples are indicative that there are parent PAHs present in the system and that they are ubiquitous. The detectable levels of the few PAH congeners are proof that there is.

Table 10: Levels of naphthalene, acenaphthene and phenanthrene (ug/kg) in wetland bird eggs sampled from Lenasia 2013

n	1	2	3	4	5	6	7	8	9	10	LOD	LOQ
Black headed heron												
Nap	17.0	247.0	17.0	0.0	17.0	17.0					34.0	113.2
Acea	5.9	5.9	0.0	0.0	0.0	0.0					11.9	39.5
Phe	7.3	7.3	7.3	7.3	7.3	7.3					14.7	48.9
ΣPAHs	30.2	260.3	24.3	7.3	24.3	24.3						
Cattle egret												
Nap	17.0	0.0	17.0	17.0	17.0	17.0	17.0	0.0	17.0	17.0	34.0	113.2
Phe	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	14.7	48.9
ΣPAHs	24.3	7.3	24.3	24.3	24.3	24.3	24.3	7.3	24.3	24.3		
Glossy ibis												
Nap	0.0	17.0	0.0	0.0	17.0	0.0	17.0	17.0	17.0	17.0	34.0	113.2
Phe	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	14.7	48.9
ΣPAHs	7.3	24.3	7.3	7.3	24.3	7.3	24.3	24.3	24.3	24.3		
Sacred ibis												
Nap	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	34.0	113.2
Phe	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	14.7	48.9
ΣPAHs	24.3	24.3	24.3	24.3	24.3	24.3	24.3	24.3	24.3	24.3		

4.2 PAH compositions

The ratios of the different size classes (based on number of rings) of PAHs were calculated to determine the composition percentages at each site. This allows for interpretation of the chemical concentrations and supplements the source identification ratios.

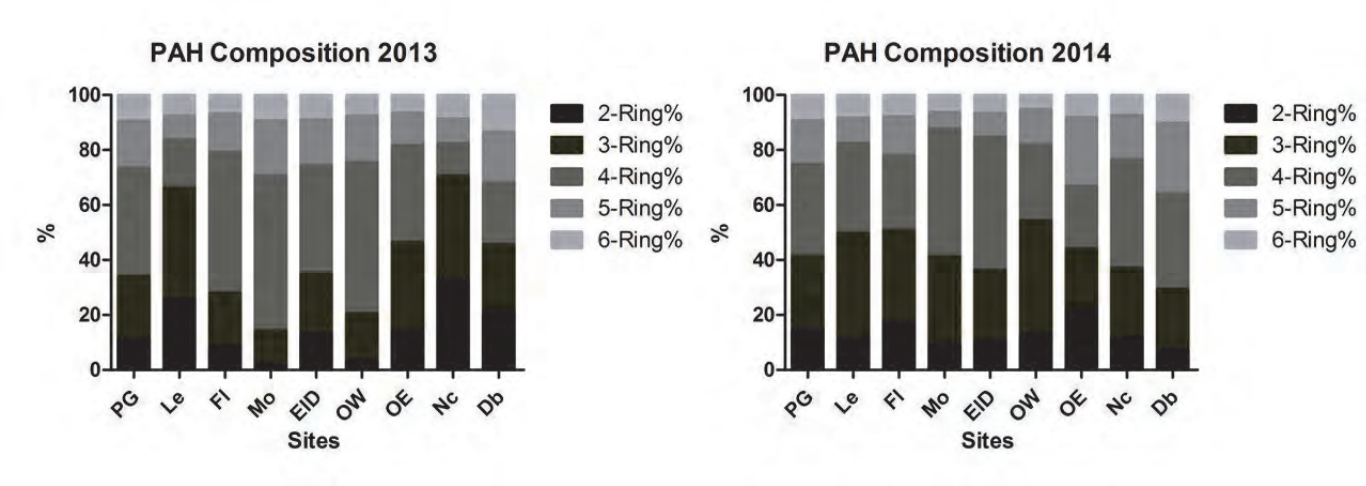


Figure 7: PAH profile composition at sites from the Soweto/Lenasia study area (2013 and 2014)

The percentage composition by the various congeners at the sites between the years varied, except for Protea Glen which seemed to have a stable contribution of similar sized PAH molecules between the two years (Figure 7). Further investigation revealed that all the sites sampled in 2014 had the most contributions from 3- and 4-ring congeners. Orlando East and Eldorado Park also had the biggest contribution from 3- and 4-ring PAHs during 2013, but the other sites showed a more random size contribution during that year (Figure 7). Lenasia, Dobsonville, and Nancefield (2013) had notably more of 2 and 3 ring congeners and this corresponds with the source identification for these sites (Figure 8, Table 11). None of the samples collected in 2013 had high percentages of the 5 and 6 ring congeners; however the sediment sampled at Orlando East and Dobsonville during 2014 had 5-ring congeners as its most prevalent PAHs (Figure 7).

4.3 PAH source identification

By calculating the ratios between various PAHs found at a site the original sources (pyrogenic vs petrogenic) can be determined. The results from the source identification are reported in Table 11 and Figure 8.

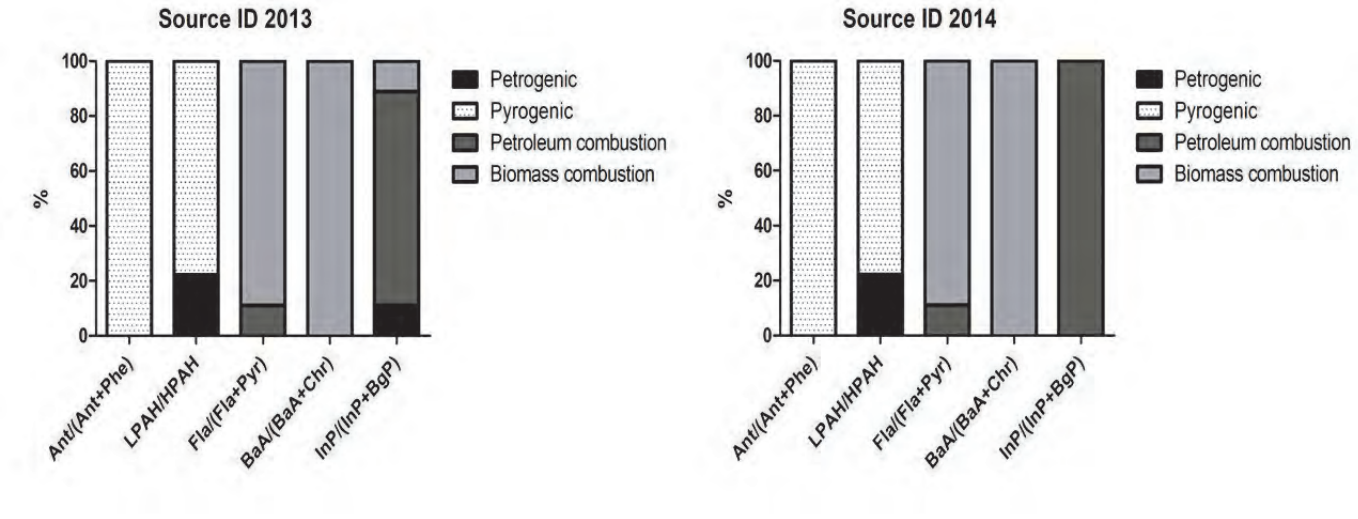


Figure 8: Source identification of PAHs in the Soweto/Lenasia sediment of 2013/2014

There is little temporal variance between the sources of PAHs in the sediment from 2013 to 2014. The sources are mainly pyrogenic as indicated by the Ant/(Ant+Phe) and LPAH/HPAH ratios (Figure 8). More specifically the type of combustion that dominated the formation of PAHs – as indicated by the Fla/(Fla+Pyr), BaA/(BaA+Chr) and InP/(InP+BgP) – is biomass combustion. Petroleum combustion is also present in the urban study area, shown by the InP/(InP+BgP) ratio (Figure 8). The only difference between 2013 and 2014 sources were measured with the InP/(InP+BgP) ratio. It indicated that 11% of the sites had PAHs that originated from petrogenic sources in 2013, and of the remaining 89% pyrogenic sites, 11% of these were due to biomass combustion.

The temporal variation observed for the InP/(InP+BgP) ratio can be attributed to the Lenasia site that seemed to have petrogenic sources for 2013 but indicated a pyrogenic source of petroleum combustion. Although Nancefield stayed in the pyrogenic category for both sampling years, its nature changed from biomass combustion in 2013 to petroleum combustion in 2014 (Table 11). Although the source distribution between petrogenic and pyrogenic showed the same percentages for the LPAH/HPAH ratio between sampling events, the particular sites that were identified with this ratio, differed between the years. In 2013 sites Lenasia and Nancefield had the petrogenic sources, but in 2014 the sites were Lenasia and Orlando West (Table 11). Similarly, the percentages shown for the Fla/(Fla+Pyr) ratio were also attributed to different sites: for 2013 Lenasia calculated for petroleum pyrogenic sources and in 2014 it was Fleurhof (Table 11). The origins identified by Roos et al. (2011) were similar to what was discovered for this study. It seems that the predominant source of anthropogenic released PAHs in SA is pyrogenic, even for the other

Table 11: Source identification ratios of PAHs in sediments at sites in Soweto/Lenasia of 2013/2014

	Sampling site	Ant/(Ant+Phe)	LPAH/HPAH	Fla/(Fla+Pyr)	BaA/(BaA+Chr)	IcdP/(IcdP+BghiP)
2013	Protea Glen	Py	Py	BioPy	BioPy	PetPy
	Lenasia	Py	Pet	PetPy	BioPy	Pet
	Fleurhof	Py	Py	BioPy	BioPy	PetPy
	Moroka	Py	Py	BioPy	BioPy	PetPy
	Eldorado Park	Py	Py	BioPy	BioPy	PetPy
	Orlando West	Py	Py	BioPy	BioPy	PetPy
	Orlando East	Py	Py	BioPy	BioPy	PetPy
	Nancefield	Py	Pet	BioPy	BioPy	BioPy
	Dobsonville	Py	Py	BioPy	BioPy	PetPy
	Sampling site	**Ant/(Ant+Phe)**	**LPAH/HPAH**	**Fla/(Fla+Pyr)**	**BaA/(BaA+Chr)**	**IcdP/(IcdP+BghiP)**
2014	Protea Glen	Py	Py	BioPy	BioPy	PetPy
	Lenasia	Py	Py	BioPy	BioPy	PetPy
	Fleurhof	Py	Pet	PetPy	BioPy	PetPy
	Moroka	Py	Py	BioPy	BioPy	PetPy
	Eldorado Park	Py	Py	BioPy	BioPy	PetPy
	Orlando West	Py	Pet	BioPy	BioPy	PetPy
	Orlando East	Py	Py	BioPy	BioPy	PetPy
	Nancefield	Py	Py	BioPy	BioPy	PetPy
	Dobsonville	Py	Py	BioPy	BioPy	PetPy

Py = pyrogenic, Pet = petrogenic, BioPy = biomass combustion, PetPy = Petroleum combustion

South African studies in Table 9 when the diagnostic ratios were calculated for them as well. Nieuwoudt et al. (2011) suggested that this is mainly from ineffective burning of organic fuels during open burning for domestic heat and cooking, and during incineration of waste.

4.4 Sediment toxicity assessment

4.4.1 PAH sediment quality guidelines

The sediment quality guidelines were used to determine ecological risk posed by the sediments from the sample sites, based on their chemical concentrations. The results of the application of the PAH sediment quality guidelines on the sediments of Soweto/Lenasia is reported in Figure 9 (for the study area) and Table 12 & 13 (for individual sites).

The CCME guidelines regarding PAHs are more protective than that set up by MacDonald et al. (2001), and are therefore lower. This is visible in the graphs in Figure 9: When the MacDonald guidelines were used only 11% of the sites had levels exceeding any of the respective guideline levels for 2013 (Figure 9a) and in 2014 between 11 and 33% of the sites surmounted the TEC guideline levels (Figure 9b). When the CCME guidelines were employed, many more sites had levels exceeding the respective guidelines (Figure 9c & 9d) and in some instances the individual guidelines were exceeded at all the sites. This was the case for naphtalene (Nap), acenapthene (Acea), and dibenzo(ah)anthracene (DBA)

(Figure 9c): In 2014 there were sites with PAH concentrations even surpassing the PEL and not merely the lower ISQG. This was true for acenapthylene (Acey), acenapthene (Acea), and fluorene (Flu) (Figure 9d).

In 2013, only Moroka exceeded the TEC guideline of MacDonald et al. (2001) for all respective PAH congener TEC levels except for naphthalene (Nap), fluorine (Fl), and benzo(a)pyrene (BaP). The ΣPAHs also exceeded its TEC guideline (Table 12). In 2014 Moroka was beyond the TEC for 9 of the 10 comparative guidelines (Table 12). In this year three more sites had levels above the respective TEC guidelines of MacDonald et al. (2001) (Table 12): Eldorado Park (6 out of 10), Lenasia (4 out of 10) and Orlando West with a single high level. No MacDonald et al. (2001) PEC guidelines were exceeded during the entire study.

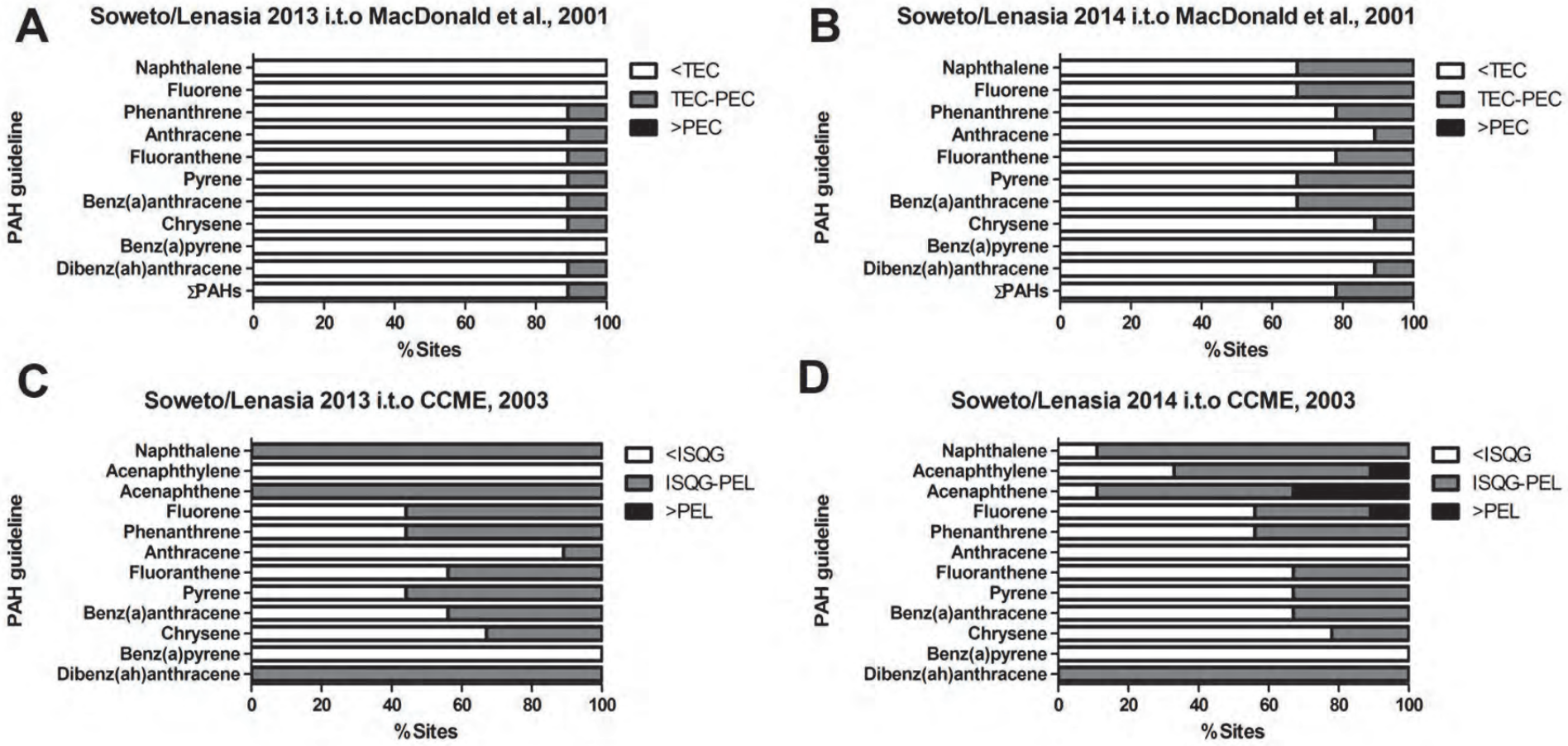


Figure 9: Soweto/Lenasia sediments compared to sediment quality guidelines of MacDonald et al., 2001 and CCME, 2012

Table 12: Sediment from the sites of Soweto/Lenasia compared to sediment quality guidelines (TEC and PEC) of MacDonald et al., 2001. Colour coordination indicates which guidelines were exceeded

	PAH	TEC	PEC	PG	Le	Fl	Mo	EID	OW	OE	Nc	Db
2013	Nap	176	561	119.8	125.4	70.1	62.4	105.7	34.7	144.2	90.4	59.5
	Flu	77.4	536	31.1	37.1	12.8	46.8	15.6	15.3	52.5	11.7	5.4
	Phe	204	1 170	112.7	56.0	99.0	326.5	79.3	126.3	138.5	32.5	29.8
	Ant	57.2	845	22.2	17.9	22.7	59.4	15.4	22.9	27.8	8.0	7.0
	Fla	423	2 230	156.3	27.5	169.0	900.3	124.2	233.1	148.1	12.0	24.5
	Pyr	195	1 520	118.4	30.6	128.6	590.1	94.2	176.6	119.9	9.2	9.2
	BaA	108	1 050	69.0	12.9	79.0	369.6	56.1	95.8	55.3	5.2	14.3
	Chr	166	1 290	79.2	13.8	41.1	214.9	39.7	58.4	36.0	6.2	14.1
	BaP	150	1 450	14.0	14.0	14.0	30.7	14.0	14.0	17.3	14.0	14.0
	DBA	33	-	23.0	23.0	23.0	76.7	23.0	23.0	23.0	23.0	23.0
	Total	1 610	22 800	745.7	358.3	659.3	2 677.4	567.2	800.2	762.5	212.2	200.8
	PAH	**TEC**	**PEC**	**PG**	**Le**	**Fl**	**Mo**	**EID**	**OW**	**OE**	**Nc**	**Db**
2014	Nap	176	561	113.1	240.9	160.0	512.7	255.8	122.8	94.3	80.6	32.3
	Flu	77.4	536	38.7	280.0	39.6	328.3	52.4	119.5	13.8	16.5	18.0
	Phe	204	1 170	77.8	184.0	78.9	385.2	207.5	57.6	42.6	105.7	57.1
	Ant	57.2	845	15.4	36.9	16.8	92.4	54.0	19.1	10.3	23.2	12.1
	Fla	423	2 230	99.7	239.2	85.5	1 086.3	479.4	103.3	37.4	105.3	67.4
	Pyr	195	1 520	74.0	212.1	95.5	786.6	358.7	80.5	30.6	81.9	30.6
	BaA	108	1 050	42.9	140.9	39.5	373.3	202.4	47.2	14.6	46.9	28.4
	Chr	166	1 290	37.3	89.7	25.1	241.9	127.3	30.3	13.8	32.0	25.0
	BaP	150	1 450	14.0	14.0	14.0	44.6	28.4	14.0	14.0	14.0	14.0
	DBA	33	-	23.0	23.0	23.0	76.7	23.0	23.0	23.0	23.0	23.0
	Total	1 610	22 800	536.0	1 460.7	577.9	3 928.0	1 788.8	617.2	294.4	529.1	307.8

Colour coordination indicates which guidelines were exceeded. TEC = threshold effects concentration; PEC = probable effects concentration

When the more sensitive Canadian guidelines (CCME, 2012) were applied, all the sites of 2013 surpassed three ISQG levels. Moroka was joined by two other sites with multiple congeners higher than the ISQG. Fleurhof had 10 out of 12 levels above the lSQG, followed by Moroka and Orlando West with 9/12. Eldorado Park and Orlando East had 6/12 and 5/12 exceedances respectively (Table 13). During the study of Roos and co-authors (2011), only 3 of the 13 sites exceeded the CCME guidelines. The congener that was exceeded by all three these sites was benzo(a)anthracene. Only one site had extra PAHs (apart from BaA) with levels higher than the ISQG for naphthalene (Nap), phenanthrene (Phe), pyrene (Pyr), and benzo(a)pyrene (BaP) (Roos et al., 2011).

Table 13: Sediment from the sites of Soweto/Lenasia compared to sediment quality guidelines of Canada (ISQG and PEL) (CCME, 2012). Colour coordination indicates which guidelines were exceeded

	PAH	ISQG	PEL	PG	Le	Fl	Mo	EID	OW	OE	Nc	Db
2013	Nap	34.6	391	38.4	88.9	167.0	48.8	132.2	65.5	69.7	40.4	68.3
	Acey	5.87	128	0.9	2.0	2.0	5.0	3.5	5.3	3.4	0.4	1.0
	Acea	6.71	88.9	24.8	58.7	54.4	17.9	79.9	12.9	48.7	22.1	26.3
	Flu	21.2	144	10.0	26.3	30.5	36.6	19.5	28.9	25.4	5.2	6.2
	Phe	41.9	515	36.1	39.7	235.7	255.1	99.1	238.3	66.9	14.5	34.3
	Ant	46.9	245	7.1	12.7	54.0	46.4	19.2	43.1	13.4	3.6	8.1
	Fla	111	2 355	50.1	19.5	402.3	703.3	155.2	439.9	71.6	5.3	28.2
	Pyr	53	875	37.9	21.7	306.2	461.0	117.7	333.3	57.9	4.1	10.5
	BaA	31.7	385	22.1	9.2	188.0	288.8	70.1	180.8	26.7	2.3	16.5
	Chr	57.1	862	25.4	9.8	97.8	167.9	49.6	110.1	17.4	2.8	16.2
	BaP	31.9	782	4.5	4.5	4.5	9.8	4.5	4.5	5.5	4.5	4.5
	DBA	6.22	135	7.4	7.4	7.4	24.6	7.4	7.4	7.4	7.4	7.4
	PAH	**ISQG**	**PEL**	**PG**	**Le**	**Fl**	**Mo**	**EID**	**OW**	**OE**	**Nc**	**Db**
2014	Nap	34.6	391	55.3	117.8	78.2	250.6	125.0	60.0	46.1	39.4	15.8
	Acey	5.87	128	21.6	49.3	23.9	205.9	45.0	57.2	0.4	0.4	0.4
	Acea	6.71	88.9	11.2	95.7	55.3	229.8	102.8	37.2	11.2	11.2	3.4
	Flu	21.2	144	18.9	136.9	19.4	160.5	25.6	58.4	6.8	8.1	8.8
	Phe	41.9	515	38.0	89.9	38.6	188.3	101.4	28.1	20.8	51.7	27.9
	Ant	46.9	245	7.5	18.0	8.2	45.2	26.4	9.3	5.0	11.3	5.9
	Fla	111	2 355	48.7	116.9	41.8	531.1	234.3	50.5	18.3	51.5	32.9
	Pyr	53	875	36.2	103.7	46.7	384.5	175.4	39.3	14.9	40.0	14.9
	BaA	31.7	385	21.0	68.9	19.3	182.5	98.9	23.1	7.1	22.9	13.9
	Chr	57.1	862	18.2	43.9	12.2	118.2	62.2	14.8	6.8	15.6	12.2
	BaP	31.9	782	6.9	7.6	9.5	29.8	26.6	19.8	18.7	10.3	15.1
	DBA	6.22	135	11.3	11.3	11.3	37.5	11.3	11.3	11.3	11.3	11.3

. ISQG = interim sediment quality guidelines; PEL = probable effects levels

An increase in concentration of PAH levels were noted in 2014. Subsequently, more guidelines were surpassed even to such an extent that some levels were higher than the PEL, e.g. Moroka, Eldorado Park and Lenasia (Table 13). Interestingly, Dobsonville seemed to have been less polluted in 2014 than in 2013 exceeding only one of the 12 guidelines.

4.4.2 Sediment assessment indices

The potential ecological risk that the sediment of the study area poses to benthic organisms, in terms of PAH exposure is expressed by the SQG-I (Table 14). In terms of the MacDonald et al. (2001) guideline, Moroka sediment sampled in 2013 and 2014, posed high probability to be toxic to biota. The only other site of 2013 that posed a moderate risk was Orlando East whereas, Lenasia and Eldorado Park scored moderate probability for 2014.

Table 14: SQG-I results for sites from Soweto/Lenasia (2013 & 2014) in terms of the MacDonald et al. (2001) guidelines and the CCME (2012) guidelines. Colour coordination according to index scale

Site	SQG-I (TEC) MacDonald et al. (2001)	SQG-I (ISQG) CCME (2012)
	2013	
Protea Glen	0.49	0.88
Lenasia	0.30	1.52
Fleurhof	0.43	3.85
Moroka	1.60	4.08
Eldorado Park	0.38	2.67
Orlando West	0.49	3.06
Orlando East	0.52	1.58
Nancefield	0.19	0.54
Dobsonville	0.18	0.86
	2014	
Protea Glen	0.38	1.09
Lenasia	1.08	3.59
Fleurhof	0.41	1.73
Moroka	2.48	9.70
Eldorado Park	1.06	3.59
Orlando West	0.49	2.10
Orlando East	0.24	0.60
Nancefield	0.37	0.76
Dobsonville	0.24	0.48

Scale	
1.5<	High probability of toxicity to biota
1.5-0.5	Moderate probability of toxicity to biota
<0.5	Low probability of toxicity to biota

Due to the more sensitive nature of the Canadian guidelines when they were applied in the calculations, more sites were deemed to have probable toxic effects (Table 14). Applying the Canadian guideline led to the deduction that only Dobsonville (2014) posed no probable toxic risk to benthic organisms. Lenasia, Fleurhof, Moroka, Eldorado Park and Orlando West had high probability of being toxic to biota for both years (Table 14). Orlando East changed from high to moderate probable toxicity between 2013 and 2014. Dobsonville also seemed to have improved as already mentioned.

From the chemical data (Table 8), and consequently the sediment quality guidelines, sediment pollution increased from 2013 to 2014. One would expect that the ecological risk would increase concurrently, but that is not the case as seen with the SQG-I (Table 14).

The sediment quality index (SQI) (Table 15) quality in terms of chemical contamination. Keep in mind that this index incorporates the magnitudes by which the determined levels exceeded the guideline levels. If no guidelines were breached, a SQI value cannot be calculated.

Table 15: Sediment quality index (SQI), in terms of PAHs contamination, for the sites in the Soweto/Lenasia area for 2013 and 2014. Colour coordination according to index scale.

Sites	PEC MacDonald et al., (2001)		ISQG (CCME, 2012)	
	2013	2014	2013	2014
Protea Glen	47.61		37.33	
Lenasia	30.90		12.75	25.83
Fleurhof	4.53		30.79	
Moroka	8.27	14.10	8.22	5.07
Eldorado Park	16.64		8.81	22.48
Orlando West	8.46		27.46	42.41
Orlando East	26.06		48.94	
Nancefield	50.53		44.74	
Dobsonville	37.69		75.61	

Scale
95< Excellent
80-95 Good
60-80 Fair
45-60 Marginal
0-45 Poor

The only site that calculated a SQI for the 2013 sediments (in terms of the MacDonald et al., (2001) guidelines) was Moroka. This site scored a poor sediment quality (14.1%). All the 2014 samples scored poorly for the sediment quality (Table 15). In contrast to the one site of 2013 using the MacDonald et al. (2001) guidelines, the SQI values for 2013 using the CCME (2012) guidelines showed four sites to have poor quality sediment: Lenasia, Moroka, Eldorado Park and Orlando West (Table 15). Similar to the SQI scores for 2014 (calculated with MacDonald et al., (2001) guidelines), all the 2014 sites had poor sediment quality when considering the CCME guidelines except for Dobsonville, which had a fair sediment quality of 75.61% (Table 15).

The toxic equivalent quotient (TEQ), calculated from the measured PAH concentrations are reported in Table 16.

Table 16: Toxic equivalent quotient (TEQ) results, calculated for the sediments of the sites from Soweto/Lenasia (2013 & 2014), compared to the TEQ guidelines of the CCME (2001). Colour coordination indicates which guidelines were exceeded.

	ngTEQ/kg	
	2013	**2014**
Protea Glen	9.72	6.10
Lenasia	1.73	6.69
Fleurhof	5.54	5.82
Moroka	39.12	9.09
Eldorado Park	7.03	7.24
Orlando West	8.44	6.35
Orlando East	5.22	5.89
Nancefield	0.60	5.26
Dobsonville	2.07	6.16
ISQG	0.85 ngTEQ/kg	**PEL** 21.5 ngTEQ/kg

The TEQ values of all the 2014 sites were higher than the Canadian interim sediment quality guideline (Table 16), indicating that the PAHs in the sediment is expected of being harmful to fish. Moroka sediment from 2014 was the only site that exceeded the PEL TEQ guideline for both seasons (Table 16). The only site that had a TEQ value below the guidelines was Nancefield (2013). These results indicate that there are AhR mediated toxic responses to be expected in fish and other high order organisms.

Dobsonville was the site of least concern, having improved quality over the two year study period (Guidelines, SQG-I and SQI). It is clear however, that there are sites that are severely affected by the PAHs in the Soweto/Lenasia study area including Lenasia, Moroka, Eldorado Park and Orlando West. The area of most concern is Moroka – the site that had the highest PAH concentrations for both years. This site has pyrogenic sources, mainly dominated by 4-ring congeners and was the site that exceeded the most guidelines (both sets). Its toxicity assessment indicated that it is likely toxic to benthic biota (from the guideline scores and the SQG-I) and that its 2013 sediment posed harmful risk to fish (Table 16). The quality assessment of this site also indicated that it is in a poor state

4.5 H4IIE-*luc* reporter gene bio-assay results

The H4IIE-*luc* reporter gene bio-assay results of the low-flow season of 2013 and 2014 are presented and discussed in this section. Cytotoxicity was seen during visual inspection of the plates, at higher concentrations of the serial dilution dosed to the cells. This was seen at all the sites except for Fleurhof and Dobsonville for 2013 (Figure 10). The only extract that

was cytotoxic to the cells for 2014 was Moroka (Figure 11).The LOD for the sediments was 20 ngTCDDeq/g (95% confidence).

In Figure 10 the dose-response curves of all nine sites (2013) are shown with %TCDDmax response ranging from 14% to 59%.

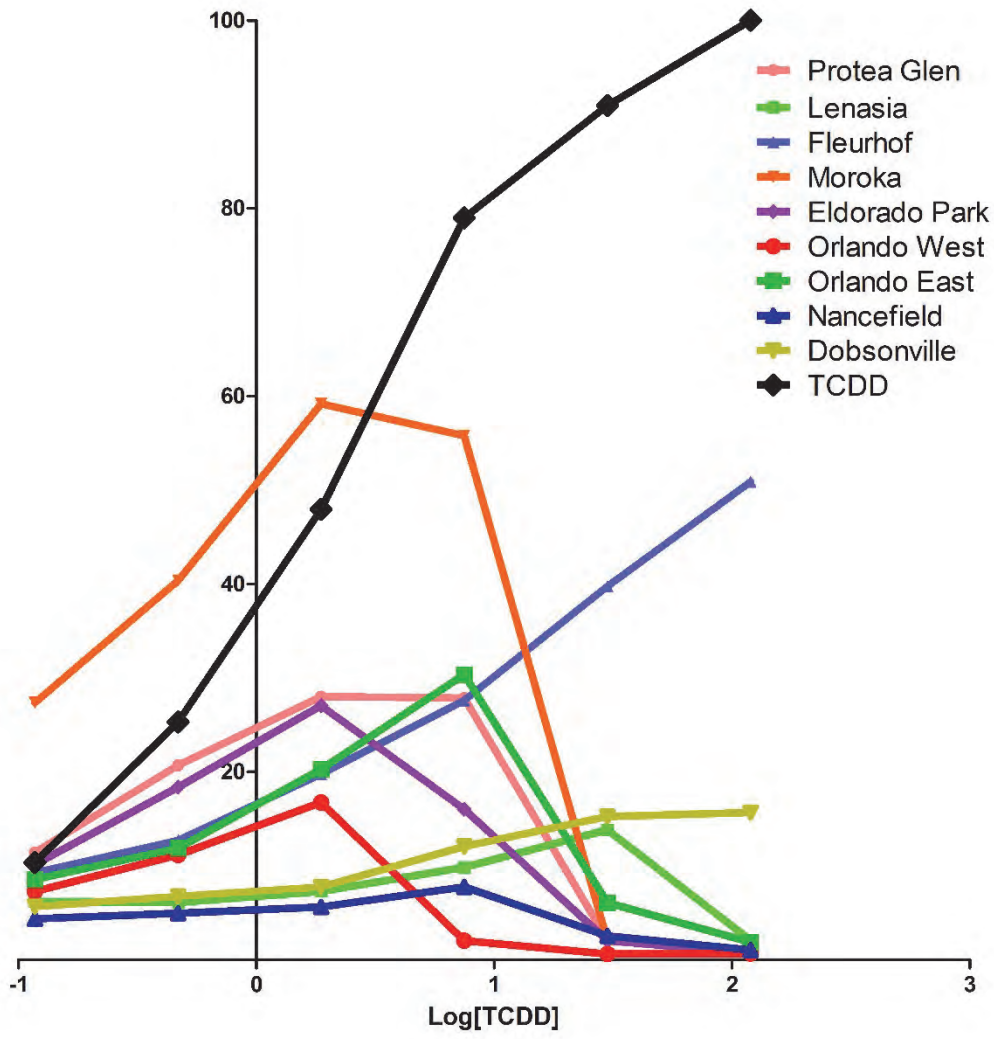


Figure 10: The dose-response curves of the nine sediment samples from the greater Soweto/Lenasia area (2013)

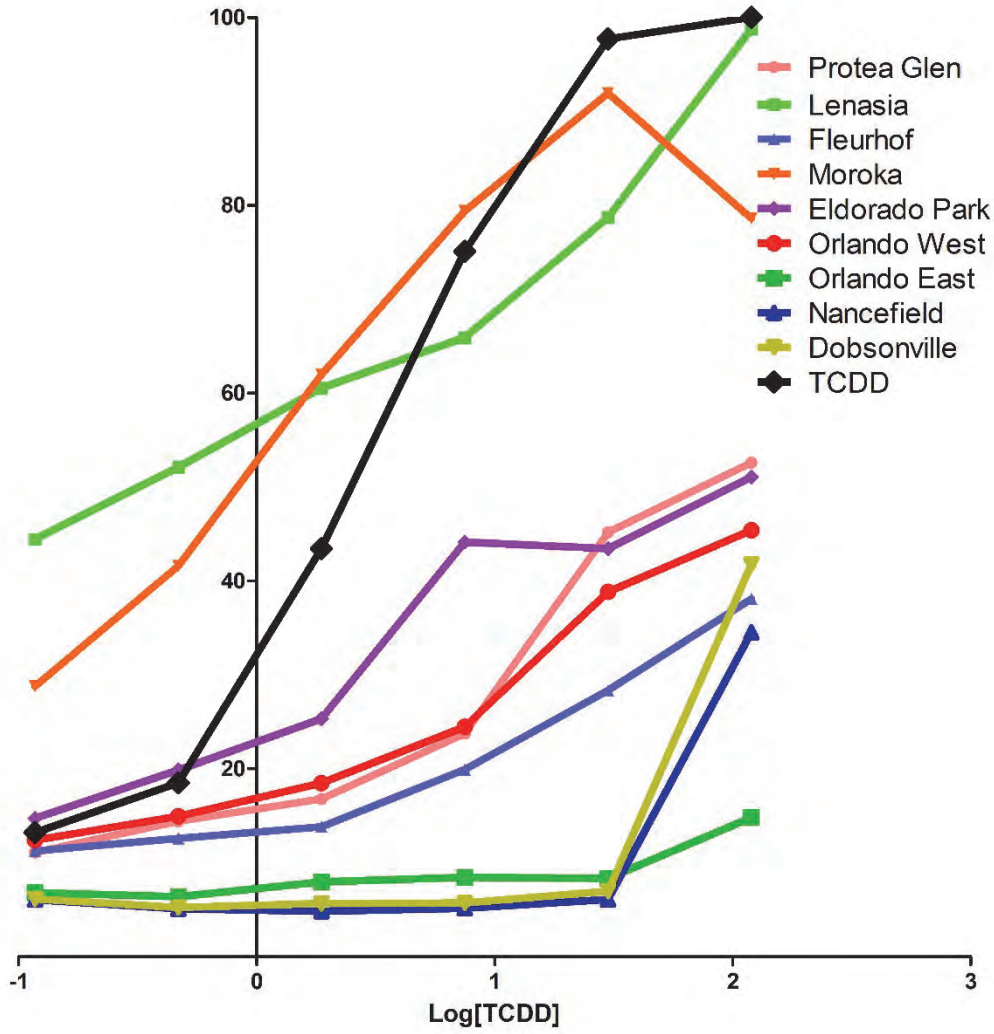


Figure 11: The dose-response curves of the nine sediment samples from the greater Soweto/Lenasia area (2014)

In Figure 11 the dose-response curves of all nine sites (2014) are shown with %TCDDmax response ranging from 15% to 98%.

Table 17: The %TCDDmax and BEQ values of the nine sediment samples from the greater Soweto/Lenasia area for 2013 and 2014

	2013 Sediment		2014 Sediment	
Sites	**%TCDDmax**	**REP 20 (pgBEQ/g sediment)**	**%TCDDmax**	**REP 20 (pgBEQ/g sediment)**
Protea Glen	28.02	46±7	52.59	2.2±5
Lenasia	13.81	0.3±0.3	98.79	415±32
Fleurhof	50.89	31±4	38.09	25±4
Moroka	59.19	138±15	91.94	261±16
Eldorado Park	27.04	41±7	51.06	70±8
Orlando West	16.74	11±7	45.4	220±10
Orlando East	30.36	6±2	14.8	<LOD
Nancefield	7.75	<LOD	34.52	<LOD
Dobsonville	15.67	<LOD	41.75	<LOD

The Lenasia 2014 result had the highest response (98.79%) of all 9 sites over the 2 sampling seasons. and had a BEQ of 415.82 pgTCDD-eq/g (Figure 11; Table 17). The highest response of the 2013 sampling season was Moroka (59.19%) with a BEQ of 138.26 pgTCDD-eq/g (Figure 10; Table 17). Other sites that also elicited high responses were Moroka 2014 (91.94%). Eldorado Park 2014 (51.06%) and Fleurhof 2013 (50.89%) (Table 17). A seasonal increase in %TCDDmax was noted for Protea Glen, Lenasia, Eldorado Park and Nancefield, and a seasonal decrease at Fleurhof and Orlando East (Table 17).

The BEQs calculated in this study are higher than those reported by Roos et al. (2011) (86 pgTCDD-eq/g) for a site in close proximity to the current study's sites. However, the same authors reported a BEQ of 161.24 pgTCDD-eq/g for a site downstream of Soweto, close to our Nancefield site. This high level matches the levels of the present study (Table 17). It is important to note that the Roos et al. study (2011) analysed a different fraction of the extract: they also treated their extracts to sulphuric acid, before running the bio-assay. This step would have destroyed most AhR-ligands including the PAHs. The only compounds that would have survived such a treatment would be the very persistent polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs). Less AhR-ligands would mostly, but not always, lead to lower BEQ levels. If Roos and co-authors were to perform bio-assays with the extract before being treated with acid, they probably would have found much higher BEQ

levels. Therefore, one would expect a higher BEQ in the Roos et al. (2011) samples if the PAHs were included, seeing that their PAHs levels were higher than this study's (Table 9).

Vogt (2013) investigated PCBs and PAHs in sediments and soils in the Durban Bay area. She prepared similar extracts as for this study – collecting a PAH containing fraction. She had both marine and estuarine sites, but for comparison purposes, only sites farthest inland, i.e. the sites least influence by the marine water. These selected sites had BEQ values lower than our study. The Umhlatuzana River had a BEQ of 4.26 pgTCDD-eq/g (ΣPAHs: 58.7 µg/kg) and the Umbilo River had BEQ of 7.7 pgTCDD-eq/g (ΣPAHs: 186.6 µg/kg) (Vogt, 2013). Closer to the harbour the BEQs were much higher (Vogt, 2013).

Hilscherova and co-authors (2001) investigated dioxin activity in a Czech river basin (Table 9). They isolated different pollutant groups by fractioning the sediment extract with a Florisil column. One of these fractions contained the PAHs (similar to our study). These extracts were dosed to the H4IIE-*luc* cells. The BEQs obtained were three orders of magnitude higher than the Soweto/Lenasia BEQs (mean of 9 ngTCDD-eq/g). This is to be expected as this study reported high levels of PAHs (Table 9) (Hilscherova et al., 2001).

4.6 Health assessment of *Clarias gariepinus*

During the sampling season of 2013, 20 per site were collected, except at the Fleurhof were only 10 fish was collected. The 2014 sampling session was less productive, where 11, 10 and 8 fish were collected from Lenasia, Fleurhof and Nancefield respectively. The Orlando site produced no fish during the 2014 sampling season. The site was heavily polluted and there was evidence of a mass fish kill. Morphological parameters of *Clarias gariepinus* sampled during the peak low flow seasons on two consecutive years, Oct 2013 and 2014 are summarised in Table 18.

Table 18: Sex ratio, mean mass, mass range and standard length range of *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

	Sex ratio (F:M)	Mean mass (g)	Mass range (g)	Range *std length (mm)
Lenasia 2013	11:9	2 363	740-4 800	400-760
Fleurhof 2013	5:5	3 024	1 720-4 840	520-770
Nancefield 2013	9:11	2 030	940-4 900	460-790
Orlando 2013	17:3	2 289	1 000-4 720	460-780
Lenasia 2014	7:4	2 223	780-3 460	430-700
Fleurhof 2014	7:3	3 120	1 080-6 640	440-870
Nancefield 2014	6:2	3 570	1 020-5 260	490-840
Control	10:10	3 337	1 100-6 040	480-800

***std length: standard length; body length excluding tail

4.6.1 Fish health assessment index

The FHAI allows for the judgement of the general health and condition of a fish population and was developed as a comparative bio-monitoring tool, to compare the same site over time and/or different sites at the same time (Heath et al., 2004). The FHAI score indicates the relative health of the fish population, when compared to the other sites (Heath et al., 2004). The standard deviation describes the health of the individual fish within the sample set: an increase in the standard deviation indicates that the health of the fish is poor. The coefficient of variance indicates the variability in health of individual fish caught within the sample set

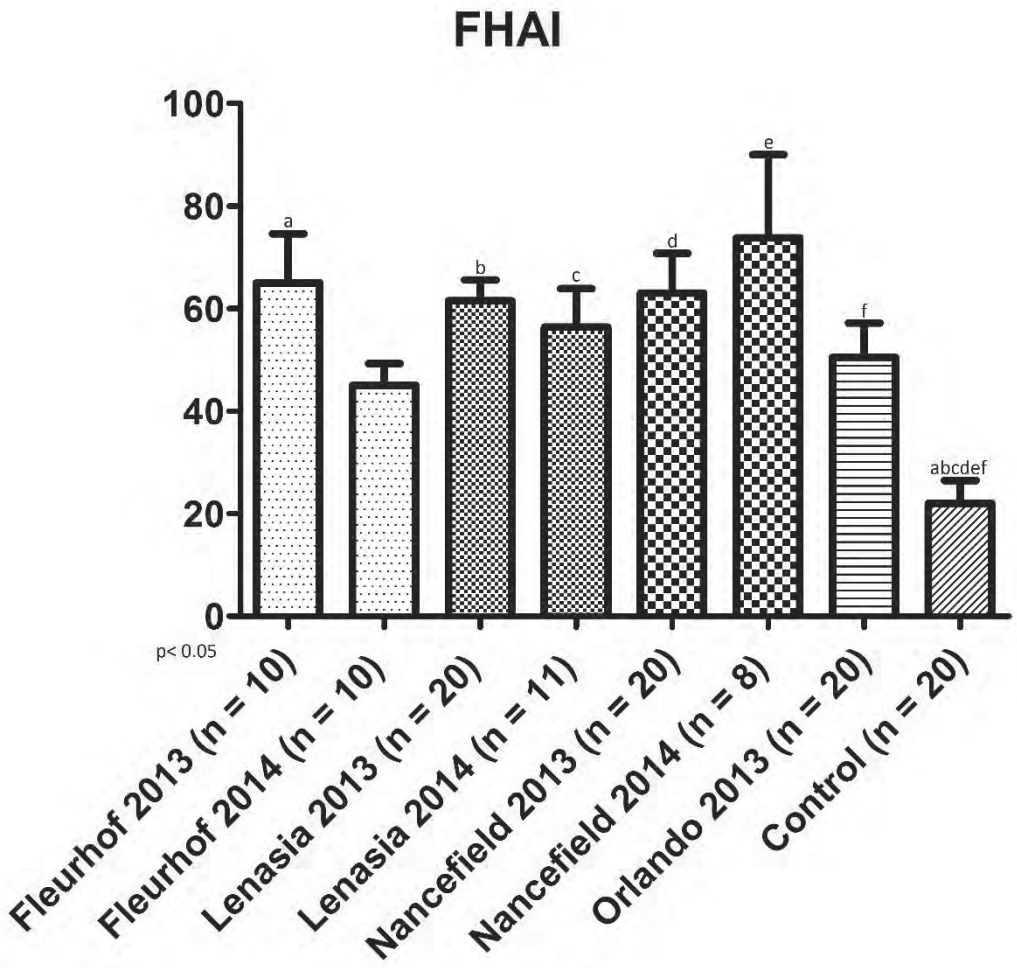


Figure 12: Fish health assessment index values for *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

The complete score sheet of the FHAI is given in the Appendix, indicating individual fish scores.

All the sites sampled for both events were significantly in poorer health compared to the control except the 2014 sample from Fleurhof (Figure 12).

The Nancefield population of 2014 had the poorest health for both seasons (FHAI: 74±46), followed by the Fleurhof 2013 (FHAI: 65±30) (Figure 12). The variability of the health of the fish within the populations was low as shown by the low CV (Appendix). The fish sampled from Nancefield (both sampling events) had high FHAI scores, 80% and 90% of the fish sampled (2013 and 2014 respectively) had liver abnormalities such as discolouration and deformations (Figure 13A), and the majority of the fish had abrasions on their skin and fins. A small percentage of the 2013 fish (15%) also showed deformities within the body cavity ranging from altered gonad structures (Figure 13B), fusion of organs to the muscle, and an increase of dense connective tissue around organs (Figure 13C). The Lenasia fish (2013), which were individually in poorer health, all fish (100%) had liver abnormalities such as discolouration and fatty deposits (Figure 13D & E). The sampled fish had no external damage, but 80% of them had gill damage: frayed gill filaments (35%), and pale gills (45%) (Appendix).

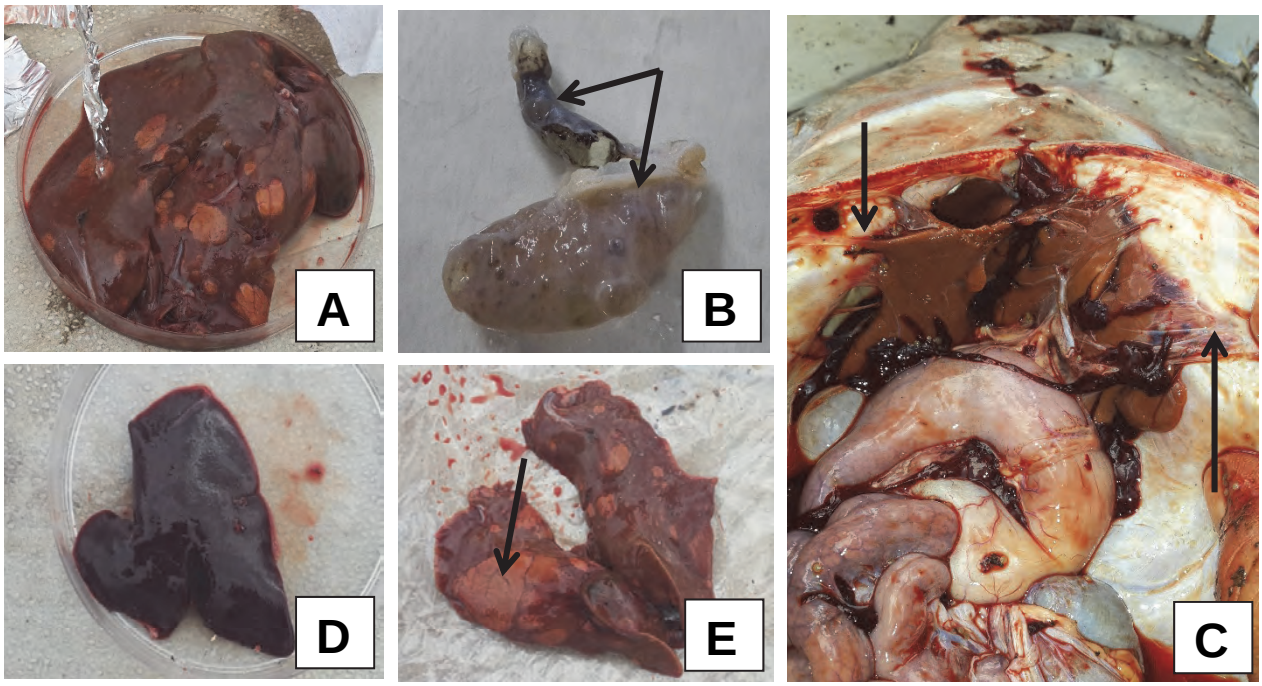


Figure 13: Observed abnormalities during necropsy: A) liver enlargement and darker disolouration B) altered testes containing vesicles [arrows] C) increase of connective tissue and fusion [arrows] D) liver discolouration E) increased fatty deposits

The fish of Fleurhof 2014 were in the best health of all sites for both sampling seasons as the FHAI for this site was the lowest (45±14) (Figure 12) However, they were still in poorer condition than the control fish whose FHAI score was even lower at 22±20 (Figure 12).

Of the fish caught at the Lenasia site in 2014 82% of them had discoloured and/or nodular livers and 36% presented enlarged spleens. Similarly, Nancefield 2014 fish scored for liver abnormalities (75%) as well as enlarged spleens (38%) and clubbed gills (38%) (Appendix).

Clarias gariepinus was sampled from the Lower Klip River during a WRC project (K5/2204) (region 3 & 4; Figure 1) by Wepener et al. (2015). These fish had significantly lower FHAI scores ($p < 0.05$) than the fish sampled upstream in Soweto/Lenasia for this study for both sampling seasons. The fish from Soweto/Lenasia were also in poorer health than the *C. gariepinus* sampled in the Okavango Delta panhandle, a pristine system (Van Dyk et al., 2009). The fish from study were in poorer condition than even those from the Phongolopoort Dam, a site known to be exposed to organohalogens (McHugh et al., 2013). Only Fleurhof 2014 and the control were in better health.

Regardless of what is causing the stress to the fish in the Soweto/Lenasia at the sites where we sampled, they are clearly under pressure when compared to the control fish and another site where there is known organohalogen pollution.

4.6.2 Fulton's condition factor

The condition factor calculates a numeric term that describes the volumetric relationship between the body mass and the total length of the fish. It expresses the condition of the fish such as well-being, relative robustness or fatness.

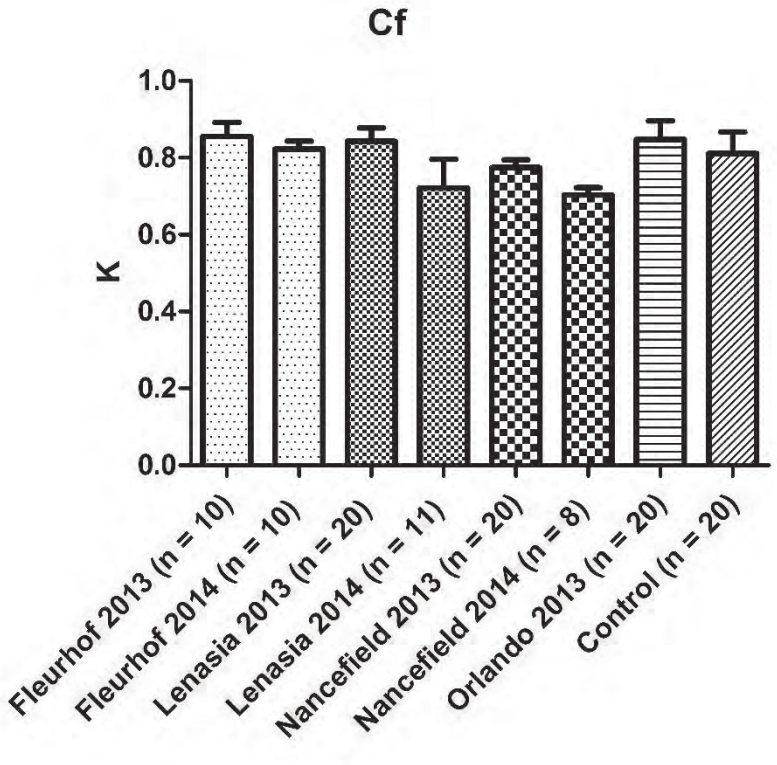


Figure 14: Condition factor values of *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

Lückhoff (2005) established a condition factor range specifically for *C. gariepinus*. If the condition factor for natural occurring catfish is above 0.85, the fish is in a good condition and if it is below 0.6 it is in a poor condition. Aqua-culturists strive for a condition factor of 1.04 in fed fish. The condition factor values of the fish sampled for both 2013 & 2014 seasons are reported in Figure 14.

All the fish sampled in both seasons were in fair condition (between 0.6 and 0.84) with exception of the Orlando 2013 (0.85±0.12) and Fleurhof 2013 (0.86±0.22) fish which were in good condition (Figure 14). The lowest Cf scores were reported at Lenasia and Nancefield (2014) (0.72±0.24 and 0.7±0.05 respectively; Figure 14). The condition factor results indicate that the fish are not short on food supply and that a decline in overall health cannot be attributed to malnutrition. The fish sampled from the Lower Klip River during the same sampling years, had comparable (no significant differences; $p>0.05$) condition factors (Wepener et al., 2015)

4.6.3 Hepato-somatic index

Fish livers are regarded as the main site of storage, bio-transformation and excretion of pollutants (Velmurugan et al., 2007; Hinton & Laurén, 1990) as well as a storage facility of energy reserves in the form of glycogen (Miranda et al., 2008). The HSI is the relationship between the liver mass and body mass and indicates the energy reserves of the fish or the effects of xenobiotics on the liver. An increase in HSI may be seen after exposure to pollutants due to an increase in hepatocyte size and numbers (Marchand et al., 2009) to increase the liver's detoxification potential (Goede & Barton, 1990). On the other hand pollution can also have a lowering effect on the HIS – decrease in cell size and number or even atrophy of hepatocytes (Marchand et al., 2009; Sanchez et al., 2008), but it is important to keep in mind that the size of the liver is affected by various other variable such as energy stores and food availability, parasites and seasonal factors (Goede & Barton, 1990; Sanchez et al., 2008). Making an interpretation of the HSI results the previously mentioned factors must be taken into account. Van Dyk et al. (2012) showed that the HSI values of *C. gariepinus* of Southern Africa – sampled from various aquatic systems – are close to 0.6%.

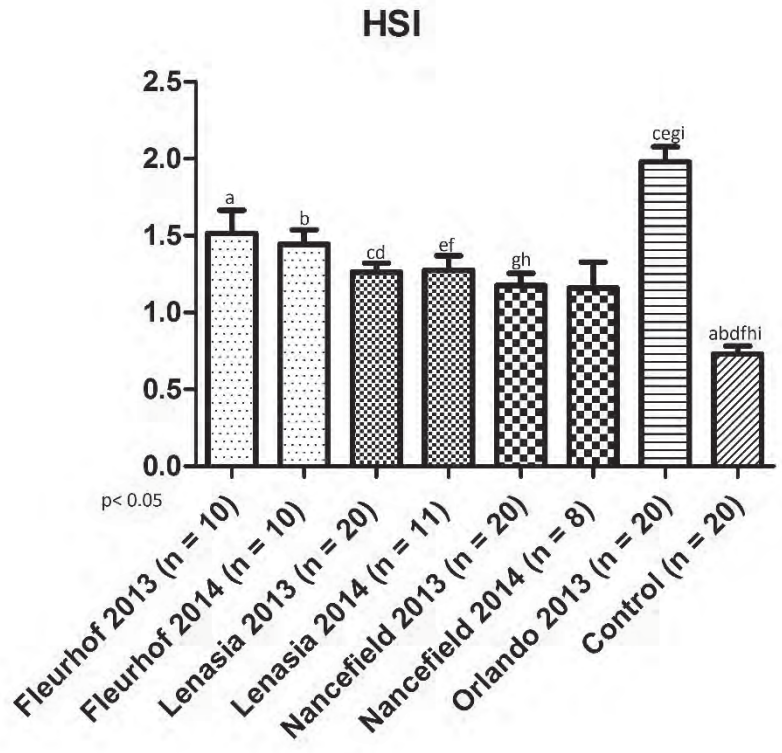


Figure 15: Hepato-somatic index values of *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

The HSI values are shown in Figure 15. The control fish population's HSI value is 0.61±0.27% (Figure 15). Orlando (1.98±0.44%) had the highest HSI value for both 2013 and 2014 (Figure 15), and was consistent with liver anomalies seen during the health assessment (Appendix). Fleurhof 2014 was the site least affected. The HSI value of Orlando was significantly higher than Lenasia [2013 & 2014], Nancefield 2013 and the control ($p<0.05$) (Figure 15). The control site was significantly lower ($p<0.05$) than all the sites excluding Nancefield 2014. A seasonal variation is seen at Fleurhof and Nancefield with a slight decrease in mean HSI over the two seasons. The range of the HSI means of the fish sampled – between 1.16±0.48% and 1.51±0.48% – was higher than the fish from the Okavango (0.5±0.1%)(Van Dyk et al. 2009), and those from the Phongolopoort Dam (0.93±0.005%)(McHugh et al., 2013).

4.6.4 Gonado-somatic index

The gonado-somatic index provides information on gonadal health and developmental stages, in response to changes, such as environmental stressors and/or natural changes (Schmitt & Dethloff, 2000).

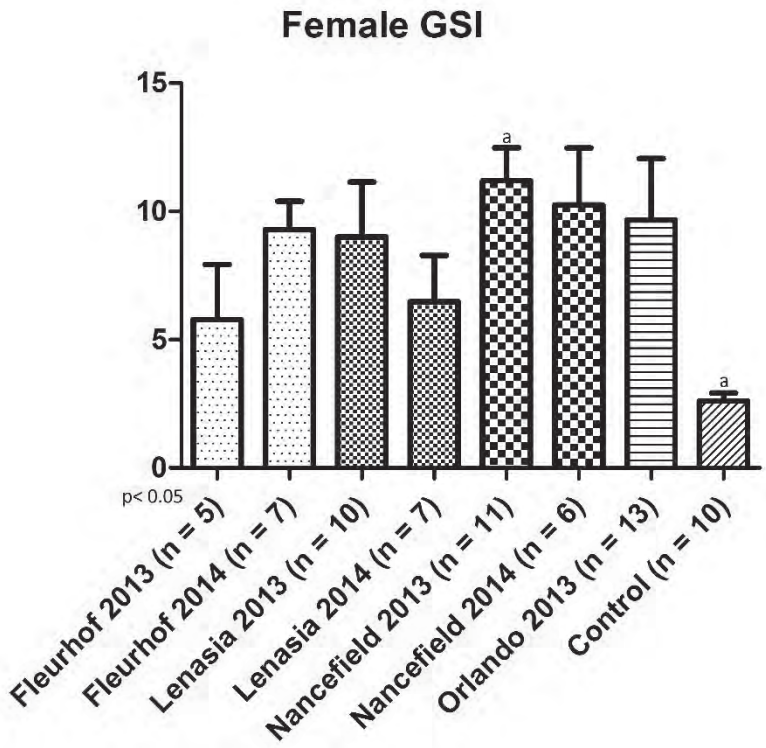


Figure 16: Gonado-somatic index values of female *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

The sampling took place during the pre-spawn season of *C. gariepinus*, and a majority of the female fish were sexually mature (Orlando had 4 fish with underdeveloped ovaries; these fish were excluded from the calculations). There is a high variation in the GSI, shown by the high standard deviation (Figure 16). This variation can be attributed to the wide range in body size and -mass of fish sampled (Figure 16). Nancefield 2013 had the highest GSI value followed by the 2014 values of the same site (11.19±4.29 and 10.25±5.44 respectively), and was the only site significantly higher than the control ($p<0.05$) (Figure 16). Nancefield (both seasons) and Orlando were the only sites significantly higher than the fish from the Lower Klip River (Wepener et al., 2015). The lowest GSI from the sampling area was Fleurhof 2013 (5.78±4.81, Figure 16) and this site had a high seasonal increase to 2014's GSI value of 9.29±2.93 (Figure 16).

The fish from Orlando that were excluded from the calculations due to underdeveloped ovaries were not necessarily juveniles. When referring to their size and mass, there were fish within the population smaller than these individuals that had developed ovaries and that

were gravid with eggs (assuming that growth within the population is constant). This underdevelopment indicates to possible endocrine disruption in these individuals. The control site had the lowest GSI of all the fish sampled. The reason for this is that the fish were processed in April 2014, when they have not yet become gravid with eggs as they would have been during October.

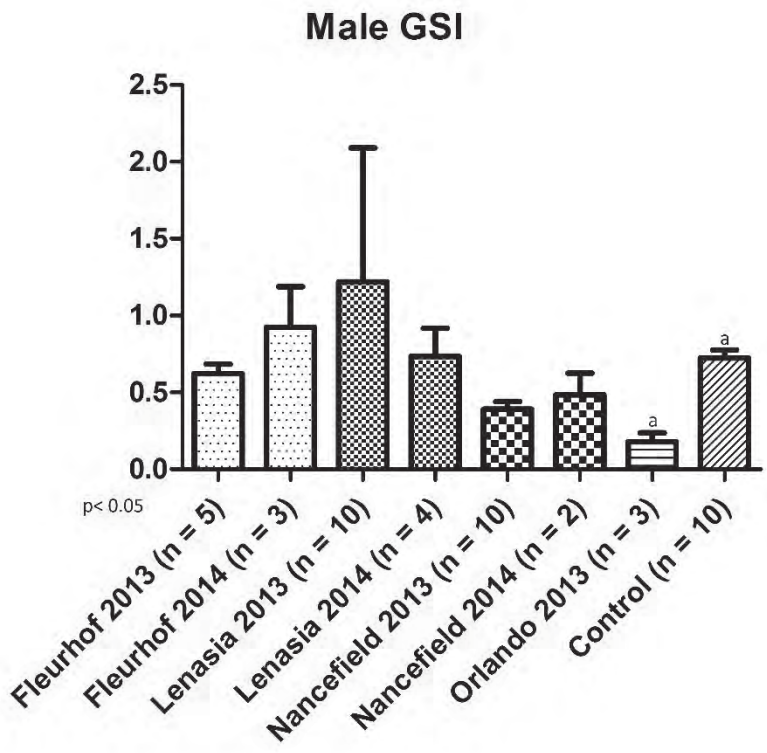


Figure 17: Gonado-somatic index values of male *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

The Lenasia 2013 fish had the highest GSI (1.22±2.61, Figure 17) and was the only site that decreased over the two seasons. The second highest GSI was from the 2014 Fleurhof fish, with a 0.92±0.46 value (Figure 17). The Orlando males had the lowest GSI (0.18±0.1), significantly lower than that of the control (0.63±0.14) (Figure 17), indicating that these male fish had smaller testes. Feminization was observed in one male from Nancefield, which had sac like vesicles growing within a deformed testis. The feminization and decrease in testes size is indicative of hormonal imbalance possibly from exposure to endocrine disrupting chemicals (Mills & Chichester, 2005).

4.6.5 Spleeno-somatic index

The spleen is a lymphatic organ which main function is to produce and store blood (Fänge & Nilson, 1985). It also that plays a role in antigen- and erythrocyte degradation and antibody production (Goede & Barton 1990; Rohlenová et al., 2011). Swelling or enlargement of the spleen can be indicative of necrosis and infection/disease in the fish or relate to immune problems (Adams et al., 1992; Goede & Barton 1990)

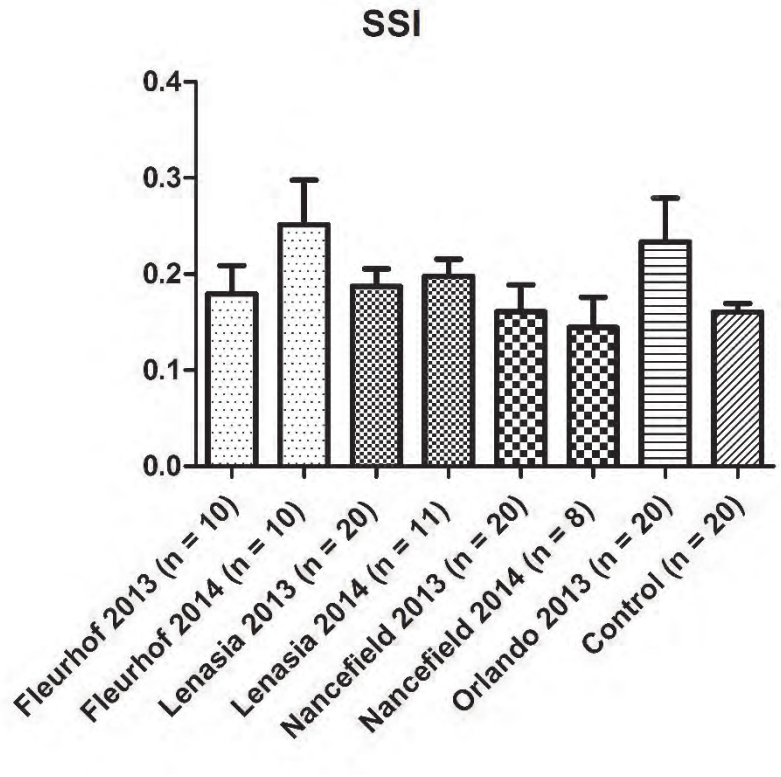


Figure 18: SSI values of *Clarias gariepinus* sampled from sites from the greater Soweto/Lenasia area for 2013 and 2014, and control fish

The SSI results show the immune responses of the fish sampled (Figure 18) and an increase in the size can be used to describe an increase in immune responses (Rohlenová et al. 2011). The control site and Nancefield 2014 had the lowest SSI of 0.14 (Figure 18). The highest SSI was at Fleurhof during 2014 (0.25±0.15) followed by Orlando (0.23±0.2) (Figure 13). The 2014 fish sampled at Fleurhof and Lenasia had swollen and/or enlarged spleens, as well as at Orlando. The high SSI can be expected from fish with enlarged spleens as it increases the mass ratio. An increase in the SSI between the 2013 and 2014 seasons was recorded at Lenasia and Fleurhof (Figure 18).

There were no parasites in/on any of the fish sampled, at any of the sites (Appendix) and thus the SSI values cannot be attributed the presence of parasites (Rohlenová et al., 2011), toxicants are suspected for causing the effect.

The SSI reported by Bester (2013) calculated for *Clarias gariepinus*, sampled from Roodekopjes-(0.18±0.11), Vaalkop-(0.13±0.05) and Marico-Bosveld Dam (0.2±0.09), were similar to this project.

4.7 Biomarker response results

The biomarker response results reported below are of the fish sampled in 2013 and 2014.

4.7.1 Acetylcholinesterase activity

Acetylcholinesterase is a neurotransmitter enzyme responsible for breaking the synaptic connections of nerve firing. The enzyme hydrolyses acetylcholine into acetate and choline. A decrease in acetylcholinesterase activity indicates an inhibition of these enzymes.

The 2014 sampling AChE results were all lower than the 2013 readings (Figure 19). The lowest 2013 results were at Fleurhof and Nancefield, whereas the highest value was at Lenasia (Figure 19).

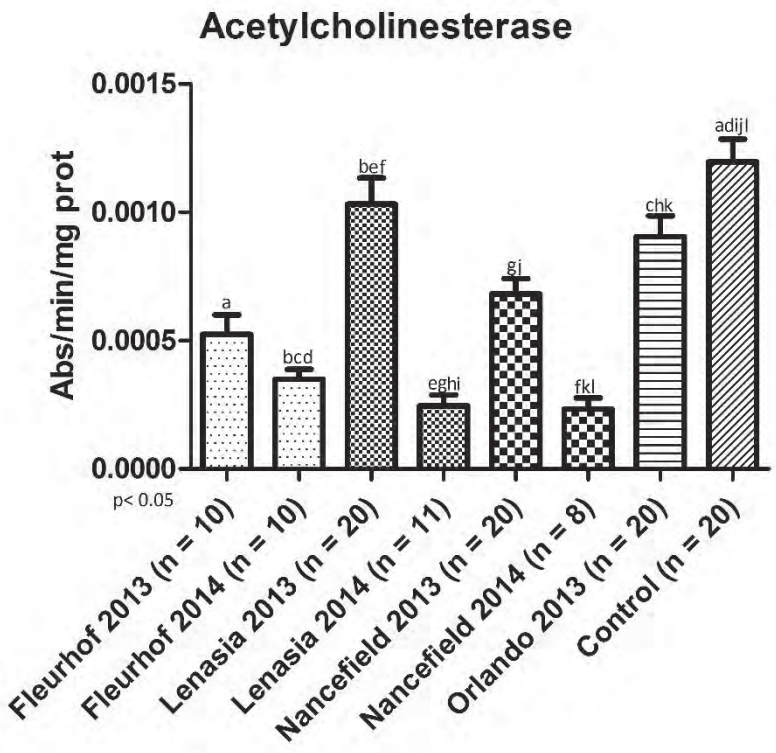


Figure 19: Acetylcholinesterase activity of fish sampled from the greater Soweto/Lenasia area (2013 and 2014)

Fleurhof 2014 had the highest value for that season and Nancefield had the lowest (Figure 19). Temporal variation shows that the 2014 samples had lower AChE activity than 2013 (Figure 19). Between the two years, only Lenasia and Nancefield's results differed significantly. There were significant differences between the control group and all the sites

(p<0.05), except for Lenasia 2013 and Orlando 2013. Those results that were significantly lower than that of the control (Figure 19) indicate inhibition of AChE activity.

PAHs have been found to inhibit acetylcholinesterase (Kang & Fang, 1997; Lau et al., 2004). The AChE measured in the fish (sampled 2014) from the Lower Klip River (Wepener et al., 2015) was significantly higher than the 2014 results of Soweto/Lenasia, indicating the presence of inhibitors up stream in 2014.

4.7.2 Superoxide dismutase

Superoxide dismutase forms the first tier of the anti-oxidant system responsible for converting reactive oxygen species (ROS) and superoxides into oxygen and hydrogen peroxide. An increased SOD level may indicate high levels of ROS present.

For the 2013 SOD results, Lenasia had the highest levels and Orlando the lowest (Figure 20). The very high standard deviation for the Orlando site is because of the large variety of responses within the samples. The second highest SOD level was at Nancefield. The 2014 SOD levels were lower than the 2013 samples with the exception of Orlando.

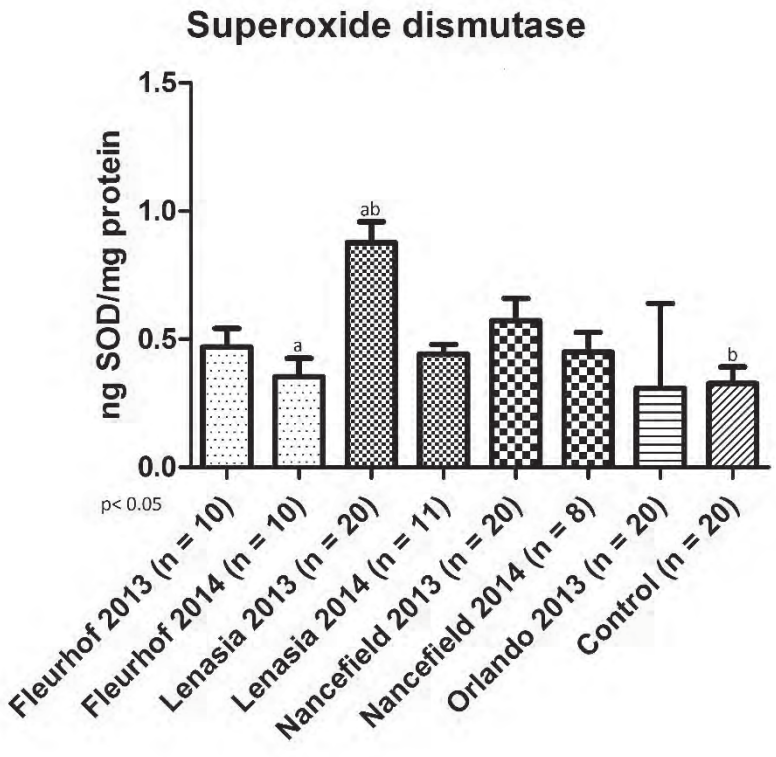


Figure 20: Superoxide dismutase levels of fish sampled from the greater Soweto/Lenasia area (2013 and 2014)

The lower SOD levels between seasons suggest that there was less antioxidant action that these fish had to undergo. The highest 2014 results were obtained at Nancefield (Figure 20).

Relative to the control, Nancefield (2013 and 2014), Lenasia (2013 and 2014), and Fleurhof (2013) showed increased SOD, indicating a possible up-regulating in the SOD system (Figure 20). Statistical difference was only seen between Fleurhof 2014 and Lenasia 2013, as well as Lenasia 2013 and the control ($p<0.05$). The increase in the SOD levels means that the organisms are experiencing possible oxidative stress and that the enzyme system is up regulated to decrease these oxidative compounds to prevent cellular damage (Pandey et al., 2003). The fish sampled from the Lower Klip River by Wepener et al. (2015), shows to be exposed to oxidative reagents. The SOD levels of the fish from the lower Klip River were all significantly lower than those upstream (our study), it shows an inhibition of the SOD system downstream.

4.7.3 Catalase activity

Catalase is the second tier in the anti-oxidant system, responsible for the hydrolysing of hydrogen peroxide formed by SOD. Catalase activity is indicative of the levels of oxidative stress compounds present in the fish. Both the 2013 and 2014 CAT activity results were higher than the control (Figure 21), indicating possible activation of the catalase system. The 2014 fish had higher catalase responses than 2013. The catalase activity of the Fleurhof 2014 fish was the highest (Figure 21). Orlando had the highest CAT levels for 2013 (Figure 21), which was statistically higher than the control group.

Similarly Fleurhof 2014, Lenasia 2014 and Nancefield of both years, were significantly different from the control. Between the 2013 and 2014, Fleurhof and Lenasia showed statistical differences ($p<0.05$). Catalase activity increases as hydrogen peroxide increases (formed by SOD), but the 2014 fish had lower SOD values compared to its increased CAT, suggesting up regulation of the CAT system of 2014 fish. The same trend was seen in the 2014 CAT results of the Lower Klip River samples as with its SOD results – significantly lower levels (Wepener et al., 2015) that indicate an inhibition of the oxidative system downstream

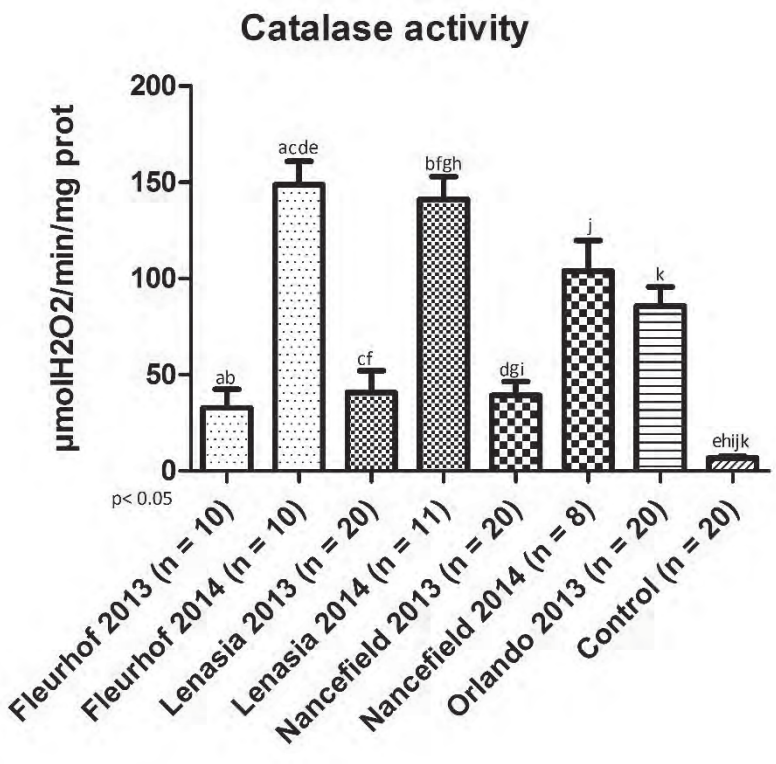


Figure 21: Catalase activity of fish sampled from the greater Soweto/Lenasia area (2013 and 2014)

4.7.4 Lipid peroxidation (Malondialdehyde content)

The levels of malondialdehyde in a sample indicates the severity of lipid peroxidation due to the presence of oxidative stress compounds. The highest MDA content was observed at Nancefield (2014) and Fleurhof (2013) and the lowest at the control (Figure 22). None of the sites differed statistically, and thus the MDA results of both years were comparable to the control group (Figure 22). The relative low MDA content indicates to very low lipid peroxidation at all the sites over the two years. The Lower Klip River samples were significantly higher than the Soweto samples, with 1.255- and 1.819 nmol/mg prot mean MDA levels (2013 and 2014 respectively) (Wepener et al., 2015). Compared to the Lower Klip River samples, the extremely low levels of MDA suggest no lipid peroxidation in these fish and that the anti-oxidant system of the Soweto sampled fish is functioning properly.

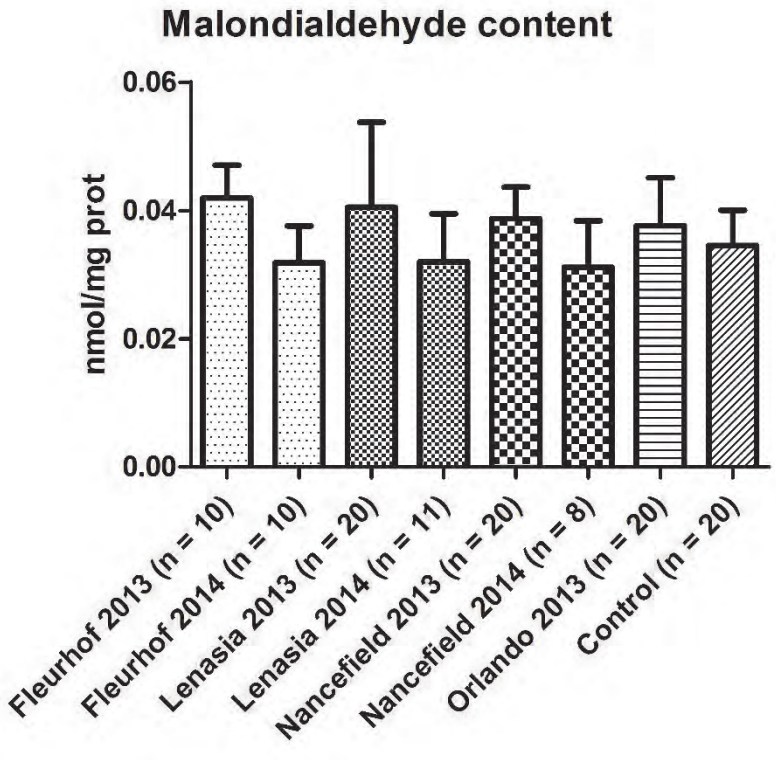


Figure 22: Malondialdehyde content of fish sampled from the greater Soweto/Lenasia area (2013 and 2014)

4.7.5 Protein carbonyls

Similar to malondialdehyde content, the amount of protein carbonyls present indicates the severity of oxidative stress. The protein carbonyls are the products of amino acid residue oxidation. Orlando fish had the highest PC values and differed significantly to all the sites ($p<0.05$). The other sites levels were well below that of Orlando 2013, the lowest being 2014 Lenasia (Figure 23).

A temporal decrease in PC values was noted, from 2013 to 2014 (Figure 23). Relative to the control, the sites (excluding Orlando) were comparable (Figure 23) and does not show any increase in protein degradation. The 2013 fish from the Lower Klip River (Wepener et al., 2015) has similar results as Orlando and suggest protein damage, similar to the other oxidative stress biomarkers.

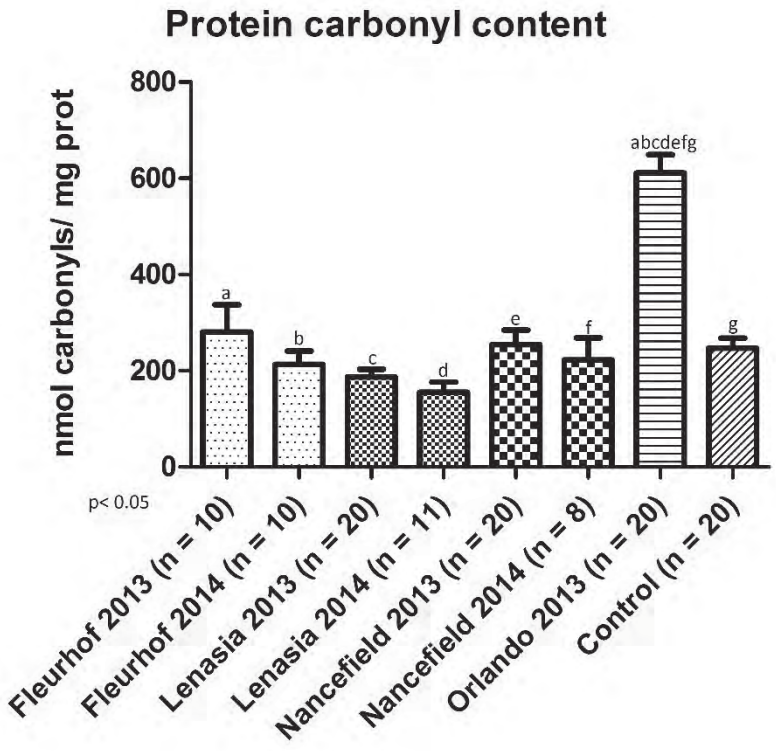


Figure 23: Protein carbonyl content of fish sampled from the greater Soweto/Lenasia area (2013 and 2014)

4.7.6 CYP450 demethylating activity

The cytochrome enzymes have detoxification responsibilities. Their activity within a sample indicates the absence or presence of xenobiotics. The CYP450 activity measured for the Fleurhof fish from both seasons were the lowest of all the study sites and were comparable to the control (not statistical different) (Figure 24). The highest CYP activity was measured for the Orlando fish and was significantly different to Fleurhof of both seasons and Nancefield 2014 ($p<0.05$).

A temporal increase in CYP induction was observed for Nancefield. In contrast cytochrome activity decreased over two years for the fish from Lenasia (Figure 24). The higher CYP activity of Orlando fish relative to that of the control indicates that there was an up-regulation of the CYP. PAHs are known AhR-ligands that induce the expression of the cytochrome enzymes (Villeneuve et al., 2002) (Cf. Biomarkers and Relevance of bioassays).

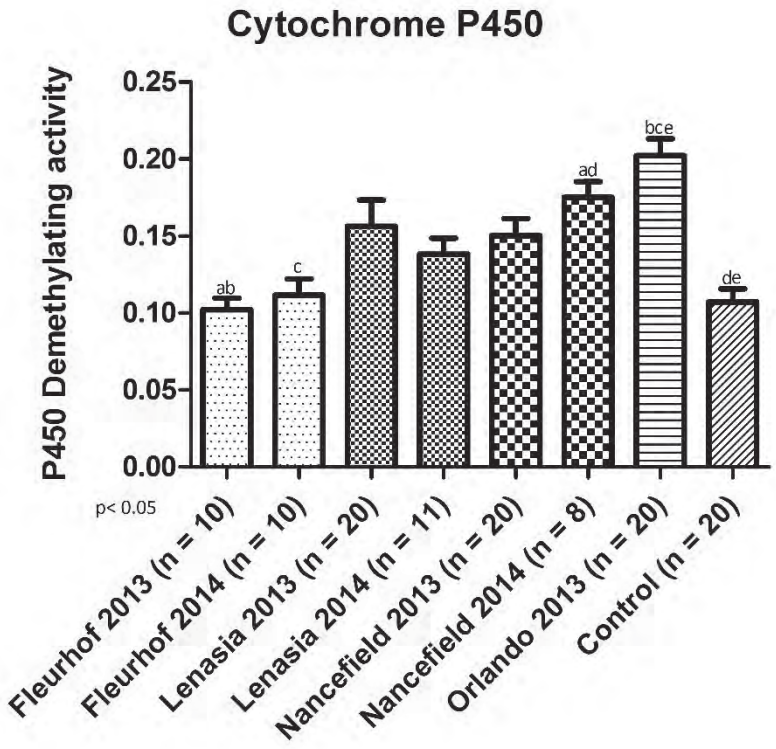


Figure 24: Cytochrome P450 demethylating activity of fish sampled from the greater Soweto/Lenasia area (2013 & 2014)

4.8 Human health risk assessment

4.8.1 Reasonable maximum PAH concentration determination

Figure 25 illustrates the "reasonable maximum" concentration of the chemicals detected in sediments at sampling sites used in the primary screening human health risk assessment. This is represented by the 95th percentile of the data measured at each of the sites. The limit of detection or LOD is presented for comparative purposes.

Levels of detection in the different media (substrates) varied significantly, with the level of detection being considerably higher in fish and eggs than in sediment (Figure 26).

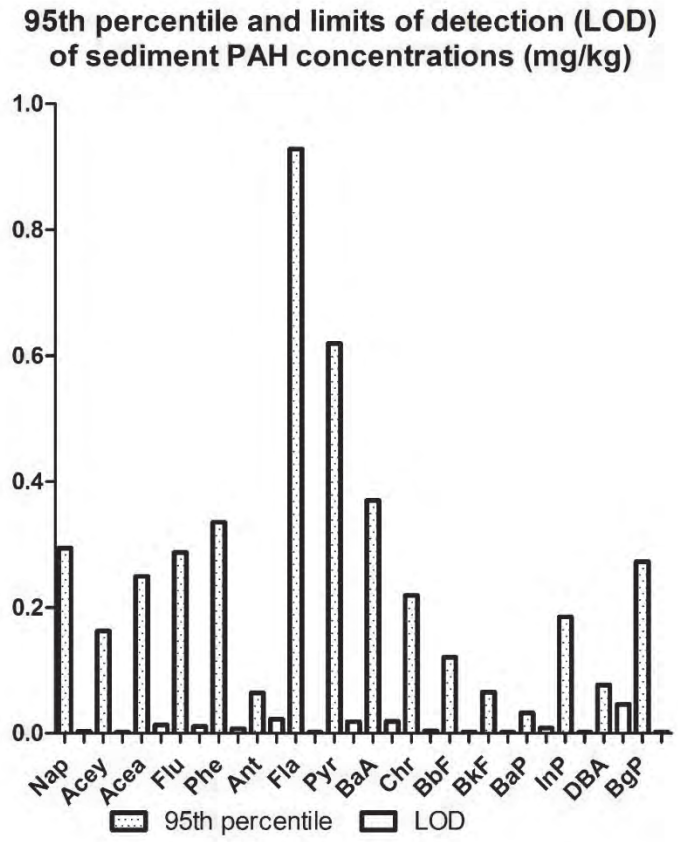


Figure 25: "Reasonable Maximum" and "Limit of Detection"/LOD of PAH concentrations detected in sediments (mg/kg)

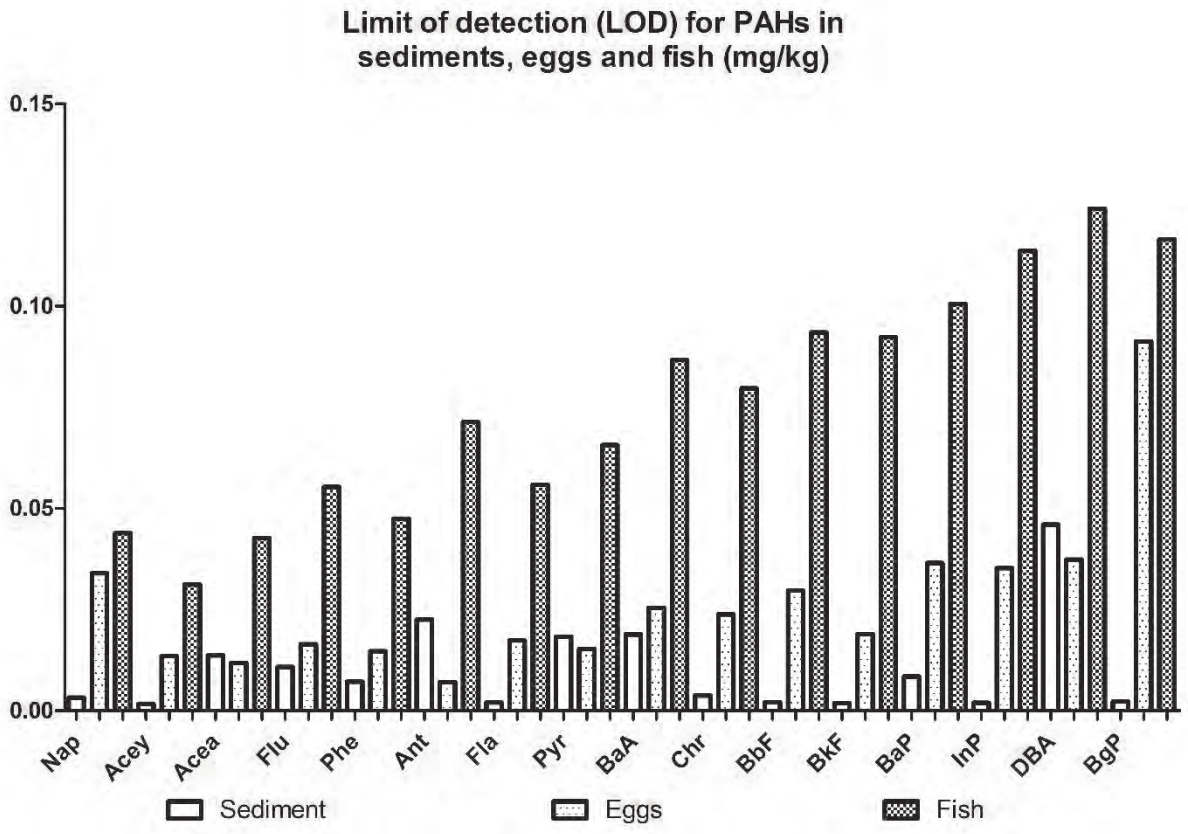


Figure 26: Level of detection of chemical concentrations (mg/kg) of sediment, eggs and fish (mg/kg)

4.8.2 Human health risk modelling

The results of the modelled fish concentrations and exposure calculations are given in the tables below (Tables 19-21) presented as Average Daily Dose (ADD) and Lifetime Average Daily Dose (LADD) in mg/kg/d.

Table 19: Calculated concentration of PAHs in fish based on the cross-media transfer equations

Chemical	PAH in sediment (mg/kg)	K_{oc}	PAH in water (mg/ℓ)	log(K_{ow})	BCF	PAH in fish (mg/kg)
Acenaphthene	0.25	3890	8.43E-04	4.15	2.879	3.15E-03
Acenapthalene	0.16	2620	2.94E-04	3.94	2.713	1.04E-03
Anthracene	0.06	7270	1.16E-04	4.35	3.037	4.59E-03
Benzo[a]anthracene	0.37	99700	0.93E-04	5.52	3.961	4.77E-04
Benzo[a]pyrene	0.03	209000	0.4E-04	6.11	4.427	2.25E-05
Benzo[b]fluoranthene	0.12	104000	0.29E-04	6.11	4.427	1.67E-04
Benzo[g,h,i]perylene	0.27	567000	0.12E-04	6.7	4.893	7.65E-05
Benzo[k]fluoranthene	0.07	201000	0.08E-04	6.11	4.427	4.68E-05
Chrysene	0.22	110000	0.5E-04	5.52	3.961	2.56 E-04
Dibenzo[a,h]anthracene	0.08	474000	0.4E-04	6.7	4.893	2.57E-05
Fluoranthene	0.93	30100	7.71E-04	4.93	3.495	3.5E-03
Indeno[1,2,3-cd]pyrene	0.18	652000	7E-06	6.7	4.893	4.5E-05
Napthalene	0.29	731	0.1E-01	3.17	2.104	2.75E-02
Phenanthrene	0.34	7420	1.13E-04	4.35	3.037	4.46E-03
Pyrene	0.62	17200	9.01E-04	4.93	3.495	4.09E-03

Koc = Octanol-Carbon Partition Coefficient; Kow = Octanol-Water coefficient; BCF = Bio-concentration factor

Table 20: Concentrations of chemicals calculated in fish, dose, hazard quotations and cancer risks based on lowest detectable concentrations in sediments

Chemical	PAH in fish (mg/kg)	Ingestion ADD* (mg/kg/d)	Ingestion LADD* (mg/kg/d)	Ingestion HQ	Ingestion Cancer Risk
Acenaphthene	0.04	2.76E-05	1.03E-05	4.61E-04	
Acenaphthylene	0.03	2.07E-05	7.69E-06		
Anthracene	0.07	4.61E-05	1.71E-05	1.54E-04	
Benz[a]anthracene	0.09	5.61E-05	2.08E-05		
Benzo[a]pyrene	0.1	6.47E-05	2.4E-05		**2.41E-04**
Benzo[b]fluoranthene	0.09	5.83E-05	2.16E-05		2.17E-05
Benzo[g,h,i]perylene	0.12	7.54E-05	2.8E-05		
Benzo[k]fluoranthene	0.09	5.97E-05	2.22E-05		2.23E-06
Chrysene	0.08	5.16E-05	1.92E-05		3.16E-06
Dibenz[a,h]anthracene	0.12	8.03E-05	2.98E-05		**2.99E-04**
Fluoranthene	0.06	3.62E-05	1.34E-05	9.05E-04	
Fluorine (Soluble)	0.06	3.59E-05	1.33E-05	5.98E-04	

Chemical	PAH in fish (mg/kg)	Ingestion ADD* (mg/kg/d)	Ingestion LADD* (mg/kg/d)	Ingestion HQ	Ingestion Cancer Risk
Indeno[1,2,3-cd]pyrene	0.11	7.38E-05	2.74E-05		2.75E-05
Naphthalene	0.05	2.84E-05	1.06E-05	1.42E-03	
Phenanthrene	0.05	3.08E-05	1.14E-05		
Pyrene	0.07	4.25E-05	1.58E-05	1.42E-03	
Total Risk/HI				*4.95E-03*	***5.95E-04***

*Average daily dose and Lifetime average daily dose

Table 21: Modelled concentrations of chemicals in fish, based on reasonable maximum sediment levels detected

Chemical	PAH in fish (mg/kg)	Ingestion ADD* (mg/kg/d)	Ingestion LADD* (mg/kg/d)	Ingestion HQ	Ingestion Cancer Risk
Acenaphthene	3.15E-03	2.43E-06	1.41E-05	4.1E-05	
Acenaphthylene	1.03E-03	8.00E-07	1.06E-05		
Anthracene	4.5E-04	3.55E-07	2.36E-05	1E-06	
Benz[a]anthracene	4.7E-04	3.69E-07	2.86E-05		
Benzo[a]pyrene	2E-05	1.74E-08	3.31E-05		5.44E-08
Benzo[b]fluoranthene	1.6E-04	1.29E-07	2.98E-05		4.04E-08
Benzo[g,h,i]perylene	7E-05	5.90E-08	3.85E-05		
Benzo[k]fluoranthene	4E-05	3.61E-08	3.05E-05		1.13E-09
Chrysene	2.5E-04	1.98E-07	2.63E-05	5E-06	1.02E-08
Dibenz[a,h]anthracene	2E-05	1.99E-08	4.10E-05	0	6.21E-08
Fluoranthene	3.50E-03	2.70E-06	1.85E-05	4.5E-05	
Fluorine (Soluble)			1.83E-05		
Indeno[1,2,3-cd]pyrene	4E-05	3.48E-08	3.77E-05		1.09E-08
Naphthalene	2.753E-02	2.12E-05	1.45E-05		
Phenanthrene	4.45E-03	3.44E-06	1.57E-05		
Pyrene	4.09E-03	3.16E-06	2.17E-05		
Total Risk/HI				9.2E-05	1.79E-07

*ADD = Average Daily Dose and LADD = Lifetime Average Daily Dose

Based on the exposure assumptions described in the section above risks of developing cancer and toxic effects were calculated for the various persistent organic chemicals where sufficient data was available. Most of the chemicals were found at concentrations to be below those where they could be detected or where they are considered “unacceptable” risks (Figure 23 & 24), as defined by both the WHO (WHO, 2001) and US EPA (US EPA, 1990).

If it is assumed that the population is exposed to concentrations daily for 30 years at the limit of detection, risks of developing cancer may be higher than the unacceptable risk of 1 in 10 000 resulting from exposure to benzo(a)pyrene and dibenzo[a,h]anthracene with a total risk of developing cancer of close to 6 in 10 000 (Table 21 and Figure 29).

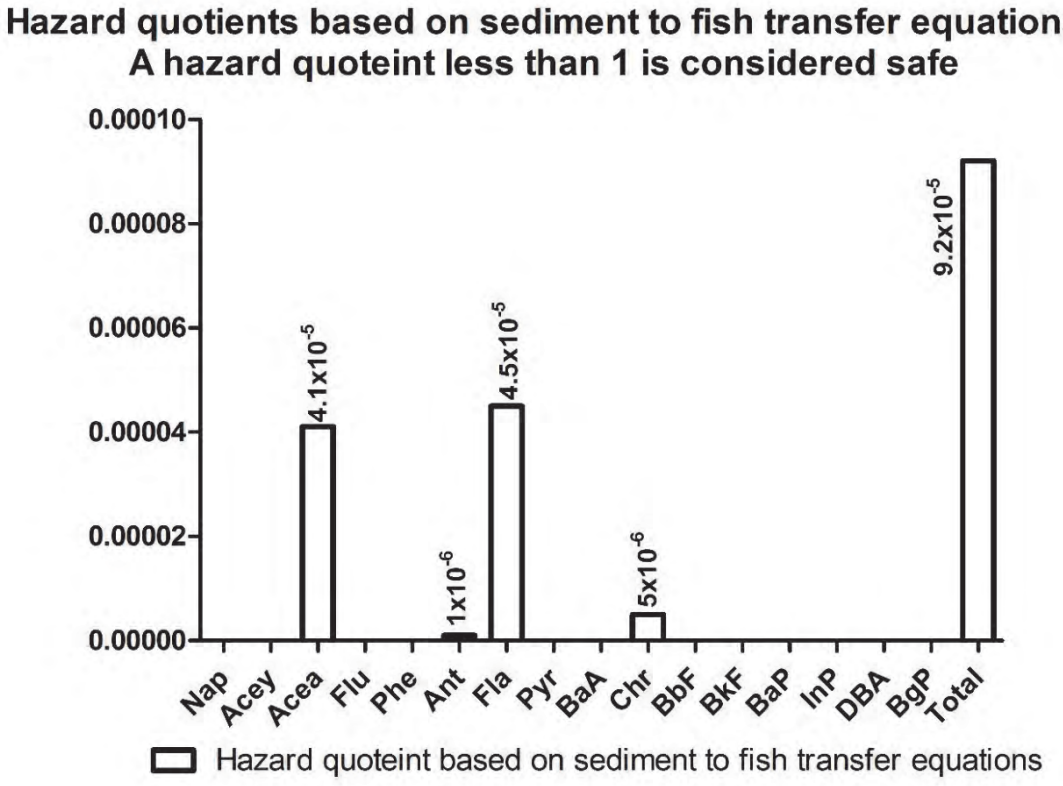


Figure 27: Hazard quotient based on sediment to fish transfer equations

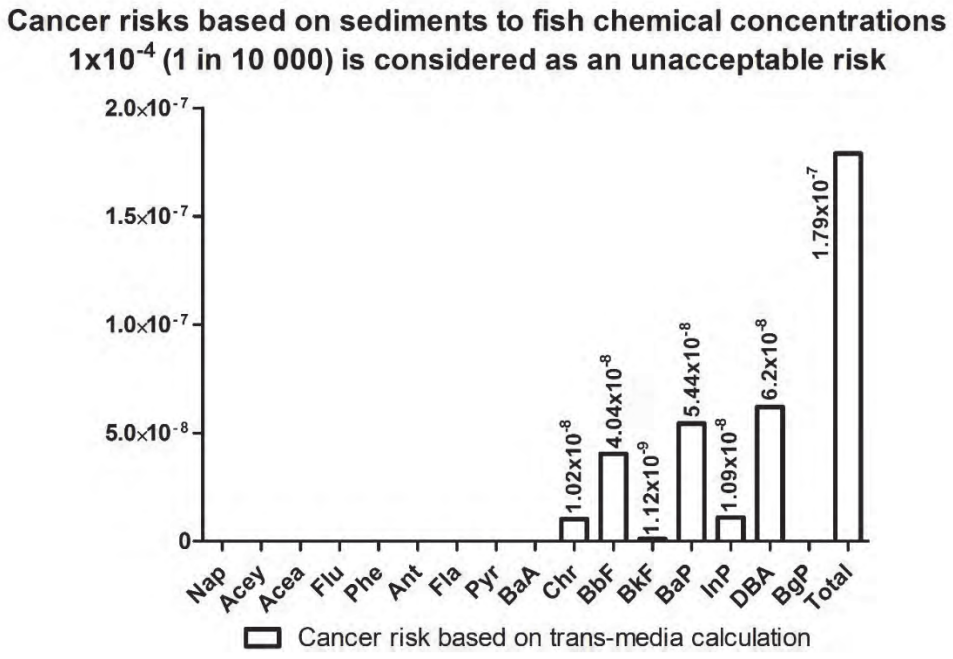


Figure 28: Cancer risks based on sediment to fish transfer equations

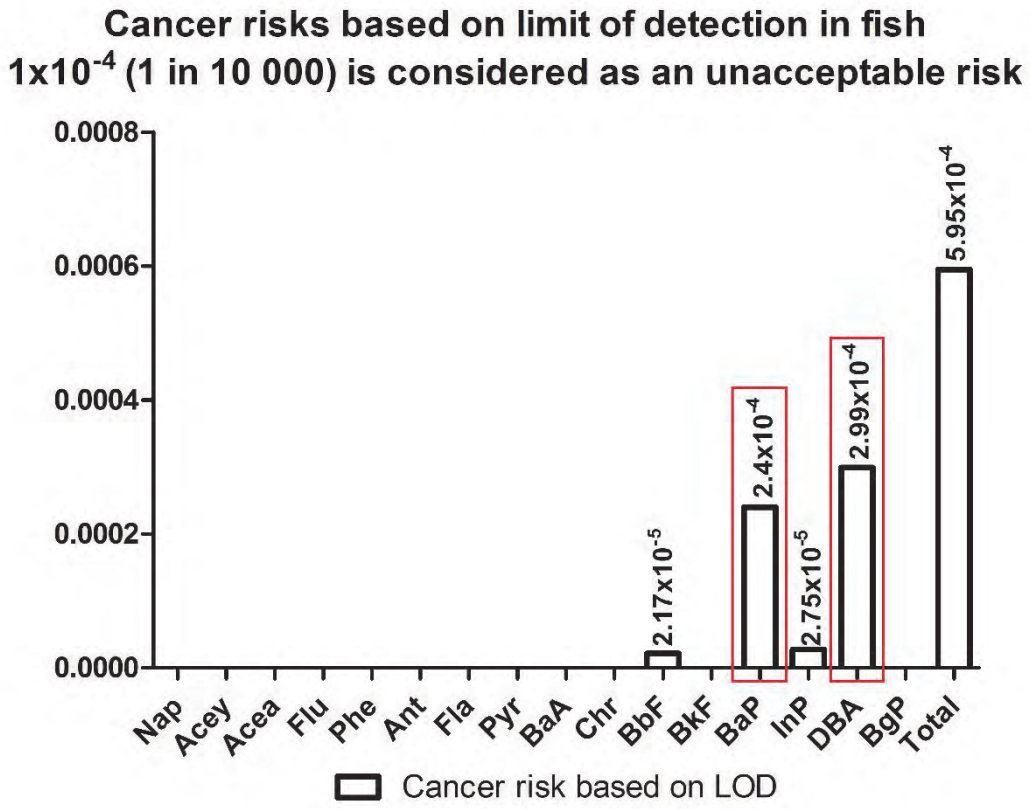


Figure 29: Cancer risks based on assuming level of detection in fish

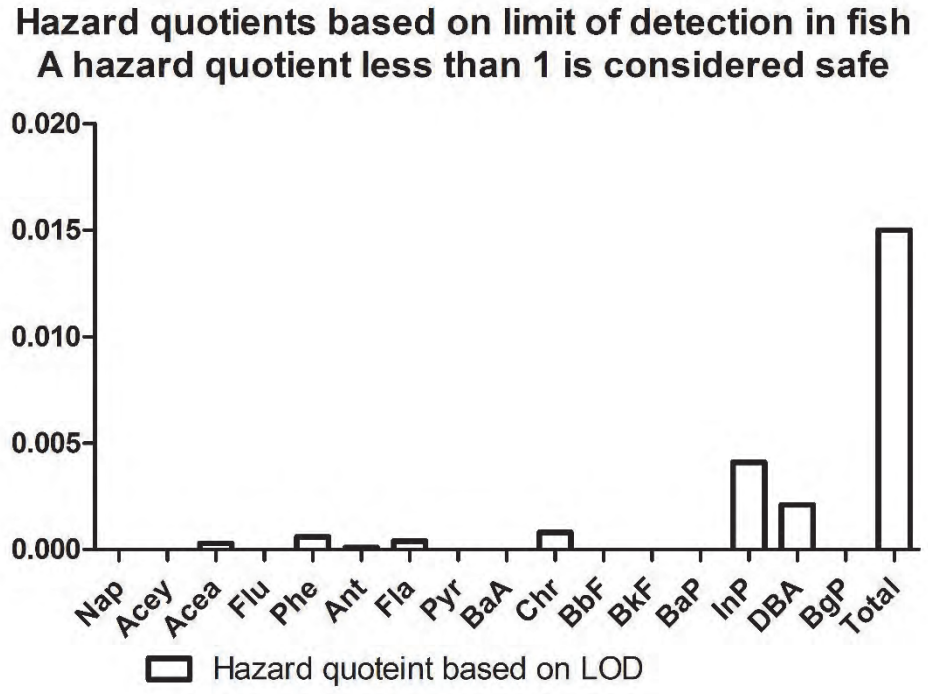


Figure 30: Hazard quotients base on assuming limit of detection in fish

The driver of potential human health risks was identified through this exercise. The chemicals responsible for possible risks include benzo(a)pyrene, and dibenz[a,h]anthracene for carcinogenic risks, and acenaphthene, chrysene and fluoranthene for potential toxicity risks.

Potential risks observed in this study were at very low levels.

Polycyclic aromatic hydrocarbons (PAHs) may be combined into one profile as they often occur together in the environment and many have similar toxicological effects, environmental fate, etc. Instances in which it is known that the various PAHs differ with regard to toxicological effects or environmental fate depend on their structure. For example, PAHs can be classified as “alternant” (e.g., benzo[a]pyrene, benz[a]anthracene, chrysene, dibenz[a,h]anthracene) or “nonalternant” (e.g., fluoranthene, benzo[k]fluoranthene, benzol[j]fluoranthene, indeno[1,2,3-c,d]pyrene). This distinction is based on the electron density associated with the molecule. Alternant PAHs have an equally distributed electron density, whereas nonalternant PAHs behave almost as if they were two different molecules because of an uneven distribution of electron density from one portion of the molecule to another. The toxicological significance of this difference is that alternant and nonalternant PAHs appear to behave differently, for example, with regard to how they are metabolized to ultimate carcinogens (US EPA, 1993, ATSDR, 1996).

Benzo(a)pyrene is one of the polycyclic aromatic hydrocarbons (PAHs) which are a group of hydrocarbons that are mainly formed by the incomplete combustion of organic materials (US EPA, 1993). There are several hundred PAHs, which usually occur as complex mixtures rather than as individual compounds. Benzo[a]pyrene (BaP) is the most potent. For the general public, the main route of exposure to PAHs is from inhalation of air or ingestion of food. Following chronic exposure in an occupational setting a decrease in lung function was reported, as well as chest pain, respiratory irritation, cough, dermatitis and depressed immune system, although in most cases it was not possible to evaluate the contribution of BaP to such effects. In animals, few adverse effects were observed in rats or hamsters exposed to BaP via inhalation. Following ingestion, myelotoxicity and hepatotoxicity was observed.

In mice, BaP has been shown to cross the placenta and cause adverse developmental and reproductive effects. Dietary administration during gestation reduced fertility and foetal abnormalities whereas administration by gavage caused an increase in foetal death and decreased fertility (McCabe & Flynn, 1990; Shugart & Matsunami, 1985). Benzo(a)pyrene

and dibenz[a,h]anthracene are considered as the most important potential chemicals of concern of risk of developing cancer in this screening health risk assessment.

5 CONCLUSIONS

- The presence of PAHs in the sediments of Soweto/Lenasia was confirmed by the chemical analysis.
- The sources of these PAHs have been narrowed down to pyrogenic sources, mainly from biomass combustion. The ratios also identified petroleum combustion as a source of the HPAHs and this is most probably from vehicles as the study areas is situated in an urban area.
- The site of greatest concern is Moroka. Other sites that are of concern are Lenasia, Fleurhof, Eldorado Park and Orlando West as well as Orlando East. Even though Protea Glen had high CPAHs, according to the toxicological assessment of this site it ranked lower than the other sites. Moroka had the highest PAHs levels (ΣPAHs, ΣLPAHs, ΣHPAHs, and ΣCPAHs) of all the sites. It was also the site that ranked the highest in all the toxicological assessments – exceeding most guidelines, especially the PEL of the Canadian guidelines.
- For both the 2013 and 2014 seasons, Moroka had the highest SQG-I score, indicating that the site's sediment posed a high ecological risk to benthic biota. The SQI corresponds to the chemical analysis and guidelines – scoring the sediment quality of this site as poor in terms of PAH pollution. Lenasia and Eldorado Park also had high levels of PAHs. The 2014 sediments of these sites exceeded both sets of guidelines.
- Fleurhof and Orlando West of 2013 exceeded the sediment guidelines and along with Lenasia and Eldorado Park posed high probability of toxic effect to benthic biota (as indicated by the SQG-I). As expected, the sediment of these sites was classified of poor quality.
- The toxic equivalent quotients (TEQs) of samples from the study area (2013 & 2014) were all higher than the lower guideline (ISQG: for the protection of fish), except Nancefield 2013. Moroka 2013 sediments had the highest TEQ – that exceeded the higher guideline (PEL) – and in conjunction with the other toxicological tests indicates that this site posed a serious threat to biota, specifically benthic organisms and fish.
- Moroka had the highest TEQ values for both years. In comparison to this, the bio-assay equivalents (BEQs) of the sites also showed that Moroka (2013) elicited the highest response when given to the H4IIE-*luc* cells. However the 2014 samples from Lenasia had the highest BEQ for that year, far higher than that of Moroka (2014), which had the second highest BEQ.

- Interestingly, Lenasia (2014) only had the 3rd highest ΣCPAHs levels of that year (CPAHs bind to the Ah-receptor). This shows that there were other Ah-ligands present at this site, which were responsible for the higher BEQ.
- Even though the chemical analysis of the fish and bird egg samples produced little to no quantifiable data in terms of the parent PAHs, there is evidence that there are PAHs present in the system – high sediment loads.
- The effective metabolism of parent PAHs by fish and birds remove these xenobiotics. However, the trace evidence of some parent congeners (nap, acea and phe) indicate that there was an influx of these compounds in these vertebrates.
- The biomarker responses are difficult to appoint to specific exposure due to the lack of chemical data in the fish. Cytochrome P450 activity in the fish can be compared to the TEQ and BEQ of the sediment data, seeing that the same mode of action is used (Ah-receptor mediated responses). One would expect the cytochrome activity to correspond to the TEQ and BEQ, but contradicting responses were observed for Fleurhof (2013) as well as Nancefield (2013): the lowest CYP450 response was in fish from Fleurhof (2013), which in turn had the highest TEQ and BEQ results for the sediment for the same year. The second highest 2013 CYP450 response was from the fish from Nancefield but its sediment had the lowest TEQ and BEQ values. Some of this discrepancy could be attributed to the fact that fish were sampled from dams and sediment from streams feeding the dams, and although the sampling sites were in close proximity to each other this might explain the observed differences. This discrepancy was unexpected as we assumed that the transportation of the PAHs to sites close together would be the same. The expectation of having high CYP450 responses from coinciding high TEQ and BEQ levels in surrounding sediment was met for Orlando: the highest CYP450 response was from Orlando, and its sediment (Orlando East) had the second highest TEQ value for the same year.
- The other biomarkers indicated that there were compounds present in the study area that elicited responses in the fish, specifically the inhibition of acetylcholinesterase activity and the up-regulation of the catalase system that could probably not be ascribed to PAH levels.

6 RECOMMENDATIONS

- The chemical analysis of the metabolised PAHs would complete the picture of what is happening to the parent PAHs after entering the animals' bodies. This would however, necessitate more funding because these analytical standards are expensive and not always readily available in South Africa. Each of the 16 parent PAHs has more than two metabolites that could be quantified chemically increasing the analytical load and associated expenses.
- The biomarker response results could not conclusively be attributed to the PAHs, and therefore a broad spectrum screening for a much larger variety of organic chemical pollutants is advised for this densely populated area of Gauteng. Chemical compounds that can be considered include: polychlorinated biphenyls, brominated flame retardants, organochlorine pesticides, plasticisers, pharmaceuticals and personal care products and perfluorinated compounds, just to name a few compound classes.
- The number of bio-assays can be broadened to include assays capable of detecting endocrine disruptive effects.
- Evaluation of fish species composition and numbers to further describe pollution effects in the system.
- Add a social component to the study in which the human population's physical interaction and dependence on the Klip River running through Soweto/Lenasia is quantified, i.e. using questionnaires and interviewing the citizens.
- Incorporating results from this study into management of this water catchment one must keep in mind that PAHs are mainly airborne. Therefore, a successful monitoring program of any water catchment for these compounds would require an integrated approach including air quality monitoring.

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8 APPENDIX

	Fleurhof 2013 (n = 10)	Fleurhof 2014 (n = 10)	Lenasia 2013 (n = 20)	Lenasia 2014 (n = 11)	Nancefield 2013 (n = 20)	Nancefield 2014 (n = 8)	Orlando 2013 (n = 20)	Control (n = 20)
				FHAI				
sum	650.00	450.00	1230.00	620.00	1260.00	590.00	1010.00	440.00
average	65.00	45.00	61.50	56.36	63.00	73.75	50.50	22.00
stdev	30.28	13.54	18.14	25.01	34.66	45.96	29.82	19.89
cv	0.47	0.30	0.30	0.44	0.55	0.62	0.59	0.90
				Cf				
sum	8.56	8.23	16.85	7.93	15.49	5.62	16.96	16.23
average	0.86	0.82	0.84	0.72	0.77	0.70	0.85	0.81
stdev	0.12	0.06	0.16	0.25	0.09	0.05	0.22	0.25
cv	0.13	0.08	0.19	0.34	0.11	0.08	0.26	0.31

	Fleurhof 2013 (n = 10)	Fleurhof 2014 (n = 10)	Lenasia 2013 (n = 20)	Lenasia 2014 (n = 11)	Nancefield 2013 (n = 20)	Nancefield 2014 (n = 8)	Orlando 2013 (n = 20)	Control (n = 20)
				H SI				
sum	15.15	14.46	25.28	14.04	23.53	9.28	39.59	14.62
average	1.51	1.45	1.26	1.28	1.18	1.16	1.98	0.73
stdev	0.48	0.29	0.26	0.31	0.35	0.48	0.44	0.23
cv	0.31	0.20	0.20	0.25	0.30	0.41	0.22	0.31
				SSI				
sum	1.80	2.51	3.74	2.17	3.22	1.16	4.67	3.21
average	0.18	0.25	0.19	0.20	0.16	0.14	0.23	0.16
stdev	0.09	0.15	0.08	0.06	0.12	0.09	0.20	0.04
cv	0.52	0.58	0.44	0.30	0.78	0.61	0.88	0.26
				Female GSI				
sum	28.90	65.03	90.07	45.36	123.14	61.49	125.72	26.06
average	9.63	16.26	16.38	11.34	20.52	17.57	17.96	4.74
stdev	10.37	19.89	25.27	14.44	32.57	20.00	32.10	7.13
cv	1.08	1.22	1.54	1.27	1.59	1.14	1.79	1.51
				Male GSI				
sum	3.11	2.77	12.19	2.94	3.52	0.97	0.54	7.23
average	0.62	0.92	1.22	0.73	0.39	0.48	0.18	0.72
stdev	0.14	0.46	2.76	0.37	0.15	0.20	0.10	0.16
cv	0.23	0.50	2.26	0.50	0.39	0.41	0.53	0.23

Lenasia 2013

FHAI

Date	Fish	Sex	EYES	SKIN	FINS	OPER	GILLS	BILE	MFAT	LIVER	SPLEEN	H/GUT	KIDNEY	PARAS	COMMENTS LIVER UNLESS OTHERWISE STATED
11-10-2013	L1	F	N	N	N	N	P	LS	<50	DISCOLOURA-TION	N	N	N	NONE	
11-10-2013	L2	F	N	N	N	N	N	LS	50	SLIGHT DISC/FATTY	N	N	N	NONE	
11-10-2013	L3	M	N	N	N	N	F	LS	<50	SLIGHT DISC/NODULES	N	N	N	NONE	
11-10-2013	L4	M	N	N	N	N	F	EMPTY	<50	SLIGHT DISC	N	N	N	NONE	
11-10-2013	L5	F	N	N	N	N	F	LS	<50	DISCOLOURA-TION	N	N	N	NONE	
11-10-2013	L6	F	N	N	N	N	F	LS	<50	DISCOLOURA-TION	N	N	N	NONE	COFFEE CREAM
11-10-2013	L7	M	N	N	N	N	F	LS	>50	SLIGHT DISC	N	N	N	NONE	
11-10-2013	L8	F	N	N	N	N	F	LS	<50	DISCOLOURA-TION	N	N	N	NONE	TAN
11-10-2013	L9	M	N	N	N	N	F	LS	>50	SLIGHT DISC	N	N	N	NONE	
17-10-2013	L10	F	N	N	N	N	N	EMPTY	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
17-10-2013	L11	M	N	N	N	N	P	LS	>50	DISCOLOURA-TION	N	N	SWOL-LEN	NONE	MOTTLED
17-10-2013	L12	M	N	N	N	N	P	DS	>50	SLIGHT DISC	N	N	SWOL-LEN	NONE	MOTTLED
17-10-2013	L13	F	N	N	N	N	P	DS	>50	DISCOLOURA-TION	N	N	N	NONE	COFFEE CREAM
17-10-2013	L14	F	N	N	N	N	P	DS	<50	DISCOLOURA-TION	N	N	SWOL-LEN	NONE	COFFEE CREAM
17-10-2013	L15	F	N	N	N	N	P	DS	<50	DISCOLOURA-TION	N	N	N	NONE	COFFEE CREAM AND OLIVE
17-10-2013	L16	M	N	N	N	N	P	LS	>50	SLIGHT DISC	N	N	N	NONE	
17-10-2013	L17	M	N	N	N	N	N	LS	<50	DISCOLOURA-TION	NODU-LAR	N	N	NONE	TAIL DEFORMED
17-10-2013	L18	F	N	N	N	N	P	LS	>50	DISCOLOURA-TION	ENLAR-GED	N	N	NONE	COFFEE CREAM
17-10-2013	L19	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
17-10-2013	L20	M	N	N	N	N	P	LS	<50	SLIGHT DISC	N	N	N	NONE	

Fleurhof 2013

FHAI

Date	Fish	Sex	EYES	SKIN	FINS	OPER	GILLS	BILE	MFAT	LIVER	SPLEEN	H/GUT	KIDNEY	PARAS	COMMENTS-LIVER UNLESS OTHERWISE STATED
23-10-2013	F1	M	N	M	N	N	N	DS	<50	DISCOLOR	S	N	N	NONE	
23-10-2013	F2	F	N	N	N	N	P	LS	<50	DISCOLOR	N	N	S	NONE	CARAMEL/COFFEE CREAM
23-10-2013	F3	M	N	N	N	N	N	LS	>50	SLIGHT DISC	S	N	S	NONE	
23-10-2013	F4	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
23-10-2013	F5	M	N	N	EROSION	N	F	LS	<50	N	S	N	N	NONE	
23-10-2013	F6	M	H1	M	N	N	P	LS	<50	SLIGHT DISC	N	N	N	NONE	FOCAL DISC
23-10-2013	F7	M	N	M	N	N	N	LS	<50	N	N	N	N	NONE	SCOLIOSIS
23-10-2013	F8	F	N	N	N	N	N	LS	<50	DISCOLOR	S	N	N	NONE	
24-10-2013	F9	F	N	N	N	N	N	LS	<50	N	NODULER	N	N	NONE	
24-10-2013	F10	F	N	N	N	N	P	LS	<50	SLIGHT DISC	N	N	N	NONE	

Nancefield 2013			FHAI												
Date	**Fish**	**Sex**	**EYES**	**SKIN**	**FINS**	**OPER**	**GILLS**	**BILE**	**MFAT**	**LIVER**	**SPLEEN**	**H/GUT**	**KIDNEY**	**PARAS**	**COMMENTS- LIVER UNLESS OTHERWISE STATED**
22-10-2013	N1	M	N	N	S	N	F	DS	>50	SLIGHT DISC	ENLAR-GED	N	N	NONE	WHISKER OFF, CUADAL FIN MISSING LIVER MOTTLED
22-10-2013	N2	F	N	N	N	N	N	DS	<50	SLIGHT DISC	N	N	N	NONE	
22-10-2013	N3	F	N	N	N	N	N	DS	>50	SLIGHT DISC	N	N	N	NONE	
22-10-2013	N4	M	N	N	N	N	DISC	LS	<50	SLIGHT DISC	N	N	S	NONE	FOCAL DISC, COFFEE CREAM
22-10-2013	N5	F	N	N	E	N	N	LS	<50	SLIGHT DISC	N	N	S	NONE	COFFEE CREAM
28-10-2013	N6	F	N	S	S	N	N	LS	0	OTHER	S	OTHER	N	NONE	SEVERE DEFORMATIES IN OVARIES, DIST TRACT, TUMOURS, LIVER DEFORMED, INCREASE IN CONNECTIVE TISSUE
28-10-2013	N7	F	N	N	M	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
28-10-2013	N8	F	N	M	S	N	N	LS	>50	SLIGHT DISC	N	N	N	NONE	
28-10-2013	N9	F	N	N	S	I	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
28-10-2013	N10	M	B1	S	S	I	C	DS	0	DISC	N	N	N	NONE	DARK LIVER- CHOCOLATE TESTIS DEFORMED, FEMINIZATION, TESTIS SACLIKE WITH TRANSPARENT VESICLES, SCOLIOSIS
29-10-2013	N11	M	N	M	M	I	N	LS	<50	N	N	N	N	NONE	COFFEE CREAM
29-10-2013	N12	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
29-10-2013	N13	M	N	M	M	I	N	LS	<50	N	N	N	N	NONE	
29-10-2013	N14	F	N	N	N	I	N	LS	>50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
29-10-2013	N15	M	N	M	S	I	N	DS	0	DISC	S	N	N	NONE	DARK LIVER- CHOCOLATE
29-10-2013	N16	M	N	N	N	I	N	LS	<50	SLIGHT DISC	N	N	N	NONE	DARK LIVER- CHOCOLATE
29-10-2013	N17	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
29-10-2013	N18	M	N	N	N	SHORTE NED	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
29-10-2013	N19	M	N	N	N	N	P	LS	>50	SLIGHT DISC	N	N	N	NONE	
29-10-2013	N20	F	N	N	N	N	N	LS	<50	SLIGHT DISC	S	N	N	NONE	

Orlando			FHAI												
Date	**Fish**	**Sex**	**EYES**	**SKIN**	**FINS**	**OPER**	**GILLS**	**BILE**	**MFAT**	**LIVER**	**SPLEEN**	**H/GUT**	**KID-NEY**	**PARAS**	**COMMENTS-LIVER UNLESS OTHERWISE STATED**
18-10-2013	O1	F	H1	M	N	N	N	LS	>50	SLIGHT DISC	ENLARGED	N	N	NONE	
25-10-2013	O2	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O3	F	N	M	N	N	F	LS	>50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O4	F	N	M	N	N	N	DS	<50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O5	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O6	F	N	N	N	N	F	DS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
25-10-2013	O7	F	N	N	N	N	F	DS	>50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O8	F	N	M	N	N	N	DS	>50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O9	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
25-10-2013	O10	M	N	M	N	N	N	LS	>50	SLIGHT DISC	SW	N	N	NONE	
25-10-2013	O11	M	N	M	N	N	F	DS	>50	N	SW	N	SW	NONE	
25-10-2013	O12	M	N	N	N	N	N	DS	>50	N	N	N	SW	NONE	
25-10-2013	O13	F	N	N	N	N	F	DS	<50	N	N	N	N	NONE	WHITE NODULES
25-10-2013	O14	F	N	M	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O15	F	N	N	N	N	P	LS	>50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O16	F	N	N	N	N	F	DS	<50	SLIGHT DISC	N	N	N	NONE	GILLS- ARCH MISSING
25-10-2013	O17	F	B1	N	N	N	P	LS	<50	N	N	N	N	NONE	
25-10-2013	O18	F	N	N	N	N	N	LS	>50	N	N	N	N	NONE	
25-10-2013	O19	F	N	N	N	N	N	DS	<50	SLIGHT DISC	N	N	N	NONE	
25-10-2013	O20	F	N	N	N	N	N	DS	<50	N	N	N	N	NONE	

Control			FHAI												
Date	**Fish**	**Sex**	**EYES**	**SKIN**	**FINS**	**OPER**	**GILLS**	**BILE**	**MFAT**	**LIVER**	**SPLEEN**	**H/GUT**	**KIDNEY**	**PARAS**	**COMMENTS-LIVER UNLESS OTHERWISE STATED**
25-04-2014	K1	F	N	N	E	N	N	LG	>50	NORMAL	N	N	N	NONE	
25-04-2014	K2	M	N	N	N	N	N	DS	<50	NORMAL	N	N	N	FEW	CYTS IN FILLET
25-04-2014	K3	F	N	M	N	N	N	DG	<50	FOCAL DISC	N	N	N	NONE	BRANCHED WHISKERS
25-04-2014	K4	F	N	N	N	N	N	DS	<50	NORMAL	N	N	S	NONE	ENLARGED GALL BLADDER
25-04-2014	K5	M	N	N	N	N	N	LS	<50	NORMAL	N	N	S	NONE	
25-04-2014	K6	M	N	N	N	N	P	DG	>50	NORMAL	N	N	N	NONE	
25-04-2014	K7	M	N	N	E	N	N	DS	>50	NORMAL	N	N	N	NONE	
25-04-2014	K8	M	N	N	N	N	N	DG	>50	FOCAL DISC	N	N	N	NONE	
25-04-2014	K9	M	N	N	E	N	N	LD	>50	NORMAL	N	N	N	NONE	
25-04-2014	K10	F	N	M	N	N	DISC	DS	<50	NORMAL	N	N	N	NONE	
25-04-2014	K11	M	N	N	N	N	N	DG	<50	NORMAL	N	N	N	NONE	
25-04-2014	K12	F	N	M	N	N	N	DS	<50	NORMAL	N	N	S	NONE	
25-04-2014	K13	F	N	N	N	N	N	LS	<50	NORMAL	N	N	S	NONE	
25-04-2014	K14	M	N	M	N	N	N	LS	>50	NORMAL	N	N	N	NONE	
25-04-2014	K15	M	N	N	N	N	N	DG	>50	NORMAL	N	N	N	NONE	
25-04-2014	K16	F	N	N	N	N	N	LS	<50	FOCAL DISC/FATTY	N	N	N	NONE	
25-04-2014	K17	M	N	N	N	N	N	DS	<50	NORMAL	N	N	S	NONE	
25-04-2014	K18	F	N	N	N	N	N	LS	<50	NORMAL	ENLARGED	N	N	NONE	
25-04-2014	K19	F	N	N	N	N	N	LS	<50	NORMAL	N	N	N	NONE	
25-04-2014	K20	F	N	N	N	N	N	DS	<50	NORMAL	N	N	N	NONE	

Lenasia 2014

FHAI

Date	Fish	Sex	EYES	SKIN	FINS	OPER	GILLS	BILE	MFAT	LIVER	SPLEEN	H/GUT	KIDNEY	PARAS	COMMENTS-LIVER UNLESS OTHERWISE STATED
08-10-2014	L1	M	N	N	E	N	N	LS	<50	FOCAL DISC/NODULES	N	N	N	NONE	
08-10-2014	L2	M	N	N	N	N	N	DS	<50	NORMAL	N	N	N	NONE	
08-10-2014	L3	F	N	N	N	N	P	LS	<50	NORMAL	N	N	N	NONE	
08-10-2014	L4	F	N	N	N	N	P	LS	<50	SLIGHT DISC	N	N	N	NONE	LIGHT TAN
08-10-2014	L5	F	H1	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
11-10-2014	L6	F	N	N	N	N	P	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
11-10-2014	L7	F	N	N	N	N	N	LS	<50	SLIGHT DISC	N	N	MOTLD	NONE	PURPLE=HAEMORAGED?
17-10-2014	L8	F	N	N	N	N	P	LS	<50	SLIGHT DISC	E	N	N	NONE	COFFEE CREAM
17-10-2014	L9	F	N	N	N	N	N	LS	<50	SLIGHT DISC	E	N	N	NONE	
17-10-2014	L10	M	N	N	N	N	P	LS	>50	SLIGHT DISC	SW	N	N	NONE	COFFEE CREAM
17-10-2014	L11	M	N	N	N	N	N	LS	<50	SLIGHT DISC/NODULES	E	N	N	NONE	

Fleurhof 2014

Date	Fish	Sex	EYES	SKIN	FINS	OPER	GILLS	BILE	MFAT	LIVER	SPLEEN	H/GUT	KIDNEY	PARAS	COMMENTS-LIVER UNLESS OTHERWISE STATED
15-10-2014	F1	F	N	N	N	N	N	LS	>50	NODULES/FOCAL/ENLARGED	E	N	N	NONE	CYSTS IN FILLET VERY LARGE LIVER
15-10-2014	F2	F	N	N	N	N	N	LS	<50	SLIGHT DISC/FOCAL DISC	N	N	N	NONE	CARAMEL/COFFEE CREAM
15-10-2014	F3	F	N	N	N	N	N	LS	<50	SLIGHT DISC	E	N	N	NONE	
15-10-2014	F4	M	N	M	N	N	N	DS	<50	N	E	N	N	NONE	
15-10-2014	F5	M	N	N	N	N	N	DS	<50	NORMAL/NODULES	E S	N	N	NONE	
15-10-2014	F6	F	N	M	N	N	N	LS	>50	SLIGHT DISC	N	N	M	NONE	CARAMEL/COFFEE CREAM
15-10-2014	F7	M	N	N	N	N	N	LS	<50	N	E	N	N	NONE	
15-10-2014	F8	F	E1	N	N	N	N	DS	<50	SLIGHT DISC	N	N	N	NONE	CARAMEL/COFFEE CREAM
15-10-2014	F9	F	N	M	N	N	N	LS	<50	FOCAL DISC	N	N	N	NONE	
15-10-2014	F10	F	N	N	N	N	N	LS	<50	DISCOLOURATION	N	N	N	NONE	

Nancefield 2014

FHAI

Date	Fish	Sex	EYES	SKIN	FINS	OPER	GILLS	BILE	MFAT	LIVER	SPLEEN	H/GUT	KIDNEY	PARAS	COMMENTS-LIVER UNLESS OTHERWISE STATED
20-10-2014	N1	F	E2	M	N	N	C	LS	<50	SLIGHT DISC/NODULES	N	N	N	NONE	NO ANTERIOR ANAL FIN (DOUBLE FINS ANTERIOR TO ANUS) ORGNS FUSED TO CEOLOM WITH CONNECTIVE TISSUE(LOTS OF CNT TISSUE)
20-10-2014	N2	M	E1	N	N	N	N	DS	>50	N	N	N	N	NONE	
20-10-2014	N3	F	N	N	N	N	N	LS	>50	SLIGHT DISC	N	N	N	NONE	
20-10-2014	N4	F	N	N	E	N	C	DS	<50	SLIGHT DISC/FOCAL	E	N	OTHER*	NONE	COFFEE CREAM INCREASED CNT TISSUE, FUSED ORGANS TO CEOLOM
20-10-2014	N5	F	N	N	N	N	C	DS	<50	SLIGHT DISC	OTHER*	N	N	NONE	COFFEE CREAM, INCREASED CNT TISSUE *OTHER= JAGGED EDGES ON SPLEEN
20-10-2014	N6	M	N	N	N	N	N	LS	<50	N	N	N	N	NONE	
22-10-2014	N7	F	H1	N	N	N	N	LS	<50	SLIGHT DISC	N	N	N	NONE	COFFEE CREAM
22-10-2014	N8	F	E1	N	N	N	N	LS	<50	FOCAL DISC	E	N	N	NONE	