Quantification of PAHs and PCBs in eThekwini aquatic systems, using chemical and biological analysis

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Summary

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are common contaminants of sediment, soils and biological tissues. These compounds pose a significant risk to biological and ecosystem health and functioning due to these compounds being mutagenic, carcinogenic and are known to disrupt the endocrine system. The bioaccumulation and biomagnification potential that these compounds possess mean that they are capable of affecting the entire food chain and are not limited to the organisms that are directly in contact with the compounds Even though there has been an increase in the attention on identifying the presence and impacts that these compounds may have in South Africa, the level of attention is lower than what it is in other countries around the world. Although South Africa has guidelines in place for other pollutants, such as metals, there are no such guidelines in place to monitor PAHs and PCBs.. Industries are known to release both PAHs and PCBs, mainly from incomplete burning processes and the release of oils and fuels in the case of PAHs, and from heat transfer fluids in the case of PCBs. Durban Bay and surrounding areas of eThekwini, KwaZulu-Natal are highly industrialised with many aquatic systems, in which these contaminants are likely to deposit. The aim of this research was to determine the concentrations of these compounds by means of chemical analysis and additionally biological analysis, using the H4IIE-*luc* bioassay and compare these levels to international guideline levels. It was found that the concentrations of the 23 analysed PAHs were 6.5–3 235.6 $ng.g^{-1}$ and the concentration of the PCBs analysed were 0–113.83 $ng.g^{-1}$. Many sites were found to be in exceedance of the guideline limits, particularly in the harbour. Toxic equivalency factors (TEF) were used to gauge the toxic equivalency (TEQ) of the PAHs and PCBs that were found. The TEQs were generally low, and were below any guideline levels. The assay revealed the extract containing the PCBs had a bioassay equivalence (BEQ) of 0–93.54 $pgTCDD\text{-}eq.g^{-1}$ and the extract containing the PAHs of 0–776.08 $pgTCDD\text{-}eq.g^{-1}$. With a proportion of the sites exceeding guideline limits. The BEQ results were two to three orders of magnitude greater than the TEQs calculated from the concentrations determined by the instrumental analysis, however, followed a similar trend. Additionally chemical analysis was not performed on a full suite of compounds that are able to elicit a response from the cells, which could be a reason why the BEQ and TEQ did not follow a similar trend among some of the samples. It would have been more beneficial to have performed chemical analysis on the 16 priority PAHs (as determined by the United States Environmental Protection Agency), the dioxin-like PCBs and polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDFs) which are all capable of

eliciting a response from the cells and have TEF values. The areas that were most affected by contamination of these compounds was the harbour and surrounding canals, and there was point source contamination along the Umhlatuzana, Umbilo, and Amanzimnyana Rivers. All dl-PCBs should be chemically analysed at all the sample areas, as these cause adverse effects to biota. In addition to this, biota should be sampled to determine concentrations of the compounds to determine bioavailability and the degree of bioaccumulation in the food chain. Utilising biomarkers it would be possible to determine stresses of fish.

Keywords: Polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbons (PAH), H4IIE-luc, eThekwini, KwaZulu-Natal, South Africa, toxic equivalency factor (TEF), bioassay equivalency (BEQ)

Acronyms and abbreviations

1MNP	1-Methylnaphthalene
1MP	1-Methylphenanthrene
2MNP	2-Methylnaphthalene
ACE	Acenaphthene
ACY	Acenaphthylene
AhR	Aryl hydrocarbon receptor
AMA	Amanzimnyana River catchment
AMP	Adenosine monophosphate
ANT	Anthracene
ARNT	Aryl hydrocarbon receptor nuclear translocator
ASE	Accelerated solvent extractor
ATP	Adenosine triphosphate
BaA	Benz[a]anthracene
BaP	Benzo[*a*]pyrene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BC	Blank control
BeP	Benzo[e]pyrene
BEQ	Bioassay equivalent
BghiP	Benzo[g,h,i]perylene
BiP	Biphenyl
BkF	Benzo[k]fluoranthene
CAN	Bayhead Canal
CHR	Chrysene
CI	Cell Index
DahA	Dibenz[a,h]anthracene
DBAY	Durban Bay harbour
DBT	Dibenzothiophene
DCM	Dichloromethane
dl-PCB	Dioxin-like polychlorinated biphenyl
DMN	2,6-Dimethylnaphthalene
DMSO	Dimethyl sulphoxide
DRE	Dioxin responsive element
EC	Effective concentration
ERL	Effects range low

FL	Fluorene
FLA	Fluoranthene
GIS	Geographic information system
GPC	Gel permeation chromatography
HMW	High molecular weight
HPLC	High pressure liquid chromatography
IcdP	Indeno[1,2,3-c,d]pyrene
ISI	Isipingo River
IVC	Island View Canal
KZN	KwaZulu-Natal
LAR	Luciferase assay reagent
LMW	Low molecular weight
MDL	Method detection limit
MNG	Mngeni River
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide
ndl-PCB	Non dioxin-like polychlorinated biphenyl
NP	Naphthalene
PAH	polycyclic aromatic hydrocarbon
PANT	Phenanthrene
PBS	Phosphate buffered saline
PCA	Principle component analysis
PCABs	polychlorazobenzenes
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PEC	Probable effect concentration
PER	Perylene
POPs	Persistent organic pollutants
Ppi	Inorganic phosphorus
PYR	Pyrene
REP	Relative potency
RLU	Relative light units
RTCA-SP	Real time cell analyser - single plate
SC	Solvent control
SPE	Solid phase extraction
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEC	Threshold effect concentration
TEF	Toxic equivalency factor

TEQ	Toxic equivalence
TMN	2,3,5-Trimethylnaphthalene
TOC	Total organic carbon
USEPA	United States Environmental Protection Agency
UV	Ultra violet

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1 Introduction

Worldwide there is growing concern relating to compounds in the environment that may adversely affect biota, specifically in relation to reproductive defects, compromised immune functionality, and cancer risks (Khim et al., 1999a). Aquatic ecosystems, and more specifically the sediment in these systems, is a sink for a wide range of contaminants (Brack, 2003) that present ecological and human health risks (Behnisch *et al.*, 2002, Giesy and Kannan, 1998). Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides are among the pollutants that form an important focus in ecological monitoring programs. Exposure to these compounds can affect the reproductive, immune and cardiovascular systems and affect the development of biota and some PAHs and PCBs are known carcinogens (Brack, 2003, Vallack *et al.*, 1998).

In catchments, contaminant levels are generally expected to be highest in dams, lakes and harbours, water circulation is minimal (Barra *et al.*, 2009). However, areas adjacent to direct runoff from canals or effluent releases will cause point source pollution (Baldwin and Howitt, 2007). Aquatic areas where these contaminants are known to settle are of importance from an ecological standpoint as these are spawning sites for fish, and polluted sediments directly expose benthic and pelagic organisms to pollutants (Barra *et al.*, 2009). Even at low doses, PCBs and PAHs are known to produce adverse effects in humans and wildlife (Behnisch *et al.*, 2002).

PCBs are categorised as one of the pollutant classes termed persistent organic pollutants (POPs), due to their persistence (Jones and De Voogt, 1999, Sinkkonen and Paasivirta, 2000). PCBs were manufactured and widely used in industries as lubricants, flame retardants, adhesives and heat transfer fluids, but have since been banned due to their toxicity (Vallack *et al.*, 1998, Giesy and Kannan, 1998). However, these compounds are formed unintentionally by combustion processes (Giesy and Kannan, 1998). These compounds are easily transported from their release site to remote areas (Giesy and Kannan, 1998). PCBs are lipophilic, resulting in their bioaccumulation and biomagnification in the food web (Vasseur and Cossu-Leguille, 2006).

PAHs are formed by incomplete combustion processes, such as the burning of coal, wood and agricultural waste. Additional sources are fossil fuels, including crude and refined oils (Shatalov *et al.*, 2004). Although there are natural sources of PAHs, such as fires and the degradation of organic matter, the main sources are anthropogenic (Nikolaou *et al.*, 2009). These compounds are not persistent because they tend to have a short half-life. However, they are introduced into the environment constantly (Sinkkonen and Paasivirta, 2000). These compounds are transported into waterways via storm water runoff drains and, due to their low solubility, tend to bind to sediment (Nikolaou *et al.*, 2009). Benthic organisms are most susceptible to exposure to PAHs. However,

PAHs bioaccumulate and biomagnify, and some are known or strongly suspected carcinogens, posing a risk to higher level consumers (Fu *et al.*, 2011, Jones and De Voogt, 1999).

Identifying possible harmful effects of chemicals such as PAHs and PCBs is often performed by analysis for these in environmental matrices (*e.g.* water, sediment) and their comparison to environmental quality guidelines. Because these compounds are lipophilic they adhere to organic carbon, hence sediment is the common matrix for analysis. However, this approach can only identify possible detrimental effects of compounds under investigation since it is unknown whether the chemicals were bioavailable (Behnisch *et al.*, 2001). The interactive effects of complex mixtures of chemicals are also unknown. Utilising a bioassay is a useful tool for determining the toxicity of complex mixtures of compounds (Behnisch *et al.*, 2001, Behnisch *et al.*, 2002). The liver, and more specifically the hepatoma cells, are responsible for the detoxification of toxicants in vertebrates. In this study genetically modified rat hepatoma, H4IIE, cells are used as an *in vitro* screening tool (Behnisch *et al.*, 2002, Brack, 2003, Giesy and Kannan, 1998). Utilising a bioassay is rapid and cost-effective, even when compared to *in vivo* methods and it is a highly sensitive method for determining toxic effects (Behnisch *et al.*, 2001, Vallack *et al.*, 1998).

The bioassay works on the principle that certain pollutants, such as PAHs, PCBs, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans (PCDD/Fs), referred to as dioxins, are able to bind to the aryl hydrocarbon receptor (AhR), which is present in the cytoplasm of most vertebrate cells, and is responsible for the initiation of the detoxification pathway (Giesy and Kannan, 1998, Behnisch *et al.*, 2001). When these compounds are bound to the AhR, the complex is translocated to the nucleus of the cell, which results in transcription of genes and subsequently the production of proteins, one being CYP1A, which is involved in the metabolising of the compounds. In the genetically modified cell line, the transcribed firefly luciferase is expressed. When this enzyme receives its substrate luciferin, a light-producing reaction is catalysed. The amount of light produced is directly proportional to the amount of AhR ligands present, which bound to the AhR. The response elicited by a sample extract is reported in relation to the response caused by a known positive control and expressed as bioassay equivalents (BEQs). In this way it is possible to semi-quantify the effect these pollutant mixtures might have on biota (Behnisch *et al.*, 2001).

The H4IIE assay has previously been used for determination of dioxin-like compounds in a freshwater aquatic environment in South Africa (Nieuwoudt et al., 2009). This will be the first time in South Africa where the assay will be utilised in a more marine based environment.

Because Durban Bay and the surrounding eThekwini region is highly industrialised it is suspected that contaminants in sediment in aquatic ecosystems in the area are above international guidelines. Additionally, the concentrations of contaminants are expected to be highest in sediments in the lower reaches of catchments.

The aim of this study was to determine the degree to which sediment in aquatic ecosystems within the eThekwini area of KwaZulu-Natal is contaminated by PAHs and PCBs, and to determine whether the concentrations are potentially harmful to aquatic organisms. The degree of harmfulness was determined by means of the H4IIE-*luc* reporter gene bioassay and toxic equivalences (TEQs).

The objectives of the study were to:

- Determine the chemical concentrations of PAH isomers and PCB congeners in order to:
    - compare the concentrations to sediment quality guidelines (SQGs),
    - calculate toxicity, in the form of TEQs, using toxic equivalency factors (TEF) based on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and benzo[*a*]pyrene and compare these to SQGs, and
    - determine toxicity by means of bioassay responses and compare bioassay equivalents to sediment quality guidelines.
- Compare toxicity estimated from chemistry data to cell toxicity measured by the reporter gene bioassay,
- Identify areas most affected by the PAH and PCB pollution.

2 Literature Review

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are organic compounds which commonly pollute aquatic environments. Due to the physicochemical nature of these compounds they accumulate in sediment (Khim *et al.*, 1999a, Otte *et al.*, 2008), therefore organisms directly exposed to the sediment can experience toxic effects. Contact with, and ingestion of the contaminated sediments can lead to hepatoxicity, weight loss, thymic atrophy, impairment of immune responses, dermal lesions, reproductive toxicity, alterations in vitamin and thyroid hormone metabolism, teratogenicity and carcinogenesis to humans and/or animals (Murk *et al.*, 1996). Benthic invertebrates and bottom-dwelling organisms are most exposed, but because compounds are capable of bioaccumulating and biomagnifying through the food web, it is possible for higher trophic levels and humans to be affected (Garrison *et al.*, 1996, Hong *et al.*, 2012).

2.1 Polychlorinated biphenyls

PCBs are classified as persistent organic pollutants due to their long half-life, and are resistant to biological and chemical degradation (Jaikanlaya *et al.*, 2009). They have been widely used around the world and it is estimated that 1–2 tons were produced worldwide between 1930 and 1993 (Shatalov *et al.*, 2004). Their production and use was banned in the 1970s (Khim *et al.*, 1999a). However, they can still be detected in various environmental compartments, such as sediment, soil and animal tissue (Khim *et al.*, 1999a, Van Ael *et al.*, 2012) because they are still being released into the environment from historical sources and because they are so persistent (Jaikanlaya *et al.*, 2009).

2.1.1 Physical and chemical properties/characteristics

PCBs are aromatic compounds containing two benzene rings bonded by a single carbon bond. Hydrogen atoms can be replaced by up to 10 chlorine atoms to form a 209 possible congeners (Figure 2.1). Each of these congener forms has its own physicochemical properties and toxicity, depending on the number and position of the chlorine atoms on the biphenyl molecule (Cardellicchio *et al.*, 2007). Usually, there are only 130 congeners analysed in environmental samples (Jaikanlaya *et al.*, 2009). Dioxin-like PCBs (dl-PCBs) lack multiple *ortho*-chlorines, but contain adjacent *meta*- and *para*-substituted chlorine atoms, and are also referred to as coplanar PCBs (Alcock *et al.*, 1998, Jaikanlaya *et al.*, 2009, Longnecker *et al.*, 1997). The number of chlorine atoms present is directly proportional to the toxicity of the congener (van Loon and Duffy, 2007).

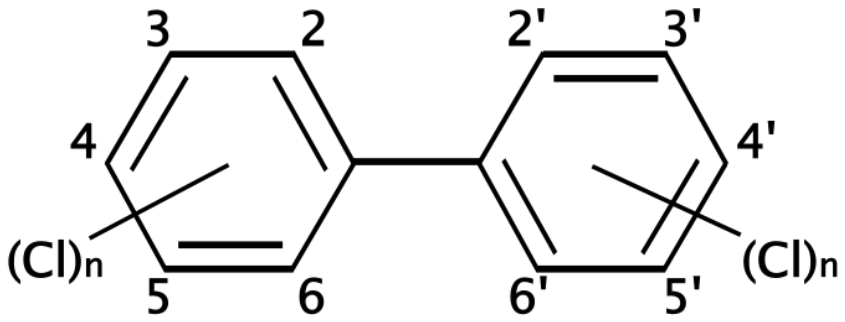


Figure 2.1. General structure of a PCB congener.

PCBs exhibit thermal and chemical stability that results in resistance to heat, light and, acid breakdown and they have low reactivity. (Stronkhorst *et al.*, 2002, Van Ael *et al.*, 2012). Their lipophilic, hydrophobic and hence its solubility and low vapour pressure, and persistent nature leads to bioaccumulation of these compounds in fatty tissues of animals. The high octanol:water partition coefficient (K_{ow}) explains their lipophilic behaviour and the octanol:carbon coefficient (K_{oc}) explains their tendency to sorb onto organic matter (Table 2.1). Compounds with higher K_{oc} tend to bind with greater affinity (Barra *et al.*, 2006, Cardellicchio *et al.*, 2007, Shatalov *et al.*, 2004). The half-life of these compounds varies according to the specific congener, and can persist for a day to as much as 70 years in soil and sediment (Jones and De Voogt, 1999, Longnecker *et al.*, 1997).

Table 2.1. Physicochemical characteristics of the PCBs targeted in this study. K_{OW} = octanol/water partioning coefficient, K_{OC} = organic carbon portioning coefficient. - = data not available, PCBs highlighted in bold are the dl-PCBs (Girvin and Scott, 1997, Hawker and Connell, 1988)

PCB	Vapour pressure	Log K_{ow}	Log K_{oc}	Solubility
PCB # 001	-	4.46	6.3	-
PCB # 008	-	5.10	4.5	-
PCB # 018	-	5.60	5.0	-
PCB # 028	-	5.80	5.2	-
PCB # 044	-	5.75	5.4	-
PCB # 052	-	6.10	5.5	-
PCB # 066	-	5.80	5.2	-
PCB # 077	**4.4×10^{-7}**	**6.60**	**-**	**1.8×10^{-1}**
PCB # 101	-	6.40	5.7	-
PCB # 105	**6.5×10^{-6}**	**6.65**	**-**	**4.3×10^{-3}**
PCB # 118	**9.0×10^{-6}**	**6.74**	**5.7**	**1.3×10^{-2}**
PCB # 126	**-**	**6.89**	**-**	**-**
PCB # 128	-	6.74	6.5	-
PCB # 138	-	6.83	6.5	-
PCB # 153	-	6.90	6.4	-
PCB # 169	**4.0×10^{-7}**	**7.40**	**-**	**1.2×10^{-2}**
PCB # 170	-	7.10	6.6	-
PCB # 180	-	7.10	6.6	-
PCB # 187	-	7.10	6.6	-
PCB # 195	-	7.56	-	-
PCB # 206	-	8.09	6.6	-
PCB # 209	-	-	-	-

2.1.2 Sources

PCBs are chemically produced, when a carbon source and chlorine in any from are incompletely burned (Alcock *et al.*, 1998), for use as lubricants, paint stabilisers, polymers and adhesives, dielectric fluids for capacitors and transformers, and heat transfer agents (Cardellicchio *et al.*, 2007, Staskal *et al.*, 2011). Very high temperatures (1200°C) are required to destroy these compounds, however, the incomplete burning of them for this purpose may lead to their release (Stine and Brown, 2006). Historical sources are still an issue due to leaking electrical transformers, hazardous waste sites, improper disposal of industrial waste, and incineration of some chemical wastes and the long degradation periods of the compounds (Jaikanlaya *et al.*, 2009). In more recent years the levels of PCBs have declined slightly in the environment, however, due to bioaccumulation and biomagnification higher trophic level animals and humans are still exposed and at risk (Longnecker *et al.*, 1997).

2.1.3 Distribution and transport

At present there is still release of PCBs, even though their production has been banned. Due to long range transport, the release of PCBs from historical production, and volatilisation from soils and vegetation, these compounds can be released into the environment and atmosphere where they can be re-deposited in areas far removed for the initial release point, which can lead to ubiquitous pollution (Jaikanlaya *et al.*, 2009, Jones and De Voogt, 1999). The levels of these compounds will be reduced slowly due to reduced rates of discharge and degradation (Stine and Brown, 2006).

2.1.4 Toxicity

The lipophilic nature of PCBs allows for their bioaccumulation and biomagnification through the food web having the potential to have a human health impact (Jaikanlaya *et al.*, 2009, Van Ael *et al.*, 2012).. The International Agency of Research on Cancer (IARC) has listed PCBs as probable human carcinogens. PCBs are able to affect the reproductive system, exhibit embryotoxic effects, cause abnormal kidney function and an increase in kidney cancer, severe weight loss, thymic atrophy, hepatotoxicity, edema, and immunotoxicity in experimental animals (Alcock *et al.*, 1998, Longnecker *et al.*, 1997, Staskal *et al.*, 2011). Exposure to PCBs has also lead to skin abnormalities like chloracne and hyperpigmentation (Longnecker *et al.*, 1997). Children whom have had exposure to PCBs while *in utero,* or postnatally through the mothers' milk, may develop hypotonia and hyporeflexia. Children also had slow cognitive development and motor functions developed at a slower rate, for the first two years of age, when compared to unexposed children (Alcock *et al.*, 1998, Jaikanlaya *et al.*, 2009, Longnecker *et al.*, 1997).

Coplanar PCBs or dl-PCBs, of which there are 12, have more potent toxicological properties than other PCBs (Shatalov *et al.*, 2004). They are capable of interacting with the aryl hydrocarbon receptor (AhR) in a similar way as 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD)—the most toxic AhR congener—causing a similar toxicological response (Cardellicchio *et al.*, 2007, Longnecker *et al.*, 1997). Even at very low concentrations and doses, dl-PCBs can cause detrimental health effects (Bhavsar *et al.*, 2007, Jaikanlaya *et al.*, 2009). Non-dioxin like PCBs (ndl-PCBs) do not exert toxicity via the AhR pathway, but affect the endocrine and neurological systems, such as reducing the serum concentrations of the thyroid hormones thyroxine and triiodothyronone (Alcock *et al.*, 1998).

2.2 Polycyclic aromatic hydrocarbons

PAHs are ubiquitous environmental contaminants, predominantly found in freshwater and marine sediments (Neff *et al.*, 2005, Willett *et al.*, 1997). These compounds are major contributors to detrimental effects on aquatic life through exposure to contaminated sources, such as sediments and soils (Neff *et al.*, 2005). The United States Environmental Protection Agency (USEPA) has regulated 16 of the PAHs and termed them priority PAHs (Achten and Hofmann, 2009). The USEPA priority PAHs are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, beo[*k*]fuoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene, indeno[*1,2,3-cd*]pyrene

2.2.1 Physical and chemical properties/characteristics

These organic compounds contain two or more fused aromatic or benzene rings, and commonly occur as complex mixtures as opposed to individual compounds (Figure 2.2) (Boström *et al.*, 2002, CCME, 2008, Haritash and Kaushik, 2009, Lee, 2010, Shatalov *et al.*, 2004). PAHs can be divided into two groups based on the number of benzene rings that are present. Low molecular weight PAHs (LMWs), which have a core structure of two or three benzene rings, such as naphthalene, acenaphthylene and phenanthrene, or high molecular weight PAHs (HMWs), which have a core molecular structure of four or more benzene rings, such as pyrene and benzo[a]pyrene (CCME, 2008, Tsymbalyuk *et al.*, 2011).

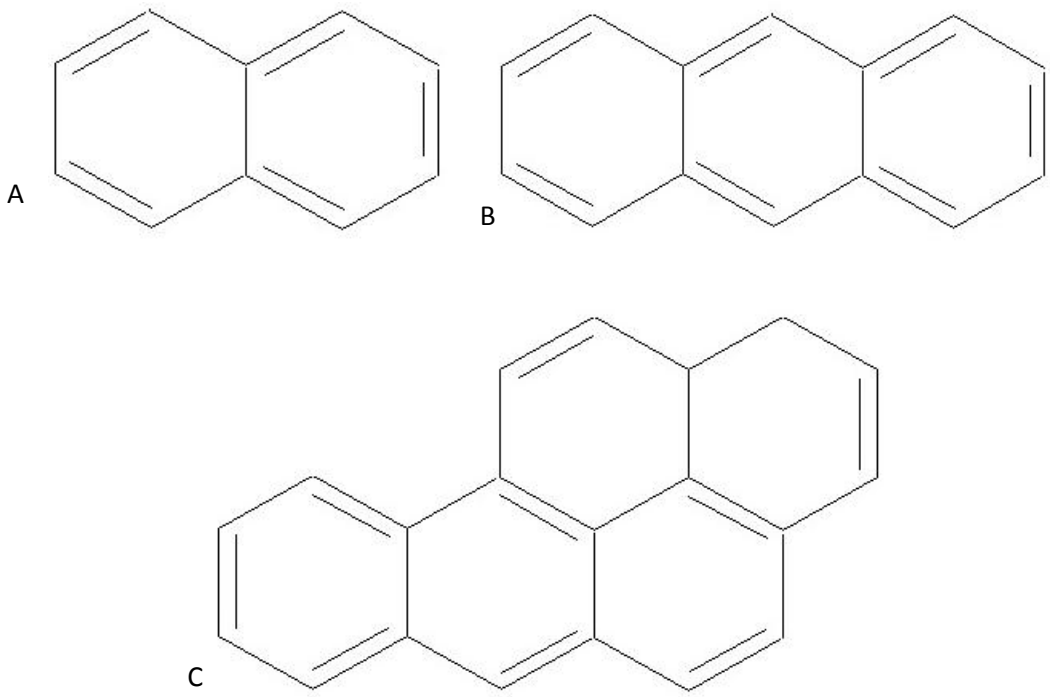


Figure 2.2. Chemical structure of naphthalene (A), anthracene (B) and benzo[*a*]pyrene (C).

These compounds generally have low water solubility, high melting points and low vapour pressure, and are semi-volatile (Ahrens and Depree, 2010, Barra *et al.*, 2009, Haritash and Kaushik, 2009). They have a tendency to bioaccumulate because of their lipophilicity, resistance to degradation and general persistence—they are discharged into the environment at a high rate (Boström *et al.*, 2002, CCME, 2008, Haritash and Kaushik, 2009). Their boiling and melting points increase with increasing molecular weight, however, solubility and vapour pressures decrease with increasing molecular weight (Haritash and Kaushik, 2009). Toxicity increases with increasing molecular weight and K_{OW}. As the K_{OW} increases the solubility in water decreases (Table 2.2). PAHs, because they are hydrophobic and lipophilic, tend to have a higher affinity to bind to organic matter (Brenner *et al.*, 2002), and because of this binding to sediments, benthic organisms are often the more directly affected by the toxicity (Walker *et al.*, 2004).

PAHs are not persistent, but degrade slowly under natural conditions. PAH degradation may be reduced in environments where there is a lack of oxygen and/or sunlight (Ahrens and Depree, 2010). Their persistence increases with an increase in molecular weight (Haritash and Kaushik, 2009). However, these compounds are continuously used and have widespread sources, and are released into the environment in high concentrations on a constant basis, resulting in PAHs being ubiquitous, with the potential for bioaccumulation and carcinogenicity (Lee, 2010, Haritash and Kaushik, 2009).

Table 2.2. Physicochemical characteristics of the PAHs targeted in this study. NC = non-carcinogenic, WC = weakly carcinogenic, C = carcinogenic, SC = strongly carcinogenic. K_{OW} = octanol/water partitioning coefficient, K_{OC} = organic carbon partitioning coefficient, - = data not available (Lee, 2010, Neff *et al.*, 2005).

PAH	Abbreviation	Number of rings	Molecular weight	Vapour pressure mmHg	Log K_{ow}	Log K_{oc}	Carcino-genicity
Naphthalene	NP	2	128.17	8.7×10^{-2}	3.29	2.97	NC
1-Methylnaphthalene	1MNP	2	142.20	5.4×10^{-2}	3.29	-	-
2-Methylnaphthalene	2MNP	2	142.20	6.8×10^{-2}	3.86	3.39	-
2,6-Dimethylnaphthalene	DMN	2	156.22	-	-	-	-
2,3,5-Trimethylnaphthalene	TMN	2	170.25	-	-	-	-
Acenaphthylene	ACY	2	152.20	2.9×10^{-2}	4.07	1.4	NC
Acenaphthene	ACE	2	152.21	4.47×10^{-3}	3.98	3.66	NC
Biphenyl	BiP	2	154.21	-	3.95	-	-
Fluorene	FL	2	166.20	3.2×10^{-4}	4.18	3.89	NC
Dibenzothiophene	DBT	2	184.26	-	-	-	-
Anthracene	ANT	3	178.20	1.75×10^{-6}	4.45	4.15	NC
Phenanthrene	PANT	3	178.20	6.8×10^{-4}	4.45	4.15	NC
1-Methylphenanthrene	1MP	3	192.30	-	4.77	-	NC
Fluoranthene	FLA	3	202.26	5.0×10^{-6}	4.90	4.58	NC
Pyrene	PYR	4	202.30	2.5×10^{-6}	4.88	4.58	NC
Benz[a]anthracene	BaA	4	228.29	2.5×10^{-6}	5.61	5.30	C
Chrysene	CHR	4	228.28	6.4×10^{-9}	5.9	-	WC
Benzo[b]fluoranthene	BbF	4	252.30	5.0×10^{-7}	6.04	5.74	C
Benzo[k]fluoranthene	BkF	4	252.30	9.59×10^{-11}	6.06	5.74	-
Benzo[a]pyrene	BaP	5	252.30	5.6×10^{-9}	6.06	6.74	SC
Dibenz[a,h]anthracene	DahA	5	278.35	1.0×10^{-10}	6.84	6.52	C
Indeno[1,2,3-c,d]pyrene	IcdP	5	276.30	-	6.58	6.20	C
Benzo[e]pyrene	BeP	5	252.30	5.5×10^{-9}	6.21	-	NC
Perylene	PER	5	252.30	-	6.21	-	NC
Benzo[g,h,i]perylene	BghiP	6	276.34	1.03×10^{-10}	6.78	6.20	NC

2.2.1 Sources

PAHs are have natural and anthropogenic sources (Haritash and Kaushik, 2009), however, anthropogenic sources far outweigh natural sources (Barra *et al.*, 2009). Anthropogenic sources include the incomplete combustion of fossil fuels, coal tar, wood and garbage. They are common components in petroleum and in lubricating oils and are released into the environment frequently from petroleum spills and discharges (Boström *et al.*, 2002, CCME, 2008, Garner *et al.*, 2009, Khim *et al.*, 1999a, Mastral *et al.*, 1996). Many human activities involving combustions and emissions, however, contribute to a greater overall concentration in the global environment of benzo[a]pyrene and other suspected carcinogenic PAHs relative to natural sources. Emission sources may affect the characterisation and distribution of the compounds (CCME, 2008).

PAHs can be sourced from two processes, petrogenic and pyrogenic; being of fuel and/or oil derivatives, or from the combustion of organic materials respectively. Petrogenic PAHs are

predominantly LMW PAHs, while pyrogenic PAHs are dominated by HMW PAHs (Neff *et al.*, 2005). The amount and composition of the total PAHs released is determined by the raw material, the combustion temperature, oxygen availability, and potential abatement technology (Shatalov *et al.*, 2004). During incomplete combustion or if the fuel being burnt is cooled too rapidly, small organic chemicals may condense to form PAHs, among other compounds (Neff *et al.*, 2005).

Industrial activities are the main source of PAH pollution (Cardellicchio *et al.*, 2007). Sources of industrial PAHs include processing of raw materials, such as aluminium, coke, petrochemical, cement, bitumen, rubber tyre, and asphalt production, wood preservation, commercial heat and power generation, and the incineration of waste (Boström *et al.*, 2002). The levels of PAHs are high due to the substantial and abundant use of fuels in the industrial sector. Oil spills from ships and tankers are also common in aquatic systems because the crews of these vessels have been known to wash out tanks into the aquatic systems (Christensen *et al.*, 2004).

Domestic sources are an important contributor to the total environmental contamination by PAHs. These sources are dominated by cooking and heating. This poses a health concern due to their presence in indoor environments (Lee, 2010). LMW PAHs originating from cooking methods dominate the PAH in residential air. Cigarette smoke is also a predominant source of PAHs in the indoor environment, and studies have found that the levels of PAHs in residences with smokers tend to be higher than those with non-smokers (Lee, 2010).

One of the important sources of PAH emissions is vehicles (Boström *et al.*, 2002). Automobile and truck exhausts and coal-fired power generation are two major sources of combustion derived PAHs to the environment (CCME, 2008). Burning of diesel and engine oils is a pyrogenic source of PAHs, characterised by HMW PAHs (Neff *et al.*, 2005). Covering parking lots with coal tar has been associated with PAH contamination in excess of what is produced from day-to-day vehicle emissions (Ahrens and Depree, 2010).

The natural burning of forests, woodlands and veld, natural oil seepages, volcanic eruptions and exudates from trees, and the decaying of organic matter causes PAH emissions (Haritash and Kaushik, 2009). The size and rate of the emissions are dependent on meteorological conditions, such as wind, temperature, humidity, as well as the fuel characteristic type, such as moisture content, green wood, and seasonal wood (Lee, 2010). Natural oil seeps, erosion of coal, peat and oil shale deposits are means whereby petrogenic PAHs enter the environment (Neff *et al.*, 2005). PAHs are also formed during the natural transformation of organic content in the environment by rapid chemical or biological processes, but these PAHs are normally simple structures and do not contribute importantly to the total mass of PAHs in sediments (Neff *et al.*, 2005), and this normally forms LMW PAHs (Barra *et al.*, 2009) The burning of organic material under suboptimum

combustion processes produces significant amounts of PAHs. Such processes include the burning of brushwood, straw, and stubble and the burning of harvested crops and waste plant matter in agricultural practices (Lee, 2010).

2.2.2 Fate

These compounds readily deposit onto sediments and soils due to their lipophilic characteristics (Khim *et al.*, 1999a), but their sorption to these particles can reduce its bioavailability. PAH composition could change within the sediment or soil due to anaerobic and aerobic biodegration (Brenner *et al.*, 2002). The degradation of PAHs is slowed in anaerobic conditions, due to the need of oxygen to cleave the rings (Cardellicchio *et al.*, 2007). PAHs undergo natural weathering processes, such as volatilisation, photo-oxidation, chemical oxidation, and microbial degradation (Haritash and Kaushik, 2009). Although most PAHs are chemically inert, they can be photochemically decomposed under strong ultraviolet (UV) light, or sunlight. They can also react with ozone, hydroxyl radicals, nitrogen and sulphur oxides, and nitric and sulphuric acids, which affect the characteristic and hence the toxicity and contamination potential (Lee, 2010).

2.2.3 Toxicity

Exposure of vertebrates to PAHs has been shown to result in detrimental effects, such as reproductive toxicity, cardiovascular toxicity, bone marrow toxicity, suppression of the immune system, liver toxicity and developmental effects (Brack, 2003, Safe et al., 2010). The main concern is that many PAHs are known carcinogens (Collins *et al.*, 1998, Khim *et al.*, 1999a). Priority PAHs—16 PAH isomers—have been identified by the US EPA due to the their mutagenic and carcinogenic properties (Garner *et al.*, 2009). The molecular mass of the PAH seems proportional to the toxicity potential, with 5 and 6 ringed PAHs being more persistent and toxic. Naphthalene, however, does not fit this rule. It is highly toxic, even though it is only a two-ringed PAH. Benzo[a]pyrene—a five-ring PAH—is regarded as being the most carcinogenic PAH (Lee, 2010).

2.3 Determination of PCB and PAH toxicity

When an organism is exposed to xenobiotics, such as dioxins and PAHs, cytochrome P450 enzymes are expressed, mainly from the liver. These enzymes act in oxidative metabolic activation and detoxification of these xenobiotics (Ellero *et al.*, 2010) (Figure 2. 3). Both dl-PCB congeners and PAH isomers share a similar toxicological mode of action as other dioxins, and more specifically TCDD, the most toxic congener (Stine and Brown, 2006, Stronkhorst *et al.*, 2002). Several compounds, including drugs such as omeprazole, flutamide, and atorvastin, and natural products like cruciferous vegetable, carotenoids, and green tea polyphenols, have been shown to activate the AhR pathway. However, these compounds do not cause any toxic responses, such as those which would be caused by TCDD and other dl-compounds (Safe et al., 2010). Due to the

persistence of the dl-compounds the liver is less likely to be able to metabolise the compounds and toxicity then occurs.

The aryl hydrocarbon receptor (AhR) in the cytoplasm of vertebrate cells occurs as a multi-protein complex with a chaperone 90-kDa heat shock protein (Hsp90) and a co-chaperone protein (p23) within the cytoplasm. When AhR ligands enter the cytoplasm of a cell and there bind with the AhR, it causes the activation of the AhR and it dissociates from the Hsp90 and p23. The ligand-bound AhR is translocated to the nucleus, where it forms a heterodimer with the AhR nuclear translocator (Arnt) resulting in AhR:Arnt. This complex interacts with the dioxin responsive element (DRE) on the DNA strand (Baston and Denison, 2011, Denison and Nagy, 2003, Denison et al., 2004, Hilscherova et al., 2000, Villeneuve et al., 1999, Whyte et al., 2000, Whyte et al., 2004). The interaction stimulates the expression of AhR-responsive genes and the production of mRNA, which is translated in the cytoplasm to form the detoxification enzymes CYP1A1, CYP1B1 and CYP1A2 (Hosoya *et al.*, 2008, Stronkhorst *et al.*, 2002, Yoshinari *et al.*, 2006).

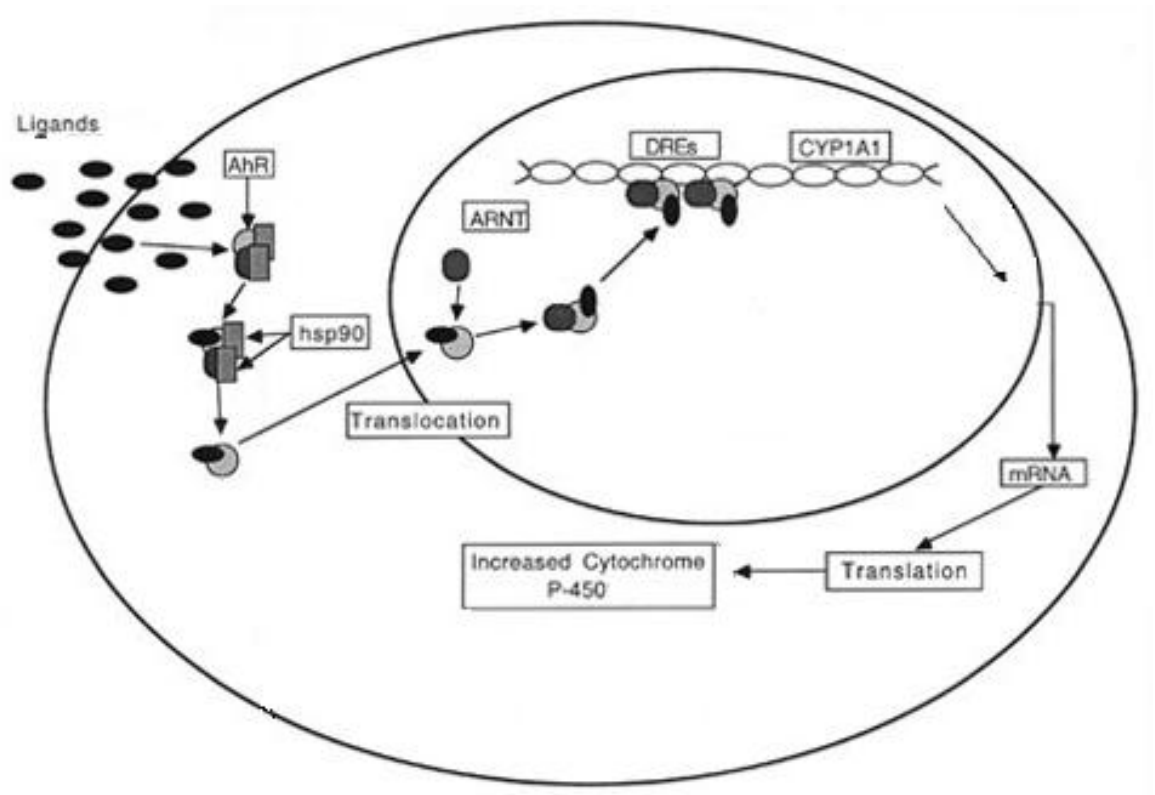


Figure 2. 3. Mechanism of the AhR detoxification pathway (adapted from Denison and Nagy (2003)).

2.3.1 Toxic equivalency factors

Dioxin-like compounds are generally found in complex mixtures in the environment, and this added to the fact that these compounds have varying degrees of toxicity and some of the toxicity has yet to be evaluated and it makes the identification of the health risks difficult to determine (Staskal *et*

al., 2011). The toxic equivalency factor (TEF) method was developed to assess their potential health risks. It is a relative potency scheme, with the most toxic and well-studied compound, TCDD, as the reference compound (Staskal *et al.*, 2011, Van den Berg *et al.*, 1998). This determination is based on *in vitro* and *in vivo* studies (Ahlborg *et al.*, 1994) and are consensus values decided upon after studying peer reviewed papers on all biota exposures to dioxins . The concept uses available toxicological and biological data to generate a set of weighting factors in an order of magnitude range to each of the dl-PCBs, PCDD/Fs congeners, which expresses the toxicity of the compounds in terms of the equivalent amount of TCDD (Alcock *et al.*, 1998). A downside to using this method is that the toxicity of compounds are assumed to be additive, however, it does not account for the possible synergism or antagonism of the compounds when in a complex mixture (Ahlborg *et al.*, 1994)

Many TEF-schemes have been developed, however, for the sake of consistency when dealing with these compounds, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) created a database based on available information to derive consensus TEFs (Ahlborg *et al.*, 1994). TEF values have been created for birds, mammals and fish, the latter being utilised in this study because fish would be the first affected by the pollutants (Table 2.3) (Van den Berg *et al.*, 2006).

PAHs have their own set of TEF values, where benzo[a]pyrene—the most toxic PAH—has been used as the reference compound. Because PAHs mediate the same type of response within an organism, TEF values utilising TCDD as a reference compound have also been developed to aid in risk identification (Table 2.4). These were defined following a similar method as the dioxin-TEFs.

The TEF for each congener or isomer is multiplied by its concentration, and the summation of each of these gives a single toxic equivalency (TEQ) for the mixture of the compounds occurring in a sample.

Table 2.3. The 2005 WHO TEF values for PCBs (Van den Berg *et al.*, 2006).

PCB number	Congener	Fish TEF
PCB 77	3,3',4,4'-tetraCB	0.0001
PCB 81	3,4,4',5-tetraCB	0.0005
PCB 126	3,3',4,4',5-pentaCB	0.005
PCB 169	3,3',4,4',5,5'-hexaCB	0.00005
PCB 105	2,3,3',4,4'-pentaCB	<0.000005
PCB 114	2,3,4,4',5-pentaCB	<0.000005
PCB 118	2,3',4,4',5-pentaCB	<0.000005
PCB 123	2',3,4,4',5-pentaCB	<0.000005
PCB 156	2,3,3',4,4',5-hexaCB	<0.000005
PCB 157	2,3,3',4,4',5'-hexaCB	<0.000005
PCB 167	2,3',4,4',5,5-hexaCB	<0.000005
PCB 189	2,3,3',4,4',5,5'-heptaCB	<0.000005

Table 2.4. TEF values for PAHs using TCCD and BaP as the reference compounds.

Compound	TEF_{TCDD} (Villeneuve *et al.*, 2002)	TEF_{BaP} (Nisbet and LaGoy, 1992)
2-Methylnaphthalene	-	1.0×10^{-3}
Acenaphthene	-	1.0×10^{-3}
Acenaphthylene	-	1.0×10^{-3}
Anthracene	-	1.0×10^{-2}
Benz[a]anthracene	1.4×10^{-6}	1.0×10^{-1}
Benzo[a]pyrene	1.3×10^{-6}	1.0
Benzo[b]fluoranthene	4.0×10^{-6}	1.0×10^{-1}
Benzo[g,h,i]perylene	-	1.0×10^{-2}
Benzo[k]fluoranthene	1.1×10^{-4}	1.0×10^{-1}
Chrysene	1.6×10^{-6}	1.0×10^{-2}
Dibenz[a,h]anthracene	4.0×10^{-6}	5.0
Fluoranthene	-	1.0×10^{-3}
Fluorene	-	1.0×10^{-3}
Indeno[1,2,3-c,d]pyrene	1.3×10^{-5}	1.0×10^{-1}
Naphthalene	-	1.0×10^{-3}
Phenanthrene	-	1.0×10^{-3}
Pyrene	-	1.0×10^{-3}

2.3.2 Cell bioassay

Soil and sediment toxicity assessment is usually conducted by comparing contaminant levels or TEQs to published guidelines. However, a limitation of this method is that environmental samples are complex mixtures of contaminants, and the biological impacts of other, unknown compounds are not taken into account (Xiao *et al.*, 2006). Chemical analysis assumes additive interactions. However, that is not always the case and non-additive interactions have been recorded (Khim *et al.*, 1999b). In the case of the compounds under investigation in this study, the bioassays that was used enabled an estimation of total biological activity of AhR ligands, which activate the AhR mediated gene expression (Khim *et al.*, 1999b, Vondráček *et al.*, 2001).

Generally, targeted chemical analysis is performed based on priority pollutants and toxicity modelling, using individual compound toxicity. However, if the suite of toxic pollutants is not known *a priory*, it is a meaningless method to determine the toxicity (Brack, 2003). So, utilising *in vitro* assays, as screening tools, can be useful to estimate environmental effects expected from the complex mix of chemicals, which can bind to the AhR, causing toxicity (

Table 2.5) (Houtman *et al.*, 2004). In the case of the H4IIE-*luc* assay, all compounds capable of activating the AhR, will be detected. This method is relatively cost effective, fast and effective in the detection of these compounds (Murk *et al.*, 1996). The ability of a compound to bind to the AhR is reported to be directly proportional to the toxicity of that compound (Behnisch *et al.*, 2001). If the bioassay elicits a response, the compounds within the extracts from the samples can be identified and then quantified using gas chromatography mass spectrophotometry (GCMS) (Garrison *et al.*, 1996, Murk *et al.*, 1996). However, this is not always that easy because for some isomers and congeners the techniques and standards for their identification do not exist (Garrison *et al.*, 1996).

Table 2.5. Compound classes which have the potential to bind to the AhR (Behnisch *et al.*, 2001, Hilscherova *et al.*, 2000, Safe *et al.*, 2010).

Class of compound	Examples
Hydrophobic aromatic compounds with a planar structure and a correctly sized molecule which can fit into the AhR binding site	Planar PCB and PCDD/F congeners, polychlorazobenzes (PCABs), polychloroxybenzes (PCAOBs), polychlorinated naphthalenes (PCNs), and high molecular weight PAHs.
Compounds with a specific stereochemical configuration	Polyhalogenated (chlorinated, brominated and fluorinated), mixed halogenated (chlorinated, brominated, and fluorinated), and alkynated analogs of the previously listed class of compounds, polychlorinated xanthenes and xanones (PCXE/PCXO), polychlorinated diphenyltoluenes (PCDPT), anisols (PCAs), anthacenes (PCANs) and flourenes (PCFLs).
Transient inducers and weak AhR ligands which deviate from the traditional criteria of planarity, aromaticity and hydrophobicity and are rapidly degraded by the detoxification enzyme	Some natural compounds such as indoles, heterocyclic amines, certain pesticides and drugs with various structures.

2.3.2.1 Mechanism of H4IIE-luc reporter gene bioassay

The reporter gene bioassay, by means of the H4IIE-*luc* rat hepatoma cells, was used in this study. The principle behind the assay (Figure 2.4) is similar to the detoxifying mechanism vertebrates use, as described earlier These cells have been stably transfected with a luciferase reporter gene under control of dioxin-responsive elements (DRE) (Houtman *et al.*, 2004, Khim *et al.*, 1999b, Koh *et al.*, 2004, Whyte *et al.*, 2004). When the endogenous AhR is ligand bound, the cytochrome P450 response is induced, and because the luciferase gene has been inserted downstream of the P450 gene, luciferase is also produced together with the already mentioned CYP enzymes (see section 2.3) (Allan *et al.*, 2006, Hilscherova *et al.*, 2000, Safe *et al.*, 2010).

Luciferin is added to the cells, and when in the presence of luciferase, a catalytic oxidisation occurs, resulting in light production (Behnisch et al., 2001, Villeneuve et al., 1999, Whyte et al., 2004). When the substrate, luciferin, together with adenosine triphosphate and oxygen reacts with luciferase and magnesium they form oxyluciferase, inorganic phosphorus (PPi), andenosine monophosphate (AMP), CO_2 and light (Alam and Cook, 1990). The amount of light produced is directly proportional to the amount of AhR ligands present within the sample to which the cells were exposed (Hilscherova *et al.*, 2000).

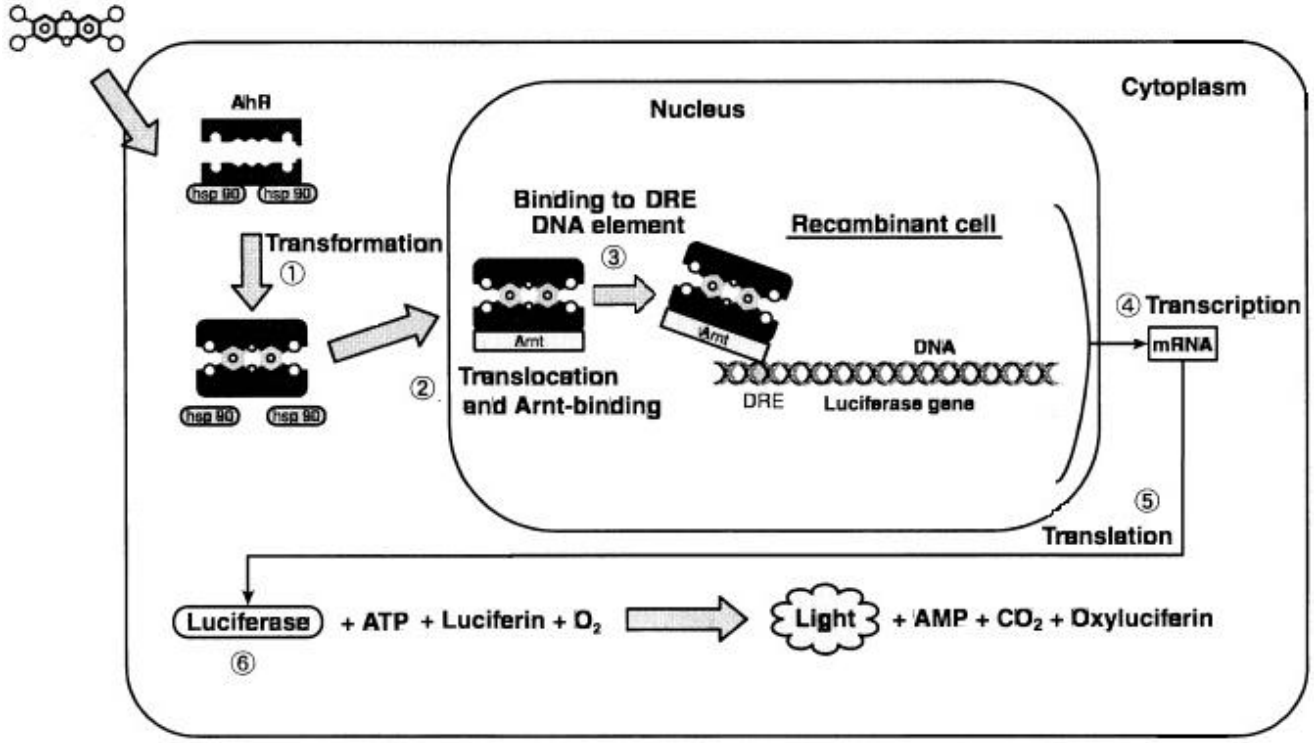


Figure 2.4. Mechanism of the H4IIE bioassay (adapted from Behnisch *et al.* (2001)).

3 Methods and Materials

3.1 Study area

The study area was situated in the eThekwini region of KwaZulu-Natal (KZN), and more specifically catchments of the Isipingo and Mngeni Rivers and Durban Bay (Figure 3.1). The catchments were identified based on the findings of a previous study that showed sediment in these catchments was the most contaminated by PAHs and PCBs in the greater eThekwini area (Newman *et al.,* 2012). Furthermore, the catchments are characterised by a wide variety of land-uses, ranging from informal settlements and high-density low and high cost housing to industry and agriculture. The flood plains of the Mngeni River (Figure 3.3) and the Isipingo River (Figure 3.2) are used for small scale subsistence farming, and water from tributaries can be used for irrigation purposes. There are recreational activities that take place in these systems, including canoeing and fishing which can contribute to dermal exposure (through contact with sediments) and eating the catch of the day.

Sampling sites were situated in upper reaches of the Durban Bay catchment, such as the Umbilo, Umhlatuzana and Amanzimyana Rivers (Figure 3.4). This served to incorporate sub-catchments with different land-uses and to determine whether land-use influences the organic chemical concentrations in sediment. Having a wide distribution of sites across the catchments assumes there will be a record of the organic pollutant make-up within the systems. Sampling sites were also positioned in the estuarine reaches of catchments because estuaries are regarded as sinks for anthropogenic contaminants that are introduced upstream (Houtman *et al.*, 2004, Rockne *et al.*, 2002). Soil samples were also collected from small garden-market farms (Figure 3.2) near the Isipingo River, to determine if organic chemicals are accumulating in these soils from an atmospheric route.

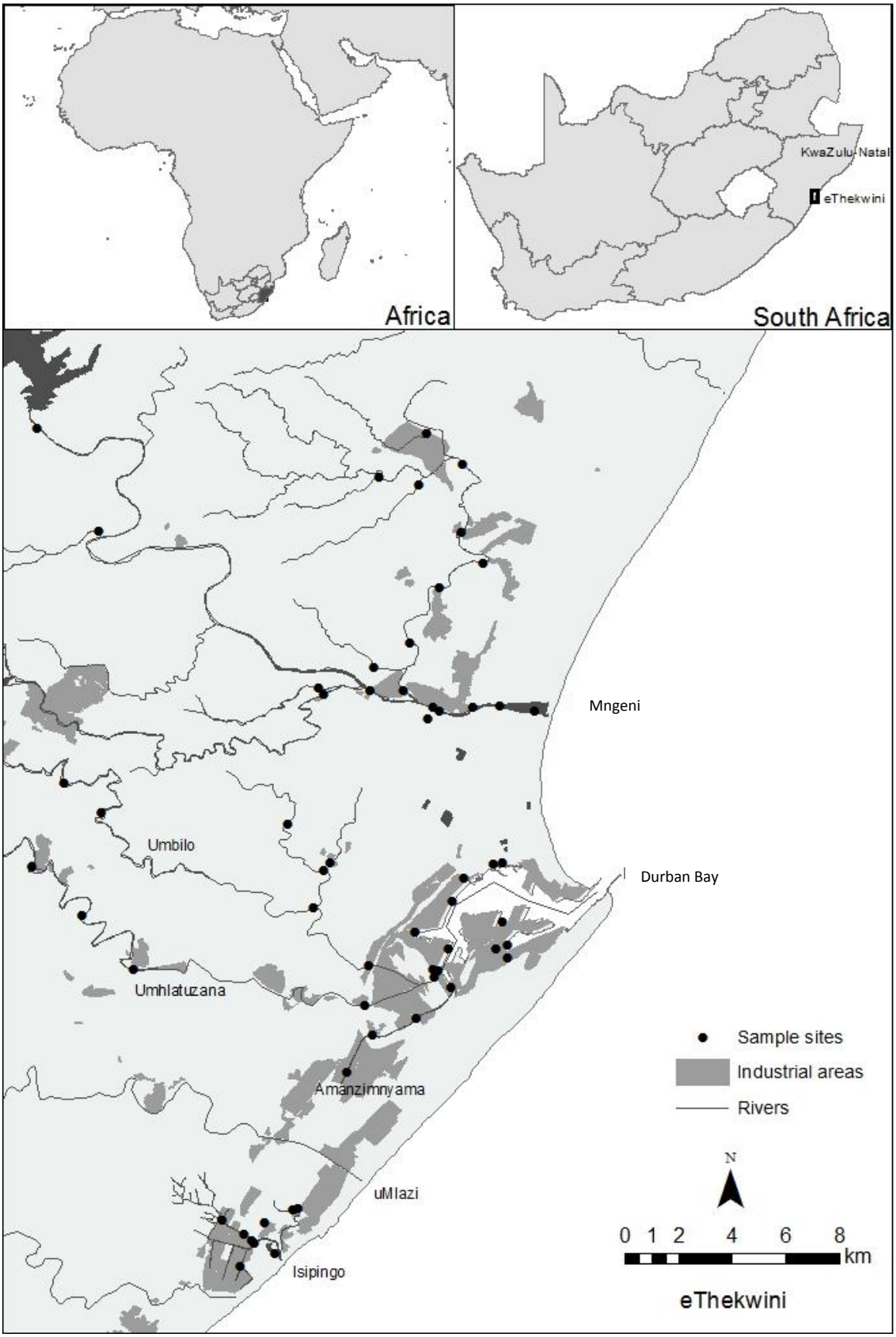


Figure 3.1. Map of the study area, showing the sites where sediment and soil was collected in the Durban Bay, Isipingo River and Mgeni River catchments.

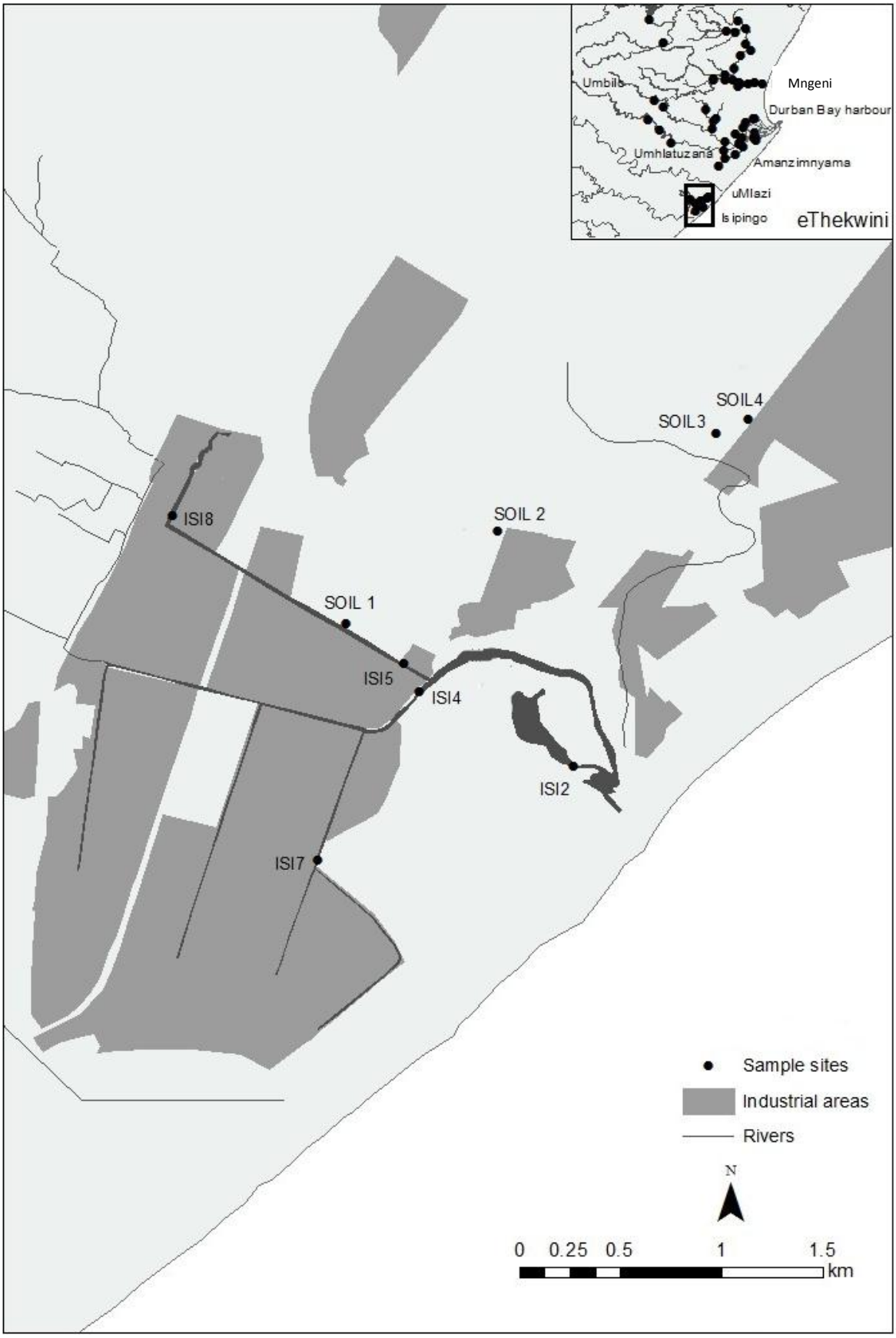


Figure 3.2. Map of the Isipingo River catchment showing sediment (ISI) and soil (SOIL) sampling sites.

Figure 3.3. Map of the Mngeni River catchment showing sediment (MNG) sampling sites.

Figure 3.4. Map showing sampling sites in the Umbilo River (UMB), Umhlatuzana River (UMH) and Amanzimnyama (AMA) River.

Figure 3.5. Map showing sampling sites in Durban Bay (DBAY) and Island View (IVC) and Bayhead (CAN 1) Canals. Also visible are parts of the Umbilo, Umhlatuzana and the Amanzimnyama Rivers.

3.2 Fieldwork

Sampling equipment included a Van Veen grab, stainless steel bowls, spoons, scoops and glass storage bottles. All equipment coming into contact with samples was scrubbed with phosphate free soap, rinsed with deionised water and sprayed with acetone followed by hexane. This was to remove both polar and apolar compounds and prevent contamination of the sample. Glass storage bottles had pre-cleaned foil liners in the lid. Cleaned equipment was stored in sealed Ziplock bags until use in the field.

The sediment samples were mainly collected from bridges that crossed the rivers of interest, as it proved to be a logistically simple way of collecting the samples. A vessel was used for collections in Durban Bay and the Mngeni River estuary. Sediment at each site was collected by means of a stainless steel Van Veen grab. Water overlaying sediment in the grab was drained through a bleeder hole, taking care not to pour out fine sediment. The sediment sample for each site consisted of a composite of three grabs, collected approximately 2 m apart. The sediment samples were mixed and material not representative of the sediment was removed, including large stones, leaves and plastic. The samples, per site, were transferred into three amber glass bottles, for biological, chemical and physical analysis. To prevent cross contamination between sites the equipment was scrubbed with distilled water and sprayed with acetone followed by hexane. The samples were stored in a cooler box on ice during sampling and immediately frozen once back at the laboratory.

3.3 Laboratory analyses

Sediment samples for PAH analysis were sent to Physis Environmental Labs Inc, California, USA, and samples for PCB analysis to Advanced Analytical, Australia. The PAH isomers and PCB congeners analysed are listed in Table 3.1.

Table 3.1. Suite of chemicals analysed in sediment samples. Bold type PCBs indicate those which are dl-PCBs, the non-bold type are ndl-PCBs.

PCB	PAH
PCB # 001	Naphthalene
PCB # 008	Biphenyl
PCB # 018	Acenaphthylene
PCB # 028	Acenaphthene
PCB # 044	Fluorene
PCB # 052	Anthracene
PCB # 066	1-Methylnaphthalene
PCB # 077	2-Methylnaphthalene
PCB # 101	Phenanthrene
PCB # 105	1-Methylphenanthrene
PCB # 118	2,3,5-Trimethylnaphthalene
PCB # 126	2,6-Dimethylnaphthalene

PCB	PAH
PCB # 128	Fluoranthene
PCB # 138	Pyrene
PCB # 153	Benz[a]anthracene
PCB # 169	Chrysene
PCB # 170	Benzo[b]fluoranthene
PCB # 180	Benzo[k]fluoranthene
PCB # 187	Benzo[e]pyrene
PCB # 195	Benzo[a]pyrene
PCB # 206	Perylene
PCB # 209	Benzo[g,h,i]perylene
	Indeno[1,2,3-c,d]pyrene
	Dibenz[a,h]anthracene
	Dibenzothiophene

3.3.1 Sample preparation

All implements to come into contact with sediment samples were cleaned as stated previously (US EPA, 1994). Solvents used were pesticide grade or higher. The sediment samples were freeze dried and ball milled to a fine powder at the CSIR in Stellenbosch. The samples were transferred into pre-cleaned glass jars with a foil lining in the lid, as discussed previously.

3.3.2 Sample analysis

Analyses for polycyclic aromatic hydrocarbons were performed by Physis Environmental Laboratories Inc. (United States of America) using USEPA method 8270C (USEPA, 1996). Analysis of procedural blanks, matrix spikes and sample replicates were used to check for laboratory contamination, accuracy and precision with each batch of 12 or less samples. Method extraction efficiency was evaluated by analysing Standard Reference Material (SRM) 1944 (National Institute of Standards and Technology). All chemicals were present in procedural blanks at concentrations below the method detection limit. With few exceptions surrogate recoveries from spiked blanks and matrix spikes fell within data quality objectives of 50 - 150%. Also with few exceptions the precision (relative percent difference) of analyses of laboratory blanks, spiked blanks, matrix spikes and certified reference material were below the data quality objective of 30%. Recoveries of isomers from SRM 1944 ranged between 75 - 125% (Table 3. 2).

Analyses for polychlorinated biphenyls were performed by Advanced Analytical (Australia). Analysis of procedural blanks, matrix spikes and sample replicates was used to check for laboratory contamination, accuracy and precision. All chemicals were present in procedural blanks at concentrations below the method detection limit. Surrogate recoveries from spiked blanks and matrix spikes fell within data quality objectives of 50–150%. Also with few exceptions the precision (relative percent difference) of analyses of laboratory blanks and matrix spikes were below the data quality objective of 30%. A Standard Reference Material was not analysed.

Table 3. 2. Recovery (%) of polycyclic aromatic hydrocarbon isomers from Standard Reference Material 1944 (National Institute of Standards and Testing).

	Replicate				
Compound	**1**	**2**	**3**	**4**	**Mean**
Anthracene	122	124	96	102	111
Benz[a]anthracene	79	76	75	76	77
Benzo[a]pyrene	77	75	80	75	77
Benzo[b]fluoranthene	76	75	77	76	76
Benzo[e]pyrene	75	75	75	75	75
Benzo[g,h,i]perylene	87	108	80	84	90
Benzo[k]fluoranthene	75	91	80	81	82
Chlordane-alpha	125	121	113	76	109
Chrysene	75	76	80	79	78
Dibenz[a,h]anthracene	125	76	84	80	91
Dibenzothiophene	125	113	123	91	113
Fluoranthene	75	79	125	98	94
Hexachlorobenzene	115	115	110	115	114
Indeno[1,2,3-c,d]pyrene	97	121	94	88	100
Naphthalene	112	79	105	96	98
Perylene	75	79	76	76	77
Phenanthrene	79	88	121	79	92
Pyrene	81	81	100	92	89

3.3.3 TEQs

PCB congeners and PAH isomers present a different toxicity, and in complex environmental mixtures it is thus difficult to quantify the risk posed to biota. In order to standardise and facilitate risk assessment, TEF values were developed. PCB congeners are compared to TCDD—the most toxic dioxin congener—ratios are derived by how similar the compounds are to TCDD. The closer the ratio is to one the more toxic the congener. Determination of the toxicity equivalency (TEQ) at a site was achieved by the summation of the product of the concentrations of individual PCB congeners and their respective TEF value, expressed as a TCDD-TEQ (Van den Berg *et al.*, 1998). In a similar method, PAH isomers were converted to a TEQ. Each isomer is assigned a ratio (TEF) by how similar it is to benzo(a)pyrene (BaP)—the most toxic PAH isomer. Again, the TEQ was calculated by the summation of the products of the TEF's and concentrations of isomers at each site.

3.4 Biological analysis

Duplicate samples for sediment at each site were extracted and their ability to elicit a response using the H4IIE reporter gene bioassay was determined. All laboratory equipment that came into contact with the samples was cleaned as mentioned before. All solvents used were high performance liquid chromatography (HPLC) grade (Burdick and Jackson).

3.4.1 Extraction and clean up

Sediment samples were air dried at North-West University (NWU), Potchefstroom, in stainless steel pans, protected from degradation by ultraviolet (UV) radiation. The samples were returned to the glass container and sent to CSIR Stellenbosch to be ball-milled to a fine consistency. The powdered sediment was stored in cleaned amber jars with foil-lined lids and returned to NWU.

The extraction process followed at NWU was similar to that followed by the laboratories responsible for the chemical analysis. The sediment was extracted using high temperature and pressure in an accelerated solvent extractor (ASE), using the Dionex 100. A mixture of 20 g of sediment and anhydrous sodium sulphate (Na_2SO_4, Merck) was placed into a 60 mℓ stainless steel extraction cylinder, between two 30 mm cellulose filters. A mixture of dichloromethane (DCM) and hexane (3:1) was passed into the cell at 100°C and 11 032 kPa. The system was set to a 10 minute static time a five minute heat. Analytes were purged from the cells into collection bottles with a 300 second purge with nitrogen gas. The extraction procedure was run twice per sample. Two separate extracts were prepared per site, one to target PCBs (persistent compounds) and the other, PAHs (less persistent compounds). The extracts were concentrated to dryness using a Turbo-Vap® II (Calpiper Lifesciences), where nitrogen gas was used to evaporate the solvents at 35°C.

An acid wash step was performed on those extracts from which PCBs were targeted. The extracts targeting the PAH compounds were not treated to this step. The sample extracts were washed with 98% sulphuric acid. The aim was to destroy most of the non-target compounds, by oxidation of compounds that are not chemically stable, such as PAHs (Behnisch *et al.*, 2001, Lamoree *et al.*, 2004). Evaporated samples were resuspended in 15 mℓ hexane within a separation funnel and repeatedly washed with an equal volume of concentrated sulphuric acid (H_2SO_4, Merck), tapping off the acid layer after approximately an hour, once the layers had separated, after approximately after an hour (Khim *et al.*, 1999a). The samples were washed with acid until the acidic layer was clear, but not exceeding six washes as this could break down target compounds. The extract was further washed with 15 mℓ of 20% sodium chloride (NaCl, Fluka), followed by 5% potassium hydroxide (KOH, Sigma-Aldrich), not exceeding a 15 minute separation time, and finally an

additional sodium chloride wash in order to remove any traces of potassium hydroxide. The samples were passed into Turbo-Vap flasks and evaporated to dryness.

It should be noted that this acid washed fraction would—apart from the targeted PCBs—also contain other persistent compounds such as the dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), and some low molecular weight PAHs. The non-acid-washed extract would also contain all of the persistent compounds as well as all the PAHs and other compounds able to act as ligands to the AhR (Behnisch *et al.*, 2001, Lamoree *et al.*, 2004).

Both types of extracts were run through gel permeation chromatography (GPC), to be able to select the fraction of the extract most likely to contain the PCBs or PAHs—similar to the method followed by the analytical laboratories. This clean-up step was also used to remove sulphur, which is toxic to the cells. Compounds are separated on the grounds of size selection using a Waters 717 plus auto-sampler, Waters 1515 isocratic HPLC pump, Waters dual λ absorbance detector, a Waters fraction collector III, and two Envirogel GPC Cleanup Columns (19 x 150 mm and 19 x 300 mm) connected in series. A GPC standard solution, containing corn oil (Sigma-Adrich), phthalate (PESTANAL), methoxychlor (PESTANAL), perylene (PESTANAL) and sulphur (PESTANAL), was used to calibrate the system and determine the collection time of the solution where PCBs are known to elute. A PAH standard (16 USEPA) was used to determine the PAH collection period.

The evaporated sample was reconstituted to 2 mℓ with DCM and passed through a 1 µℓ glass fibre filter (Sigma-Aldrich) into a recovery vial before injection into the GPC. The recovery vials were weighed before and after filling with the sample as well as after injection, in order to determine the mass fraction lost to the GPC process. The fraction that would contain the PCBs was collected from 9.5 to 20.5 minutes, while the fraction containing the PAHs was collected from 15.5 to 20.5 minutes. The system was set to a flow rate of 5 mℓ per minute for 30 minutes, with DCM as the mobile phase. The fraction of sample was collected in a Turbo-Vap flask and evaporated to dryness, as described above.

In order to further target the compounds of interest the samples were passed through Dual Layer Superclean silica Florisil columns (LC-Si, 2 g/2 g, Supelco), which trapped polar compounds and allowed apolar, target compounds through the column. The columns were conditioned with 6 mℓ hexane and followed by 6 mℓ of the sample, suspended in hexane. The column was washed with a 12 mℓ DCM and hexane mixture (1:1) and followed by 2 mℓ of DCM to elute the column, to ensure all the compounds of interest were removed. The sample was evaporated to dryness and reconstituted to 1 mℓ with hexane into an amber glass vial and stored at -80°C.

3.4.2 Bioassay

The H4IIE cells were donated by Professor John Giesy, currently at the University of Saskatoon, Canada. The tissue culture was maintained in a sterile environment, where all work areas were routinely cleaned with 70% ethanol. The cells were grown in tissue culture dishes (100/20 mm, LASEC) with Dulbecco's Modified Eagles Medium (DMEM) containing L-glutamine, 1 000 mg.ℓ^{-1} glucose without phenol red, and supplemented with 10% foetal bovine serum (FBS, Sigma-Aldrich). They were maintained in a humidified environment with 5% CO_2 and 95% air at 37°C within an incubator.

Prepared sample extracts had to be diluted to different concentrations in order to create a dose response curve. In this case a three times dilution factor was used. Known concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 120, 30, 7.5, 1.9, 0.5 and 0.1 pg TCDD per well were used as a reference compound to convert the data into bioassay equivalency (BEQ) values.

The assay takes five days to complete. On the first day the cells were trypisinised (0.25% trypsin and 0.1% versene ethylene-diamine-tetra-acetic-acid (EDTA) in Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) (Highveld Biological)) from the tissue culture dishes. A suspension of the cells were made using hormone-free FBS supplemented DMEM. This was done because the hormones could influence the response of the cells. A 96-microwell plate, with white walls and a clear base (LASEC), was seeded with a cell suspension with approximately 20 000 cells per well into the interior 60 wells, while the outer wells were filled with PBS (Sigma-Aldrich), to create a homogenous microclimate across all wells. The plates were incubated for 24 hours.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Sample 1			Sample 2			Hexane	TCDD 120 pg/well			
C		1:3			2:3			Hexane	TCDD 30 pg/well			
D		1:9			2:9			Hexane	TCDD 7.5 pg/well			
E		1:27			2:27			Blank	TCDD 1.88 pg/well			
F		1:81			2:87			Blank	TCDD 0.47 pg/well			
G		1:243			2:243			Blank	TCDD 0.12 pg/well			
H												

Figure 3.6. Layout of a 96-well plate used for the H4IIE bioassay. Grey wells indicate PBS containing wells.

The cells received 2.5 µl of the extract dilution, in triplicate, in descending concentration. TCDD was dosed in the same way. Each plate contained a solvent control (SC, hexane) and blank controls (BC) (Figure 3.6). The plates were incubated for 72 hours. A visual inspection of the cells was performed to determine the degree of confluency of the cells and, whether cytotoxicity occurred or bacteria had infected the wells. The media was removed and the cells washed with PBS containing added Ca^{2+} and Mg^{2+}. The added salts were a precaution to ensure absence of

limiting factors during the light producing reactions. Lysis buffer for mammalian cultured cells (Sigma-Aldrich) was added to cell-containing wells before the plates were frozen at -80°C, to ensure complete rupture of the cell membranes.

The plates were subsequently thawed and placed into a plate reading luminometer (Berthold multi-mode micro-plate reader, model-LB941). The thawing and reading of luminescence was performed in a darkened laboratory to prevent false excitation by UV rays. The injector automatically added luciferase assay reagent (LAR), containing 20 mM of tricine, 1.07 mM $Mg(CO_3)_4Mg(OH)_2.5H_2O$, 2.67 mM $MgSO_4.7H_2O$, 0.1 mM EDTA-disodium salt, 33.3 mM dithiothreitol, 270 µM coenzyme A, 530 µM ATP and 470 µM beetle luciferin (Villeneuve et al., 1999)**,** to each of the wells. Luciferin was digested by the luciferase, during which light was emitted. The luminescence of the wells was measured as relative light units (RLU). The amount of light produced is directly proportional to the amount of AhR agonists to which the cells were exposed (Giesy *et al.*, 2002, Hong *et al.*, 2012).

3.4.3 BEQ

The luminescence created by samples was expressed as a percentage of the maximum luminescence elicited by the positive reference compound and was labelled %TCDD-max. The dose response curves for the positive reference compound and samples were created with the logarithm of the TCDD concentration (or log of µℓ sample per well) on the x-axis and %TCDD-max on the y-axis. The effective concentration (or effective volume for the sample) was calculated for those concentrations (all volumes) responsible for the 20%, 50%, and 80% (EC 20–80) luminescence. The relative potency of a sample was calculated by dividing the samples EC by the corresponding EC of the reference. The REPs were back calculated to take the mass of the sediment initially extracted into consideration. Doing so resulted in a TCDD-eq.g^{-1} soil. TCDD-eq are commonly referred to as bio-assay equivalence or BEQ (Nieuwoudt et al., 2009, Villeneuve et al., 1999). A limit of detection (LOD) was calculated by determining the mean of all the EC_0 of the TCDD dose responses, the 95% confidence interval was added to this and converted to ngTCDD.g^{-1} LOD value (Nieuwoudt *et al.*, 2009, Thomsen *et al.*, 2003).

3.4.4 Cell viability through MTT

In order to determine viability of the cells once they had been exposed to a sample, a hydrogen accepter 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay was used. This assay serves to prove that where the luminescence assay had “below level of detection” results or low responses, it was not a result of cytotoxicity, but rather low concentrations of AhR agonists.

The methods for the MTT are identical to that of the reporter gene bioassay up until day five, with the exception that the cells were seeded into clear 96-well microplates. After washing the cells with PBS, the cells received MTT solution (0.5 mg.m$ℓ^{-1}$ MTT in non-supplemented DMEM), prepared

that day. This step and subsequent steps were performed in a darkened room. The plates were incubated for 30 minutes under normal growing conditions. The living cells metabolised the yellow MTT solution to form blue formazan crystals. The MTT solution was removed from the wells and dimethyl sulphoxide (DMSO, Sigma-Aldrich) was added to the cell containing wells, to dissolve the formazan crystals. The plates were left at room temperature for 30 minutes. The optical density (OD) of the solutions in the well was measured at 560 nm (Berthold multi-mode micro plate reader) (Vistica *et al.*, 1991)**.**

To determine the viability of the cells, the ODs from the sample containing wells were divided by the mean of the solvent containing wells. This was expressed as a percentage value. Low cell viability could result in the responses of the cells in the reporter gene assay to be reduced.

3.4.5 Cell viability through xCELLigence

The MTT assay is labour intensive and is only able to measure the end point of the cell's fitness at the end of the exposure. Due to the assay utilising optics based detection and absorbance, there may be distortions and compound interferences (Urcan *et al.*, 2010). It was decided to utilise an automated system, which has high reproducibility and is capable of determining the real-time physiological state of the cells, thereby allowing monitoring of proliferation, viability and cytotoxicity of the cells, before and during exposure to the extracted sample. This method was used to monitor any changes in the cell growth during the exposure period, which would be lost as an endpoint reading, such as in the MTT assay (Urcan *et al.*, 2010).

This technique utilises the Real-Time Cell Analyser Single Plate (RTCA SP®) (Roche) developed by biosensor technologies (Quereda *et al.*, 2010). The system consists of a 96-well microtiter plate, on with the bottom of each well, being 80% covered by incorporated gold sensor arrays. The plate fits inside the RTCA SP® station, inside the incubator, in the same conditions as previously mentioned for cell growth. The station is connected to the RTCA analyser and a computer loaded with the RTCA integrated software, by cable, which is outside the incubator (Urcan *et al.*, 2010, Zhu *et al.*, 2006).

The sensors allow the contents of the wells to be monitored by measuring the impedance of the electrodes. Voltage is applied, approximately 20 mV, and the impedance between electrodes is measured. These data were represented as cell index (CI), which is calculated by the difference between the impedance at a particular point in time and the impedance at the start point, divided by 15 (Urcan *et al.*, 2010, Wu *et al.*, 2010). The impedance measured depends on electrode geometry, ion concentration in the well and whether cells have attached to the electrodes in the well (Urcan *et al.*, 2010, Zhu *et al.*, 2006). A high CI value represents a great number of healthy

attached cells, whereas a low CI value corresponds to cell death, cytotoxicity and morphological changes (Urcan *et al.*, 2010, Quereda *et al.*, 2010)**.**

A background reading was obtained by placing 100 µℓ of supplemented media (DMEM) into each of the wells, and placed into the station, and set to do 6, 1 minute sweeps (Urcan *et al.*, 2010, Quereda *et al.*, 2010)**.** The cells were seeded at 80 000 cells.mℓ$^{-1}$, as in the luminescence assay, in a 96 well E-plate. Three wells contained only supplemented media, and served as a negative control. Proliferation was monitored for a 24 hour period, with a reading interval of 15 minutes. After the 24 hour period, the cells were dosed in triplicate with the most concentrated sample extract. A SC and BC were also present on every plate. The xCELLigence was left to monitor the cells for a 72 hour exposure period, the same period of exposure as the luminescence and MTT assay.

As an additional measure of quality control and viability a MTT assay was conducted using the xCELLigence plate, once the 72 hour exposure was completed (Zhu *et al.*, 2006). The method followed for this was identical for the ordinary MTT.

Data from the xCELLigence was exported into an Microsoft Office Excel spread sheet. The data was expressed as a CI per time period per individual well—these were also graphically represented on the RTCA software. Triplicate wells, per sample exposure were analysed. A mean, standard deviation and coefficient of variation (CV) of the replicate wells was determined. If the CV was greater than 15%, the well causing the high CV was removed for further analysis. To determine the viability of the cells the individual sample exposed well's CI was divided by the CI of the SC cells and multiplied by 100, expressed as percentage viability (Quereda *et al.*, 2010, Wu *et al.*, 2010, Zhu *et al.*, 2006).

3.5 Physical sediment analysis

3.5.1 Grain size composition

PAHs and PCBs preferentially adsorb to finer grain sizes, mostly to the mud fraction (Wang *et al.*, 2001), and therefore, grain size analysis was performed. The grain size of the sediment was determined by means of dry and wet sieving into seven grain size classes, mud (<0.063 mm), very fine-grain (VFG) sand (0.063–0.125 mm), fine-grained (FG) sand (0.125–0.250 mm), medium-grained (MG) sand (0.25–0.50 mm), course-grained (CG) sand (0.5–1.0 mm), very course-gained (VCG) sand (1.0–2.0 mm), and gravel (>2.0 mm). The contribution of each grain size class is expressed as a percentage of the bulk weight of the dried sediment. Sand is an aggregate of very fine-grained, fine-grained, medium-grained, coarse-grained and very coarse-grained sand.

3.5.2 Total organic carbon (TOC)

PAHs and PCBs also have a strong tendency to adsorb to organic matter within the sediment. This could influence the abundance of PAHs or PCBs that have accumulated at a sample site. Approximately 1–2 mg of dried sediment was weighed into silver weighing boats. A small volume of 10% hydrochloric acid (HCl) was added to the sediment and continued until foaming ceased, in order to remove the inorganic carbon. Samples were subsequently oven dried overnight at 65°C. The weighing boats were crimped closed and total organic carbon and total organic nitrogen were measured using an Exeter CHN Model 440 analyser at 985°C. In order to determine the recovery CRM BCCS-1 was used. Blanks and the CRM were analysed with every batch of 10 samples. The method detection limits were 0.03% for total organic carbon and 0.014% for total organic nitrogen. The total organic carbon and nitrogen were expressed as a fraction of bulk sediment dry weight (USEPA, 2004).

3.6 Data analysis

Statistica 11 (Statsoft) software was used for the statistical analysis of data, at the $\alpha = 0.05$ level of confidence. Normality was checked using Kolmogorov-Smirnov test—if the data was not normally distributed a Box-Cox transformation was used to approximate a normal distribution. Thereafter, parametric or non-parametric (if data was still not normally distributed) testing was used. The Spearman rank R was used for non-parametric correlations and Kruskal-Wallis ANOVA for comparisons between treatments.

Principle component analysis (PCA) was used to aid in making underlying similarities or characteristics in the data clearer. The individual congeners or isomers were expressed as a proportion of the sum of all the congeners or isomers at that site. The compositional data was log-ratio transformed by dividing each proportion by the geometric mean of the sample. PCA was performed using Canoco 4.5 for Windows. The PCA results were exported to Excel and bi-plots for factors one to three were prepared showing both the factor loadings of the congeners and the factor scores of the sites, using Excel.

4 Results and discussion[1]

4.1 Results of chemical analysis

4.1.1 PAHs

In this study, a distinction is made between the 16 so-called USEPA priority PAHs (ΣPAH_{16}), characterised so due to their high toxicity potential (Bojes and Pope, 2007), and the full suite of PAHs analysed—23 isomers (ΣPAH_{23}) (Table 4.1). Where isomer concentrations were lower than the method detection limit (MDL) a surrogate value of one half the detection limit was used for calculations and the investigation of relationships between PAHs and the properties of sediment. According to Helsel (2005), there are a number of ways to treat non-detects. Using the detection limit would have presented inflated mean levels rather than more realistic values. Helsel (2005) showed that using half the detection limit yields approximately the same descriptive statistics as the maximum likelihood and Kaplan-Meier estimates. In the data, a large amount of data was below the detection limit—so that neither the maximum likelihood nor Kaplan-Meier estimates could be determined; thus half the detection limit was substituted for data below the detection limit.

Table 4.1. PAH concentrations (ng.g^{-1} dw) in sediment and soil samples, indicating the sum of low and high molecular weight isomers (ΣLMW and ΣHMW) and total PAH (ΣPAH) concentrations, distinguishing between the 16 USEPA priority PAHs and the 23 PAHs analysed in this study.

Site	ΣLMW		ΣHMW		ΣPAH	
	ΣLMW_{16}	ΣLMW_{23}	ΣHMW_{16}	ΣHMW_{23}	ΣPAH_{16}	ΣPAH_{23}
AMA 1	358.1	904.8	671.0	702.5	1 029.1	1 607.3
AMA 2	350.0	745.3	378.1	405.7	728.1	1 151.0
AMA 3	3.0	32.9	135.2	144.8	138.2	177.7
CAN 1	1 387.6	3 235.6	3 798.7	4 243.9	5 186.3	7 479.5
DBAY 1	178.5	377.6	345.1	373.7	523.6	751.3
DBAY 2	156.0	367.1	195.2	216.6	351.2	583.7
DBAY 3	1 003.8	1 356.5	3 693.9	3 855.3	4 697.7	5 211.8
DBAY 4	322.1	489.7	942.9	985.4	1 265.0	1 475.1
DBAY 5	457.7	676.1	2 302.5	2 419.3	2 760.2	3 095.4
DBAY 6	177.1	324.5	665.9	788.9	843.0	1 113.4
DBAY 7	444.7	709.2	1 852.2	2 078.8	2 296.9	2 788.0
DBAY 8	163.8	320.6	575.5	713.6	739.3	1 034.2
DBAY 9	167.6	242.5	1 041.7	1 162.1	1 209.3	1 404.6
DBAY10	173.9	466.3	573.8	606.9	747.7	1 073.2
ISI 2	103.0	236.7	208.9	240.4	311.9	477.1
ISI 4	70.7	169.3	185.2	209.4	255.9	378.7
ISI 5	339.1	464.8	1 715.9	1 935.2	2 055.0	2 400.0
ISI 7	139.9	242.3	558.0	608.0	697.9	850.3
ISI 8	312.9	599.6	1 055.7	1 178.6	1 368.6	1 778.2
IVC 1	883.6	1 750.4	3 016.9	3 210.0	3 900.5	4 960.4
IVC 2	172.8	495.9	279.8	297.3	452.6	793.2
MNG 1	39.6	78.5	100.5	111.5	140.1	190.0
MNG 2	46.9	80.5	136.1	166.1	183.0	246.6
MNG 3	40.0	65.4	83.7	92.2	123.7	157.6
MNG 4	47.2	84.2	145.7	174.9	192.9	259.1

[1] Please see foldout table at the end of the dissertation.

Site	ΣLMW		ΣHMW		ΣPAH	
	ΣLMW_{16}	ΣLMW_{23}	ΣHMW_{16}	ΣHMW_{23}	ΣPAH_{16}	ΣPAH_{23}
MNG 5	19.6	33.7	24.7	30.9	44.3	64.6
MNG 6	96.7	180.5	444.7	488.4	541.4	668.9
MNG 7	146.0	276.8	346.6	619.9	492.6	896.7
MNG 8	30.8	50.5	58.7	67.6	89.5	118.1
MNG 9	19.6	33.0	19.8	22.5	39.4	55.5
MNG10	19.3	32.0	14.3	17.0	33.6	49.0
MNG 11	171.7	270.3	490.1	535.3	661.8	805.6
MNG 12	69.1	117.4	155.5	191.8	224.6	309.2
MNG 13	48.0	89.9	87.0	107.5	135.0	197.4
MNG 14	34.6	56.5	77.1	88.2	111.7	144.7
MNG 15	41.0	142.9	39.7	42.9	80.7	185.8
MNG 17	40.5	81.4	55.5	67.7	96.0	149.1
MNG 18	597.4	2 425.6	1 301.7	1 341.5	1 899.1	3 767.1
MNG 19	183.7	273.6	338.5	378.8	522.2	652.4
MNG 20	27.1	48.8	45.0	53.9	72.1	102.7
MNG 21	26.0	40.9	38.9	44.4	64.9	85.3
MNG 22	19.4	31.2	15.1	16.1	34.5	47.3
SOIL 1	9.1	25.6	5.0	6.0	14.1	31.6
SOIL 2	108.0	236.6	215.7	231.2	323.7	467.8
SOIL 3	81.9	148.0	272.9	294.7	354.8	442.7
SOIL 4	735.0	1 719.1	1 496.8	1 590.5	2 231.8	3 309.6
UMB 1	75.2	189.4	111.1	120.7	186.3	310.1
UMB 2	43.2	69.7	105.1	117.5	148.3	187.2
UMB 3	27.3	48.2	38.7	42.5	66.0	90.7
UMB 4	3.0	6.5	183.8	200.1	186.8	206.6
UMB 5	3.0	6.5	103.6	112.3	106.6	118.8
UMB 7	3.0	6.5	83.9	92.2	86.9	98.7
UMB 8	54.5	93.9	223.2	236.4	277.7	330.3
UMB/UMH	37.7	69.5	83.3	95.6	121.0	165.1
UMH 1	29.0	49.7	60.3	64.9	89.3	114.6
UMH 3	18.8	32.1	28.5	34.8	47.3	66.9
UMH 5	37.6	72.1	66.9	76.0	104.5	148.1
UMH 6	33.1	47.7	25.6	27.6	58.7	75.3

The mean (± standard deviation) concentration for the ΣLMW_{16} PAHs was 179.8 ± 268.2 $ng.g^{-1}$. The lowest concentration was 3 $ng.g^{-1}$ at sites UMB 4, 5, 7 and AMA 3—this was due to concentrations at these sites being < MDL. The highest concentration was at site CAN 1, at 1 987.6 $ng.g^{-1}$. The mean (± standard deviation) concentration for ΣLMW_{23} PAHs was 371.1 ± 607.2 $ng.g^{-1}$. The lowest ΣLMW_{23} PAHs concentration was 6.5 $ng.g^{-1}$, at the same sites mentioned for the lowest ΣLMW_{16} PAHs concentrations, and the highest was again at site CAN 1, at 3 235.6 $ng.g^{-1}$. The mean (± standard deviation) concentration for the ΣHMW_{16} PAHs was 539.9 ± 682.0 $ng.g^{-1}$. The lowest concentration was at SOIL 1, at 5.0 $ng.g^{-1}$, and the highest again at site CAN 1, at 3 798.7 $ng.g^{-1}$. The ΣHMW_{16} PAHs had a mean (± standard deviation) concentration of 592.7 ± 929.1 $ng.g^{-1}$. Site SOIL 1 again had the lowest concentration of 6 $ng.g^{-1}$, and the highest concentration was again at site CAN 1, at 4 243.9 $ng.g^{-1}$. ΣPAH_{16} had a mean (± standard deviation) concentration of 719.7 ± 1 119.7 $ng.g^{-1}$. The site with the lowest concentration was SOIL 1, at 14.1 $ng.g^{-1}$, while the highest concentration was site CAN 1, at 5 186.3 $ng.g^{-1}$. The mean (± standard deviation) ΣPAH_{23} concentration was 963.8 ± 1 469 $ng.g^{-1}$. The lowest concentration was 31.6 $ng.g^{-1}$, at site SOIL 1, and the highest concentration was at site CAN 1, at

7 479.5 ng.g^{-1}. Thus, site CAN 1 had the highest concentrations and site SOIL 1 the lowest concentrations (Table 4.1).

4.1.2 PCBs

The PCBs were separated into two categories (Table 4.2), namely dl-PCBs—these are capable of interacting with the AhR and have adverse health implications (Giesy and Kannan, 1998)—and the ndl-PCBs which also might have adverse health effects but through other pathways. Where congener concentrations were lower than the MDL a surrogate value of zero was used for calculations and the investigation of relationships between PCBs and the properties of sediment. This is because congener concentrations at many sites were < MDL and replacing these with a value of one half the MDL would have skewed the results.

Table 4.2. PCB concentrations (ng.g^{-1} dw) in sediment and soil samples, indicating the sum of PCB congeners (ΣPCBs) and the sum of dioxin like (Σdl-PCBs) and non-dioxin like PCBs (Σndl-PCBs).

Site	ΣPCB	Σdl-PCBs	Σndl-PCBs
AMA 1	13.41	5.16	8.25
AMA 2	0.00	0.00	0.00
AMA 3	2.00	0.00	2.00
CAN 1	10.08	4.03	6.05
DBAY 1	26.00	8.00	18.00
DBAY 2	20.00	6.00	14.00
DBAY 3	113.83	39.79	74.04
DBAY 4	28.93	10.99	17.94
DBAY 5	8.00	1.50	6.50
DBAY 6	25.00	8.00	17.00
DBAY 7	105.67	35.54	70.13
DBAY 8	25.38	9.85	15.53
DBAY 9	6.00	1.50	4.50
DBAY10	34.56	14.21	20.35
ISI 2	1.00	0.00	1.00
ISI 4	71.27	27.75	43.52
ISI 5	6.96	2.61	4.35
ISI 7	0.00	0.00	0.00
ISI 8	57.31	19.46	37.85
IVC 1	21.19	7.98	13.21
IVC 2	0.00	0.00	0.00
MNG 1	0.00	0.00	0.00
MNG 2	2.00	0.00	2.00
MNG 3	0.00	0.00	0.00
MNG 4	2.00	0.00	2.00
MNG 5	0.00	0.00	0.00
MNG 6	1.08	0.00	1.08
MNG 7	13.51	0.00	13.51
MNG 8	0.00	0.00	0.00
MNG 9	0.00	0.00	0.00
MNG10	0.00	0.00	0.00
MNG 11	6.00	1.00	5.00
MNG 12	0.00	0.00	0.00
MNG 13	0.00	0.00	0.00
MNG 14	0.00	0.00	0.00
MNG 15	0.00	0.00	0.00
MNG 17	0.00	0.00	0.00
MNG 18	5.00	3.00	2.00
MNG 19	1.25	0.00	1.25
MNG 20	0.00	0.00	0.00
MNG 21	0.00	0.00	0.00
MNG 22	0.00	0.00	0.00
SOIL 1	0.00	0.00	0.00

Site	ΣPCB	Σdl-PCBs	Σndl-PCBs
SOIL 2	0.00	0.00	0.00
SOIL 3	4.00	0.00	4.00
SOIL 4	28.26	13.86	14.40
UMB 1	0.00	0.00	0.00
UMB 2	0.00	0.00	0.00
UMB 3	0.00	0.00	0.00
UMB 4	1.00	0.00	1.00
UMB 5	0.00	0.00	0.00
UMB 7	0.00	0.00	0.00
UMB 8	0.00	0.00	0.00
UMB/UMH	0.00	0.00	0.00
UMH 1	0.00	0.00	0.00
UMH 3	0.00	0.00	0.00
UMH 5	0.00	0.00	0.00
UMH 6	0.00	0.00	0.00

The mean (± standard deviation) concentration of the ΣPCBs was 11.05 ± 23.6 ng.g^{-1}, the Σdl-PCBs was 2.8 ± 8.44 ng.g^{-1}, and the Σndl-PCBs was 7.25± 15.26 ng.g^{-1} (Table 4.2). The highest concentration across all three PCB categories was at site DBAY 3 in Durban Bay, at a concentration of 113.83 ng.g^{-1} for the ΣPCBs. The highest Σdl-PCBs was 39.79 ng.g^{-1} and the Σndl-PCBs was 74.04 ng.g^{-1}.

4.2 Sediment characteristics

The physical properties of the sediment and soil sampled is summarised in Table 4.3. PAHs and PCBs tend to preferentially adsorb to mud and organic carbon matter (Barra *et al.*, 2006, Cardellicchio *et al.*, 2007). Some studies report a positive correlation between organic chemical concentrations and the mud and/or TOC content of the sediment (Hiller *et al.*, 2009, Pait *et al.*, 2008, Vane *et al.*, 2007b, Vane *et al.*, 2007a). However, other studies have reported no such correlation (Kilemade *et al.*, 2004, Tao *et al.*, 2010)**.** The correlation between ΣPAH and %TOC and %mud for this study was investigated. The same relationship was explored between ΣPCB and %mud and %TOC respectively. A value of one half the MDL was used to represent ΣPAHs < MDL, whereas < MDL for PCBs were treated as zero.

Table 4.3. Grain size composition and total organic carbon (TOC) content of the sediment and soil sampled.

Site	Gravel (%G)	Very coarse grained sand (%VCG)	Coarse grained sand (%CG)	Medium grained sand (%MG)	Fine grained sand (%FG)	Very fine grained sand (%VFG)	Mud (%)	TOC (%)
AMA 1	12.77	2.93	3.99	21.79	45.40	5.21	7.91	1.45
AMA 2	0.00	5.53	12.38	41.22	32.20	3.49	5.18	2.34
AMA 3	3.43	5.43	11.57	50.42	27.97	0.71	0.47	0.6
CAN 1	5.87	2.97	4.86	30.68	40.21	4.23	11.18	6.84
DBAY 1	0.10	0.17	0.34	11.20	41.80	4.16	42.23	1.97
DBAY 2	1.38	1.26	4.25	24.69	33.65	5.08	29.69	0.96
DBAY 3	2.50	3.78	7.26	15.12	31.00	3.89	36.45	5.33
DBAY 4	4.04	5.69	9.48	39.24	35.07	2.26	4.22	1.35
DBAY 5	13.10	16.61	28.91	26.06	12.08	1.17	2.07	1.22
DBAY 6	0.00	0.12	0.40	1.33	14.32	5.31	78.52	2.45
DBAY 7	12.13	18.37	21.65	17.18	15.06	2.27	13.34	3.17
DBAY 8	1.72	2.65	7.05	13.96	21.48	9.36	43.78	2.46

Site	Gravel (%G)	Very coarse grained sand (%VCG)	Coarse grained sand (%CG)	Medium grained sand (%MG)	Fine grained sand (%FG)	Very fine grained sand (%VFG)	Mud (%)	TOC (%)
DBAY 9	0.00	0.06	0.13	0.89	5.08	12.01	81.83	2.97
DBAY10	0.00	0.00	0.33	1.97	9.86	2.71	85.13	3.11
ISI 2	1.58	7.29	23.27	26.31	18.98	2.65	19.92	2.5
ISI 4	0.00	0.00	1.79	6.32	17.58	3.85	70.46	6.36
ISI 5	0.00	0.97	5.81	34.08	38.87	4.54	15.73	2.36
ISI 7	0.00	1.17	6.21	44.87	40.94	2.25	4.56	1.29
ISI 8	0.37	0.43	1.58	12.19	19.74	6.95	58.74	4.07
IVC 1	7.10	3.70	8.28	59.73	18.36	0.93	1.90	7.07
IVC 2	0.13	0.45	1.70	22.92	22.02	7.03	45.75	2.03
MNG 1	0.22	0.76	2.76	47.89	34.87	5.45	8.05	1.95
MNG 2	0.00	0.15	0.97	49.33	26.57	3.52	19.46	1.13
MNG 3	0.11	0.34	1.85	46.11	43.42	5.33	2.84	0.4
MNG 4	4.42	15.83	40.13	18.90	3.69	0.55	16.48	1.71
MNG 5	0.00	12.95	57.22	25.01	3.00	0.34	1.48	0.25
MNG 6	0.03	0.34	5.27	43.56	28.21	2.37	20.22	2.75
MNG 7	1.10	1.42	2.41	1.84	2.73	3.88	86.62	2.99
MNG 8	3.83	6.23	20.15	52.64	11.69	3.44	2.02	0.32
MNG 9	0.32	5.58	56.85	35.42	1.64	0.16	0.03	0.16
MNG10	0.00	10.55	44.76	34.72	6.33	2.15	1.49	0.19
MNG 11	2.20	1.72	6.97	38.91	21.16	5.56	23.48	2.6
MNG 12	0.00	0.12	2.42	18.24	20.81	6.05	52.36	1.84
MNG 13	0.79	2.42	13.28	45.04	15.06	4.27	19.14	0.91
MNG 14	1.43	1.43	10.59	52.39	29.37	1.89	2.90	0.38
MNG 15	2.60	3.92	20.13	55.68	11.94	3.42	2.31	0.37
MNG 17	0.00	0.68	19.16	45.09	22.23	6.42	6.42	0.4
MNG 18	0.00	0.06	0.12	0.70	7.78	12.14	79.20	5.89
MNG 19	5.07	4.19	15.72	19.06	7.84	4.29	43.83	3.76
MNG 20	0.52	1.34	9.11	27.65	19.11	8.90	33.37	0.76
MNG 21	0.18	0.95	7.26	52.83	19.09	4.94	14.75	1.01
MNG 22	0.05	7.30	34.28	42.58	14.16	1.18	0.45	0.11
SOIL 1	0.11	0.18	0.46	9.47	21.42	2.42	65.94	2.08
SOIL 2	5.05	3.11	4.11	17.31	34.62	8.57	27.23	6.75
SOIL 3	1.84	2.27	8.16	28.90	20.57	5.43	32.83	2.62
SOIL 4	0.00	0.00	0.24	1.31	5.26	4.54	88.65	9.46
UMB 1	1.25	2.65	16.65	58.26	16.77	3.26	1.16	0.34
UMB 2	1.58	4.61	24.55	48.95	13.16	3.21	3.94	0.67
UMB 3	0.07	1.75	26.80	54.34	10.92	2.22	3.90	0.61
UMB 4	0.00	9.03	24.62	33.55	22.75	4.03	6.02	0.79
UMB 5	0.00	6.60	31.44	44.93	13.28	2.44	1.31	0.33
UMB 7	1.12	5.64	29.50	53.70	8.45	0.94	0.65	0.18
UMB 8	14.99	17.13	36.27	29.60	1.56	0.07	0.38	1.41
UMB/UMH	0.64	6.51	34.50	44.38	10.62	0.77	2.58	0.54
UMH 1	0.32	2.78	7.60	63.08	23.17	1.86	1.19	0.22
UMH 3	0.24	1.61	26.45	60.75	9.09	0.86	1.00	0.28
UMH 5	0.00	3.09	21.38	56.83	11.53	2.22	4.95	1.03
UMH 6	5.02	15.57	29.24	34.17	9.49	2.67	3.84	0.27

The ΣPAHs correlated significantly with the %TOC ($r = 0.81$ and $p < 0.05$). PAH concentrations at five sites deviated greatly from the relationship for the bulk of the sites (Figure 4.1). Three of these sites—CAN 1, DBAY 3 and DBAY 5—had high PAH concentrations despite a low %TOC, to which the PAHs could partition. This indicates these sites were considerably more polluted than other sites. Sites ISI 4 and SOIL 2 had low PAH concentrations. When the outliers were removed the correlation was stronger, at $r = 0.86$ ($p < 0.05$).

When the ΣPAH concentrations were correlated to %mud, the relationship was statistically significant but weak ($r = 0.52$; $p < 0.05$) (Figure 4.2). In this instance, three sites were outliers.

Again, sites CAN 1 and DBAY 3, and additionally IVC 1, seemed to be more contaminated than other sites because the ΣPAH concentration was high while the %mud was low. When the outlier sites were removed, the correlation coefficient improved slightly to $r = 0.58$ ($p < 0.05$). The relationship between the ΣPAH and %TOC was thus stronger than the relationship between the ΣPAH and %mud, indicating the PAHs were preferentially associating with TOC.

The ΣPCB concentrations correlated significantly with %TOC ($r = 0.70$, $p < 0.05$). Sites DBAY 3 and 7, which had the highest PCB concentrations of all the sites (Table 4.2), also contained high concentrations of PCBs in comparison to the %TOC, and were above the 95% predictive limit (Figure 4.3). However, when these outliers were removed the correlation coefficient was reduced to $r = 0.68$ ($p < 0.05$).

When ΣPCB and %mud were correlated there was a positive relationship with a correlation coefficient of $r = 0.55$ ($p < 0.05$). DBAY 3 and 7 were again outliers, having a higher PCB concentration compared to other sites based on the %mud (Figure 4.4). When these outliers were removed the correlation coefficient increased slightly to $r = 0.56$ ($p < 0.05$).

The general trend among both PAHs and PCBs in comparison to the %TOC and %mud was that high concentrations of these chemicals were detected in sediment or soil with a high %mud or %TOC. %TOC was found to be more strongly correlated to PAH and PCB concentrations, implying these chemicals were preferentially associating with this fraction of the sediment.

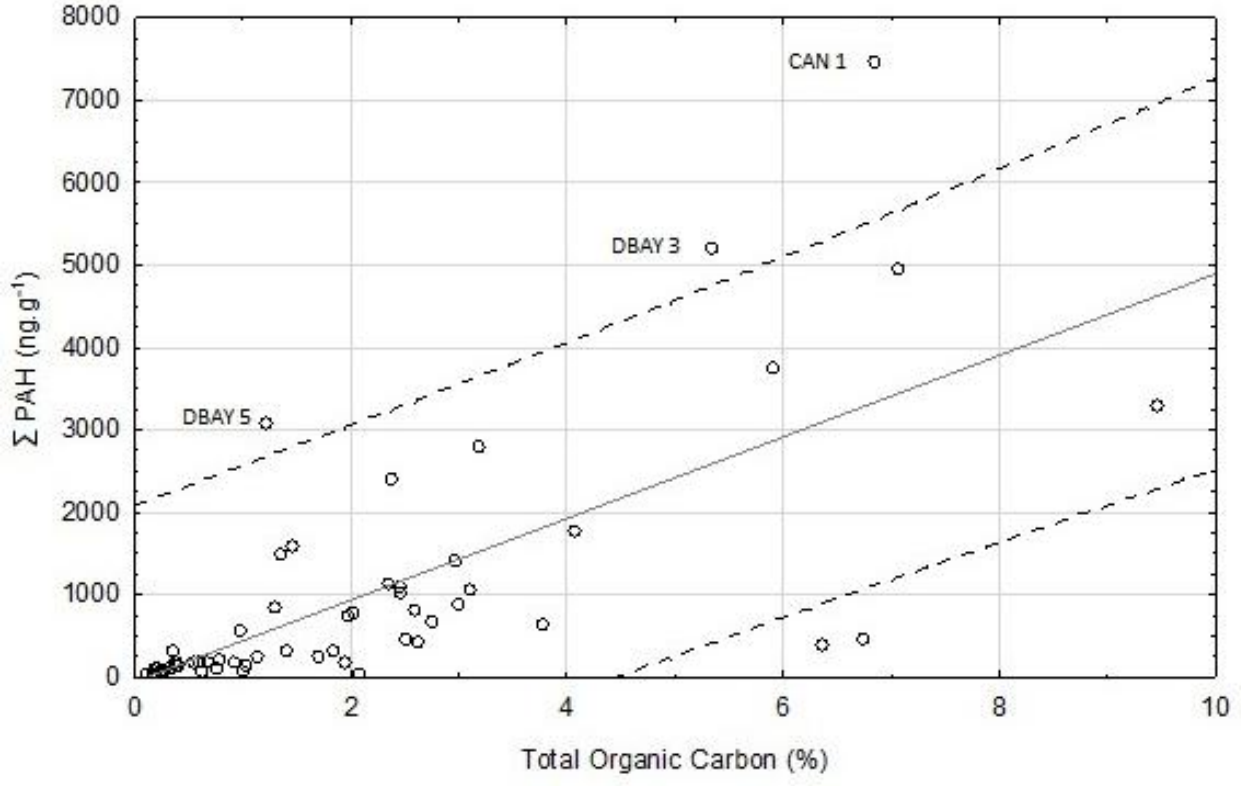


Figure 4.1. Correlation between ΣPAH_{23} concentration and %TOC, indicating the 95% prediction limits. r = 0.81, p < 0.05.

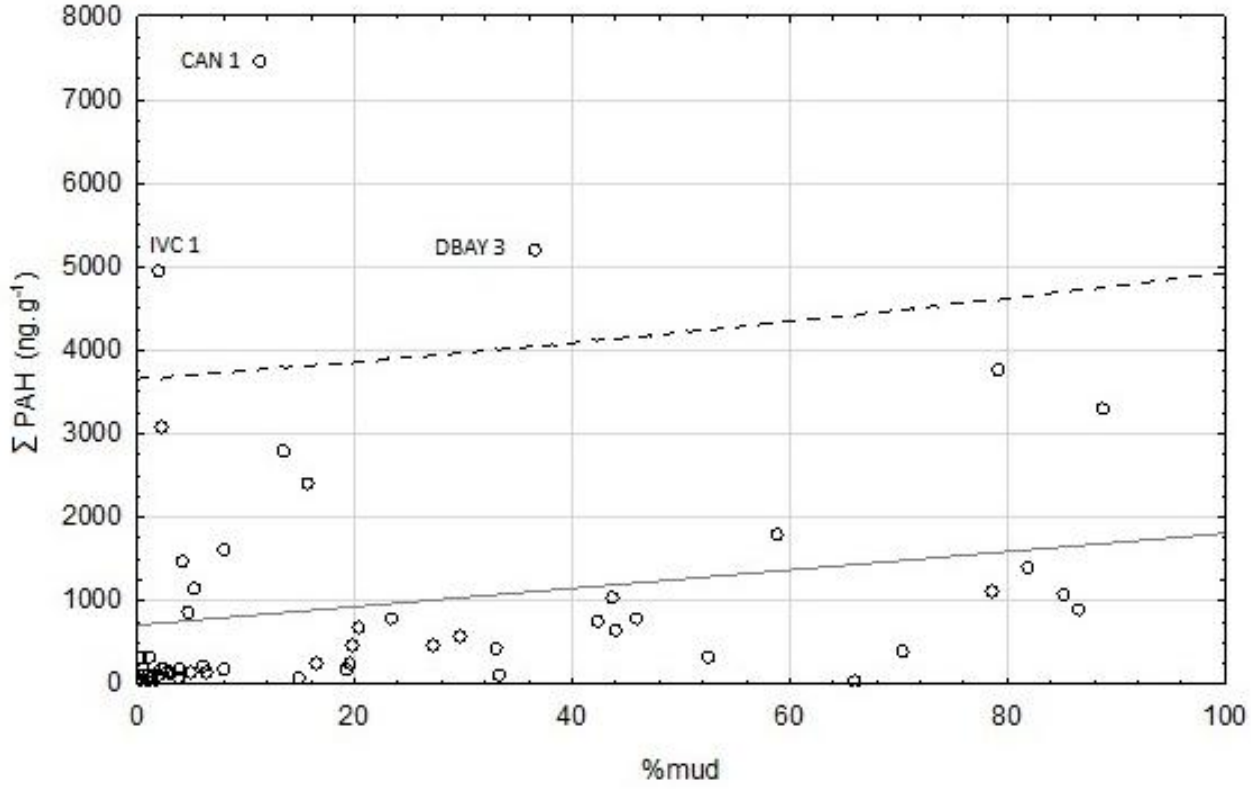


Figure 4.2. Correlation between ΣPAH_{23} concentrations and %mud, indicating the 95% prediction limits. r = 0.52, p < 0.05.

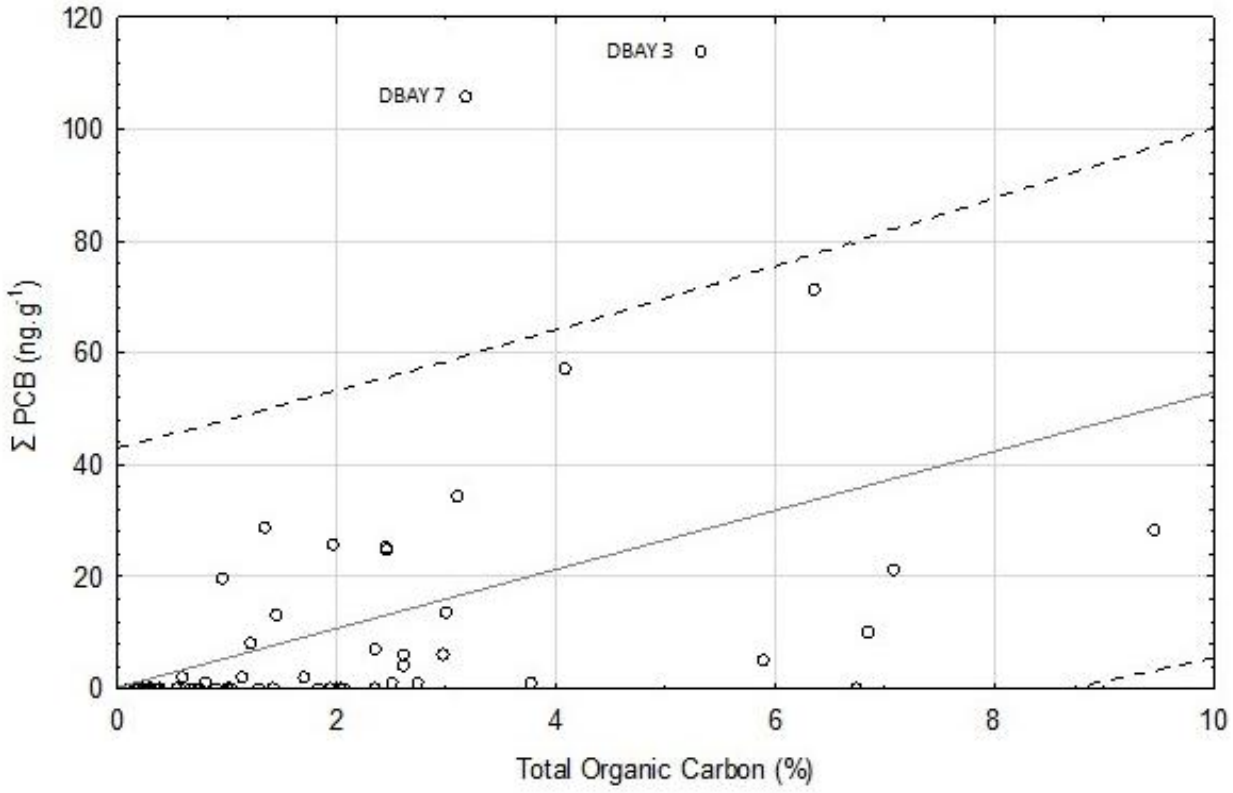


Figure 4.3. Correlation between ΣPCB concentrations and %TOC, indicating the 95% prediction limits. r = 0.70, p < 0.05.

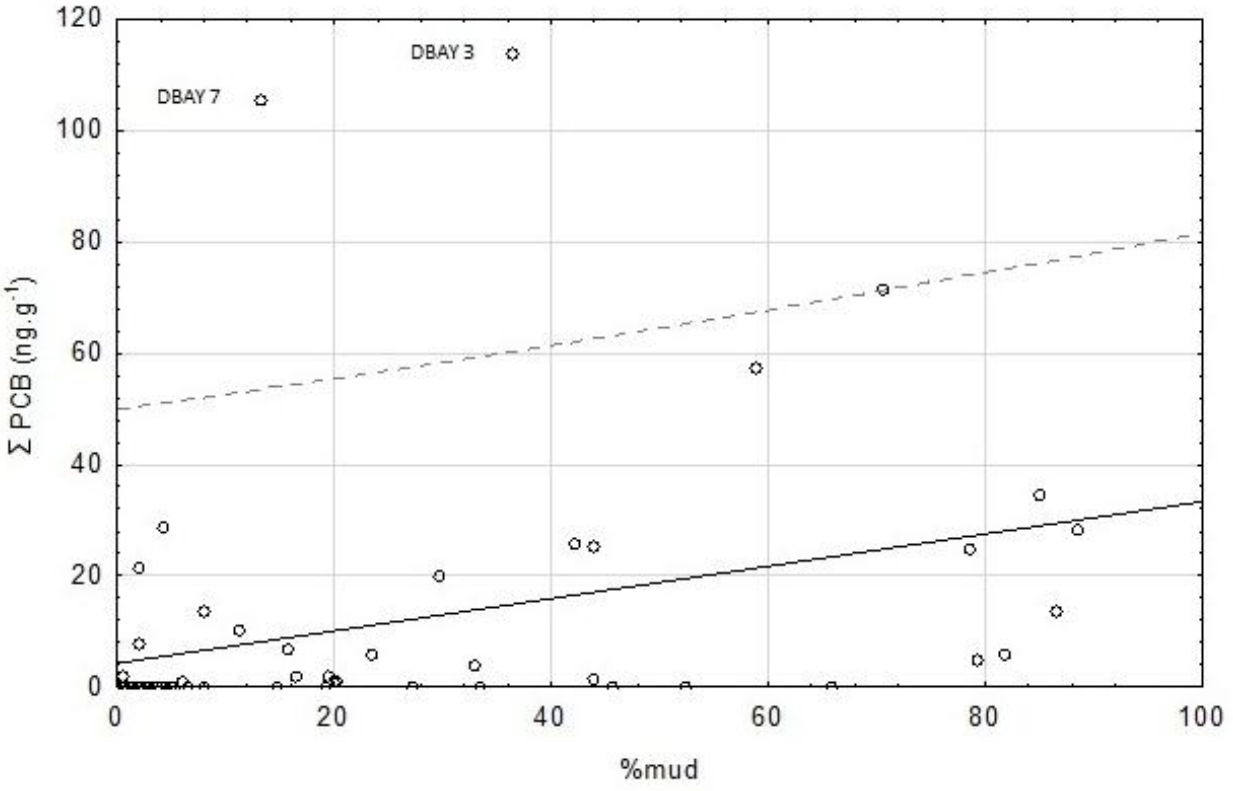


Figure 4.4. Correlation between ΣPCB concentrations and %mud, indicating the 95% prediction limits. r = 0.55, p < 0.05.

4.3 Sediment quality screening using sediment quality guidelines

Sediment quality guidelines (SQGs) are used as a screening tool to identify contaminants that are at concentrations of potential ecological concern. When the guidelines are used in conjunction with sediment toxicity testing it is possible to adequately assess the health of an aquatic system, specifically its sediment (MacDonald *et al.*, 2000). South Africa does not have SQGs for PAHs and PCBs, and because the study was based in both marine and freshwater environments, SQGs defined for North American freshwater ecosystems by MacDonald *et al.* (2000) and estuarine and marine ecosystems by Long *et al.* (1995) were used. Both of these SQGs have lower and upper effect levels. The Threshold Effect Concentration (TEC) (MacDonald *et al.*, 2000) and Effects Range Low (ERL) (Long *et al.*, 1995) describe a lower effect level, where chemicals at concentrations equal to or below these guidelines are unlikely to cause adverse effects to sediment-dwelling organisms. The upper effect level is represented by the Probable Effect Concentration (PEC) (MacDonald *et al.*, 2000) and the Effects Range Median (ERM) (Long *et al.*, 1995), where concentrations equal to or exceeding these guidelines are likely to frequently cause adverse effects to sediment-dwelling organisms. Chemical concentrations between the lower (TEC or ERL) and upper (ERM or PEC) effects levels will result in adverse effects occurring occasionally, but with increasing frequency the closer the concentration is to the upper effect level. Figure 4.5—Figure 4.7 compare the chemistry data to the marine and freshwater SQGs.

4.3.1 PAHs

The ΣLMW and ΣHMW PAHs (Figure 4.5a and Figure 4.5b) were only compared to the SQGs of Long, *et al.* (1995), because the MacDonald *et al.* (2000) SQGs do not provide guidelines for ΣLMW and ΣHMW PAHs. Concentrations of ΣLMW PAHs (Figure 4.5b) at most sites were below the ERL, which leads to the assumption that sediment-dwelling organisms would not experience detrimental effects due to PAH exposure. It is expected that adverse effects to sediment-dwelling organisms would occasionally occur at sites AMA 1 and 2, DBAY 3, 5 and 7, ISI 8, IVC 1, MNG 18 and SOIL 4, due to the ΣLMW PAH concentrations that exceeded the ERL. CAN 1 was the only site at which the ΣLMW PAH concentration exceeded the ERM. Sediment-dwelling organisms at this site are thus expected to have experienced adverse effects due to exposure to PAHs.

ΣHMW PAHs (Figure 4.5b) at sites CAN 1, DBAY 3, 5 and 7, ISI 5 and IVC 1 were predicted to possibly pose a risk to sediment-dwelling organisms because the concentrations fell between the ERL and ERM. ΣHMW PAHs at the remaining sites fell below the ERL, indicating that sediment-dwelling organisms would not experience detrimental effects due to PAH exposure. The HMW PAHs contributed more to the ΣPAHs than LMW PAHs (Table 4.1). The HMW PAHs generally

have longer half-lives than the LMW PAHs and pose a higher toxicological risk to sediment-dwelling organisms (Chen *et al.*, 2005, Yamada *et al.*, 2003).

Comparing the ΣPAHs to the marine and freshwater SQGs (Figure 4.6), only the lower effect level of the guidelines was exceeded. The TEC prescribes a lower concentration compared to the ERL. ΣPAH concentrations at most sites were below the lower effects level of both SQGs. The exceptions were DBAY 5 and 7, ISI 5 and 8, MNG 18 and SOIL 4, where the ΣPAH concentration exceeded the TEC, and sites CAN 1, DBAY 3 and IVC 1, where the ΣPAH concentration exceeded the ERL.

4.3.2 PCBs

ΣPCB concentrations were compared to the marine and freshwater SQGs. ΣPCB concentrations at the majority of the sites were below the ERL and adverse effects to sediment-dwelling organisms were thus unlikely. Sites where the ΣPCB concentration exceeded the ERL were DBAY 1, 4, 6, 8, 10, ISI 8 and SOIL 4. ΣPCB concentrations at sites DBAY 3, 7 and ISI 4 exceeded both the ERL and the TEC. ΣPCB concentrations at none of the sites exceeded the ERM or PEC.

Sites DBAY 3, 7, 8 and SOIL 4 thus seem to be sites of concern as the ΣLMW, ΣHMW and ΣPAHs, and ΣPCBs exceeded marine and/or freshwater SQGs (Figure 4.5 and Figure 4.7).

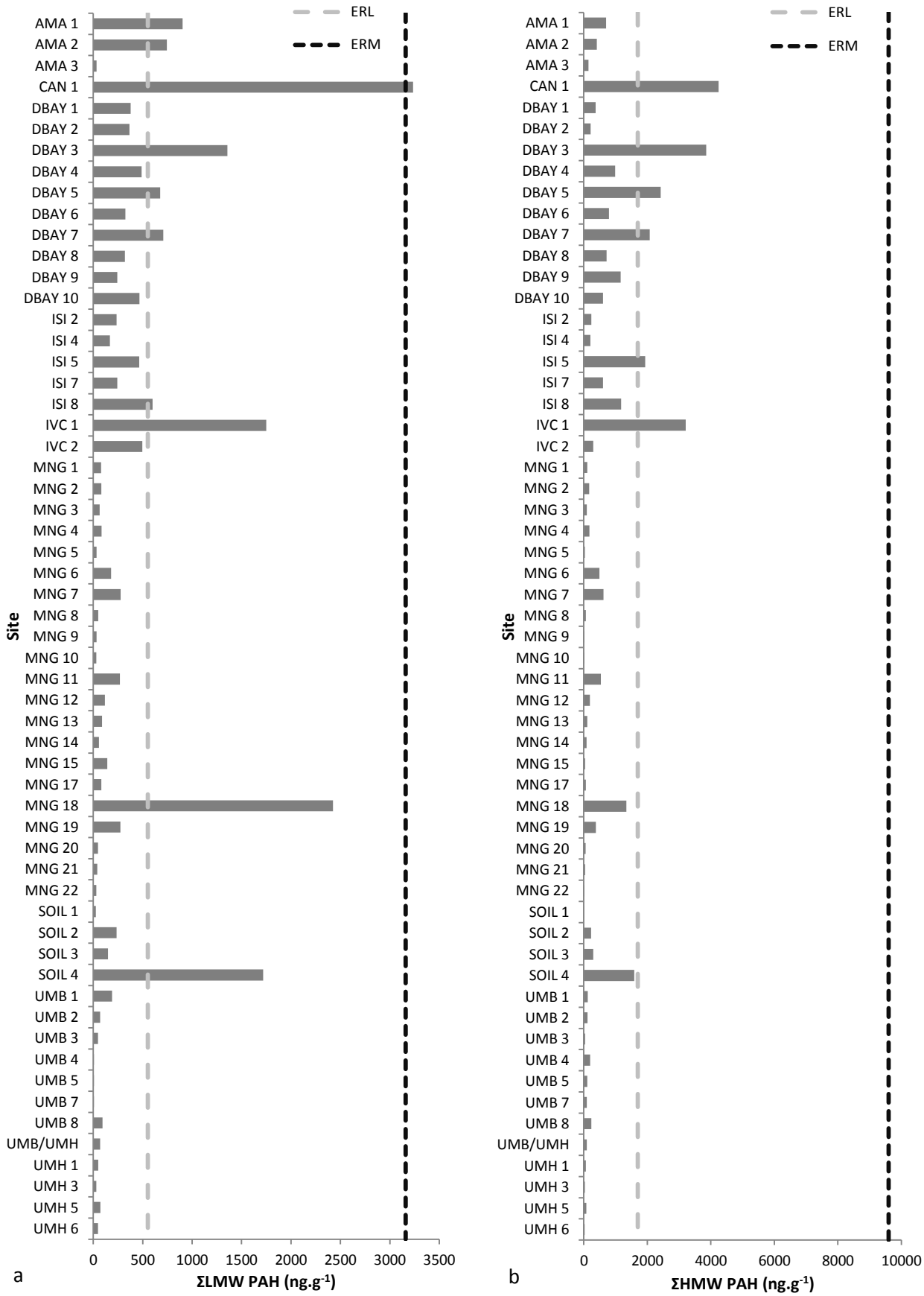


Figure 4.5. Sum of low molecular weight (ΣLMW) PAHs (a) and high molecular weight (ΣHMW) PAHs (b) in sediment and soil samples. Sediment quality guidelines derived by Long *et al.* (1995) are indicated.

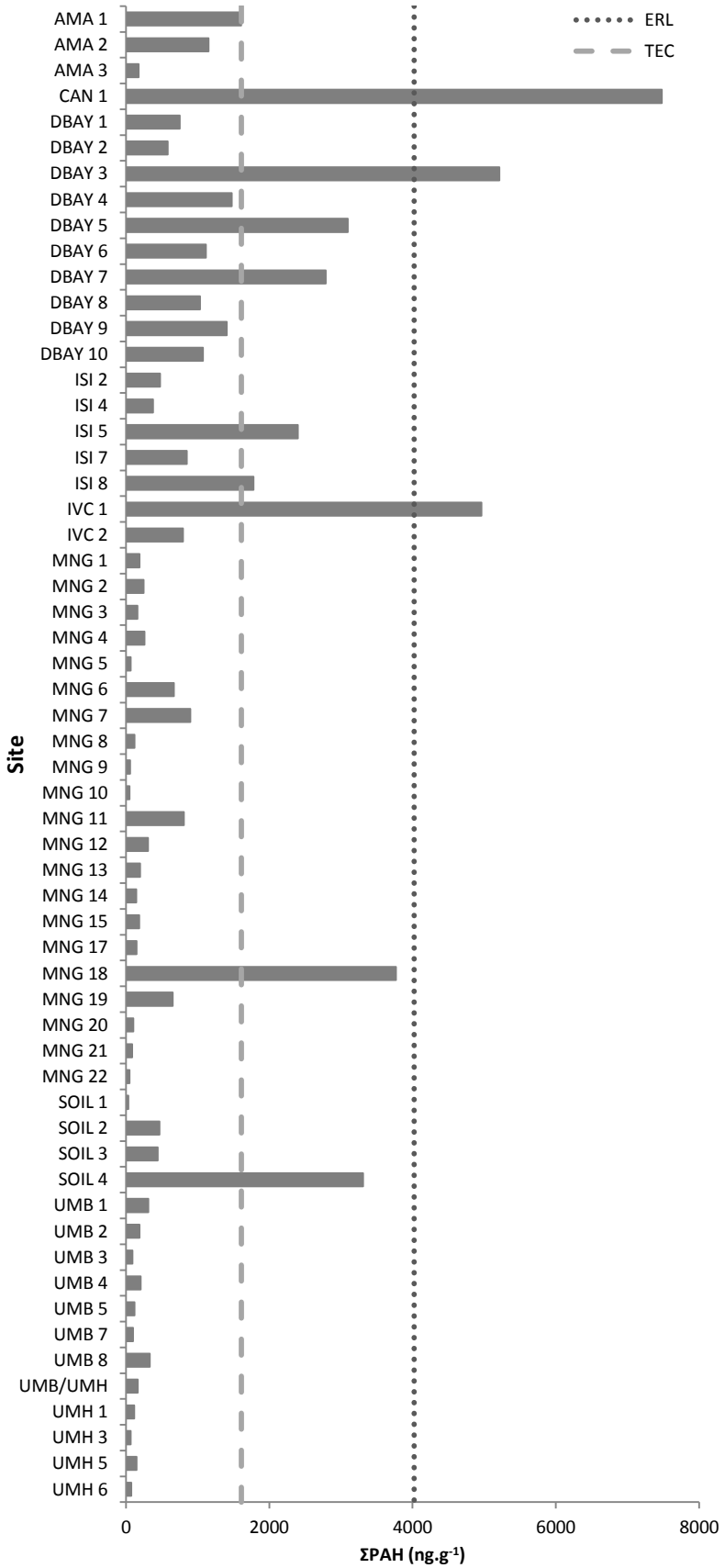


Figure 4.6. The sum PAH (ΣPAH) concentrations in sediment and soil samples. Sediment quality guidelines derived by Long *et al.* (1995) and MacDonald *et al.* (2000) are indicated.

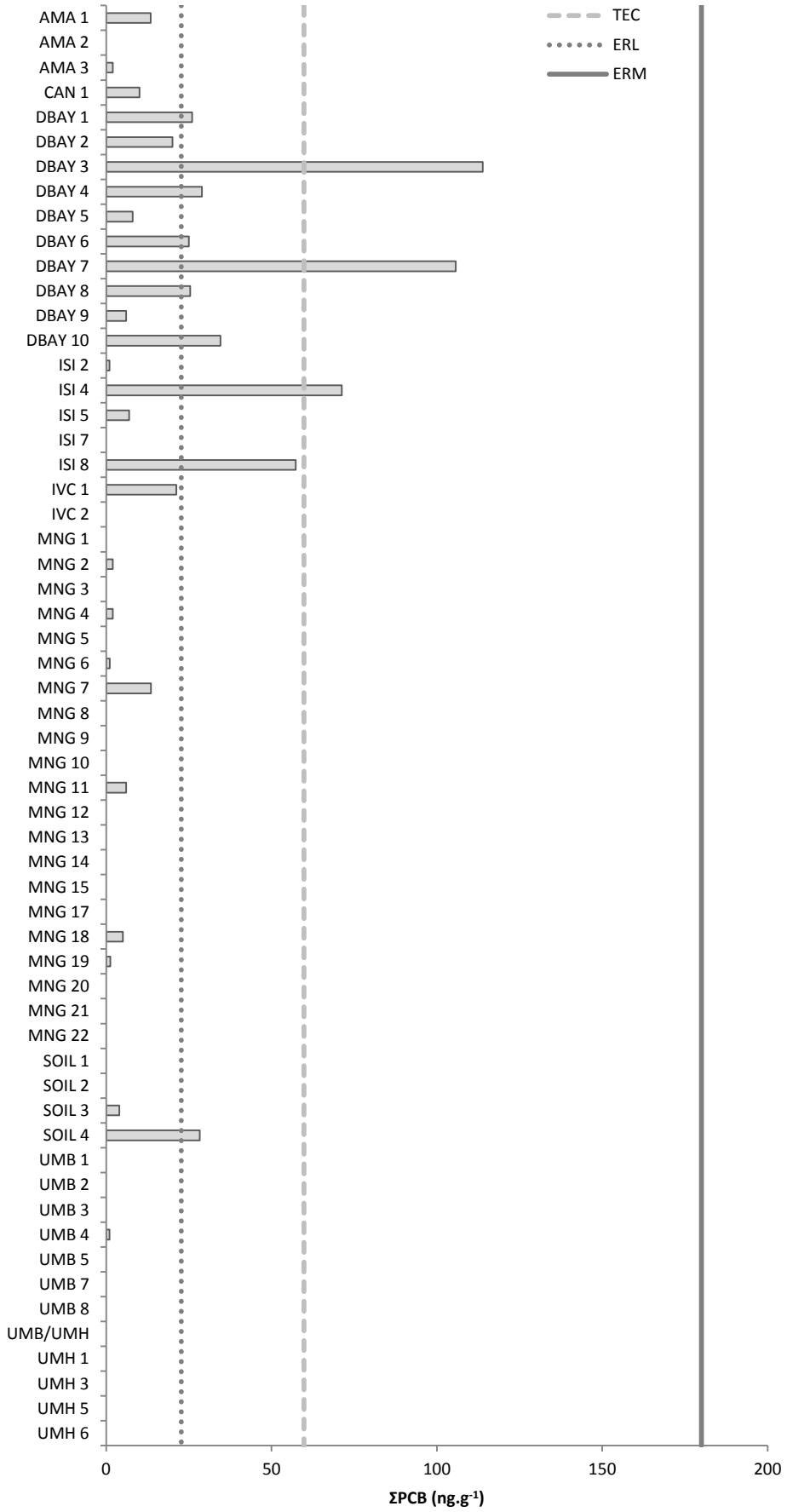


Figure 4.7. The ΣPCB congeners in sediment and soil samples. Sediment quality guidelines derived by Long *et al.* (1995) and MacDonald *et al.* (2000) are indicated.

4.4 Source determination

PAHs enter the environment as a complex mixture of isomers. The process leading to the production of PAH emission profiles depends on the source of the PAH. LMW PAHs are usually sourced directly from fossils fuels and oils or from the combustion of wood and grass amongst others, at low temperatures. HWM PAHs are typically formed from the combustion of fuels, such as in engines, at high temperatures (Tobiszewski and Namieśnik, 2012). The ratio between certain PAH isomers can be used to diagnose the emission source.

Table 4.4 lists ratios that have been used by other workers to identify PAH sources as either petrogenic or pyrogenic. The ANT/(ANT+PHE) ratio best determines whether a source is petrogenic (Tobiszewski and Namieśnik, 2012). For the most accurate determination of pyrogenicity either the FLA/(FLA+PYR), BaP/(BaP+CHR) or IcdP/(IcdP+BghiP) ratio has been used (Table 4.4). By plotting the petrogenic ratio—ANT/(ANT+PHE)—against each of the pyrogenic ratios, the distribution of the sites on biplots was investigated to determine the likely source of the PAHs (Figure 4.8–Figure 4.10). Again, isomers at concentrations < MDL were substituted by a value of one half the MDL for ratio calculations.

Table 4.4. PAH ratios used to diagnose PAH sources.

PAH ratio	Range	Source	Reference
ANT/(ANT + PHE)	< 0.1	Petrogenic	(Pies *et al.*, 2008)
	> 0.1	Pyrogenic	
FLA/(FLA + PYR)	< 0.4	Petrogenic	(De La Torre-Roche *et al.*, 2009)
	0.4–0.5	Petroleum combustion	
	> 0.5	Grass/wood and coal combustion	
BaA/(BaA + CHY)	< 0.2	Petrogenic	(Akyüz and Çabuk, 2008)
	0.2–0.35	Petroleum combustion	
	> 0.35	Grass/wood and coal combustion	
IcdP/(IcdP + BghiP)	< 0.2	Pyrogenic	(Yunker *et al.*, 2002)
	0.2–0.5	Petrol combustion	
	> 0.5	Grass/wood and coal combustion	

Of the 58 sites, 19% were categorised as having mixed PAH sources, and were categorised as such in each of the biplots (*i.e.* petrogenic and pyrogenic). These were sites DBAY 2 and 4, AMA 1 and 2, SOIL 2 and 4, MNG 6, 15 and 18, and IVC 2 and 4 (Figure 4.8—Figure 4.10).

The PAH source at site IVC 2 was categorised as petrogenic by both ratios in only one instance, when ANT/(ANT + PHE) was plotted against FLA/(FLA + PYR) (Figure 4.8). This ratio also characterised 64% of the sites as having PAHs derived from the combustion of grass, wood and coal. Petroleum combustion was determined to be the source of PAHs at 12% of sites, while 5% of

sites bordered between petroleum combustion and grass, wood, and coal combustion, and 19% were mixed sources (Figure 4.8). When the BaA/(BaA + CHR) ratio was used (Figure 4.9), 7% of sites had petroleum combustion as the source of the PAHs and 74% had the source as grass, wood, and coal combustion, and 19% as mixed sources. The IcdP/(IcdP + BghiP) ratio identified PAHs at 15% of sites as having a petroleum combustion source and 59% as having a grass, wood, and coal combustion source. At 7% of sites, the PAH source bordered between the two sources and 19% were of mixed sources (Figure 4.10).

According to the biplots, PAHs at the majority of the sites thus originated predominantly from grass, wood and coal combustion, and at a small proportion of sites from the combustion of petroleum. Although all three combinations of ratios (Figure 4.8–Figure 4.10) had a category for "petroleum combustion", there were no similarities between the sites grouped in this category with the exception of site DBAY 7, which fell into this category in two of the biplots (Figure 4.8 and Figure 4.10). PAHs at site SOIL 1 bordered on the petroleum combustion and grass, wood, and coal combustion categories in both Figure 4.8 and Figure 4.10. Within the grass, wood and coal combustion category, the same 33 sites appeared in Figure 4.8 and Figure 4.9, 32 of the sites in Figure 4.8 and Figure 4.10, and 30 of the sites in Figure 4.8 and Figure 4.10. Due to the fact that there was a distribution of the sites between the two categories it is likely these sites were polluted by a mixture of PAHs from both emission sources.

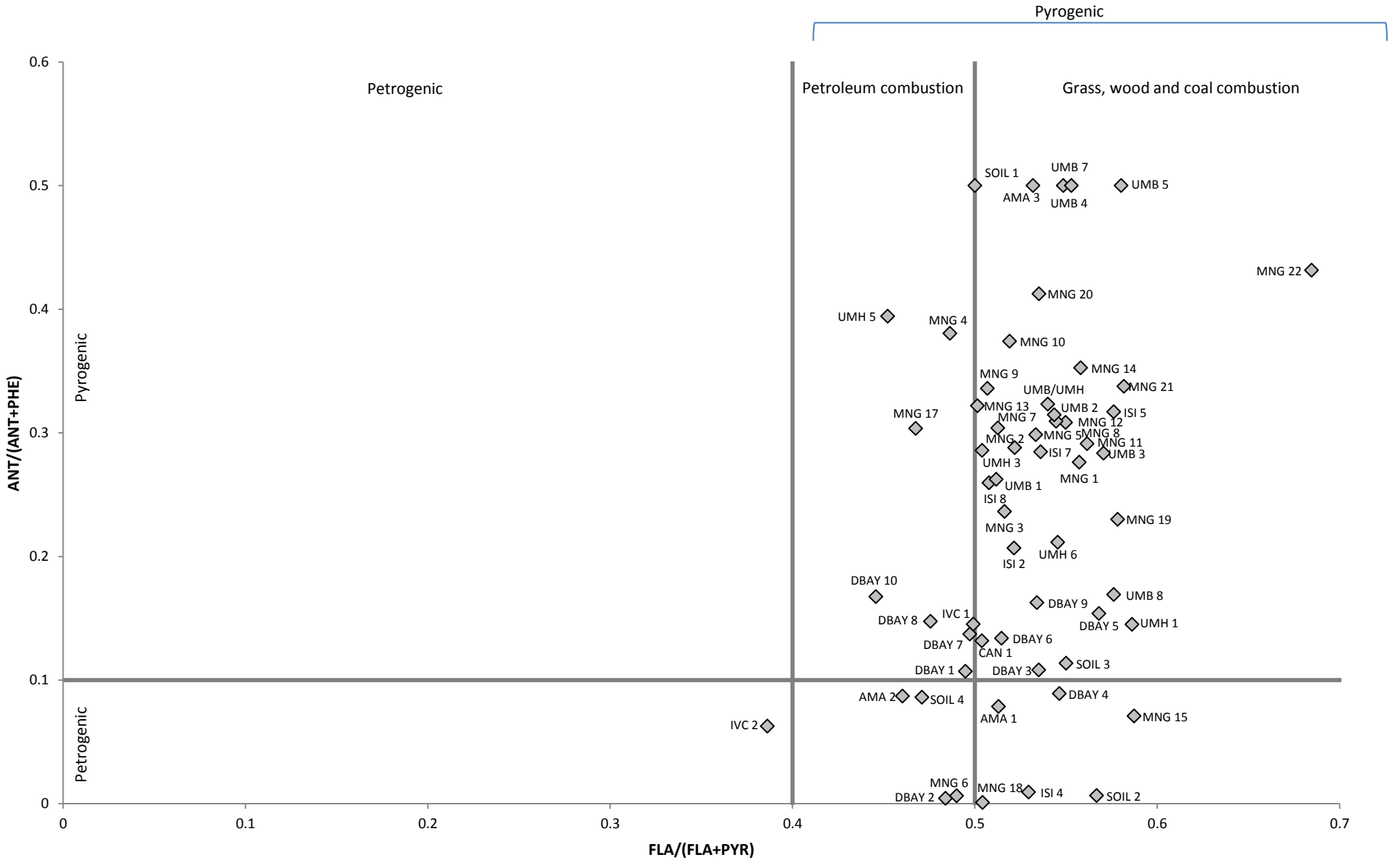


Figure 4.8. Source determination of PAHs using ANT/(ANT+PHE) and FLA/(FLA+PYR) ratios as indicators of petrogenicity and pyrogenicity.

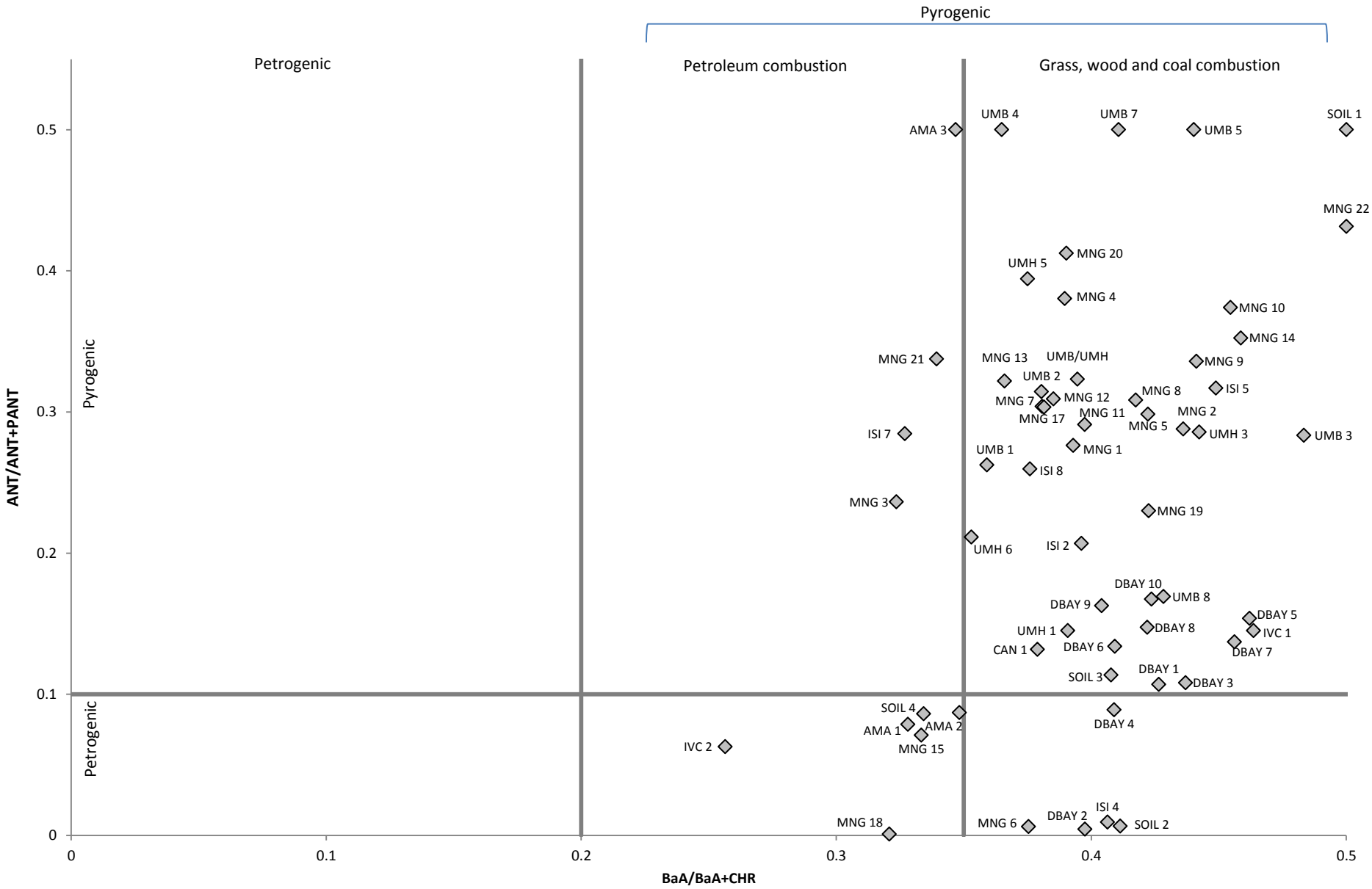


Figure 4.9. Source determination of PAHs using ANT/(ANT+PHE) and BaA/(BaA+CHR) ratios as indicators of of petrogenicity and pyrogenicity.

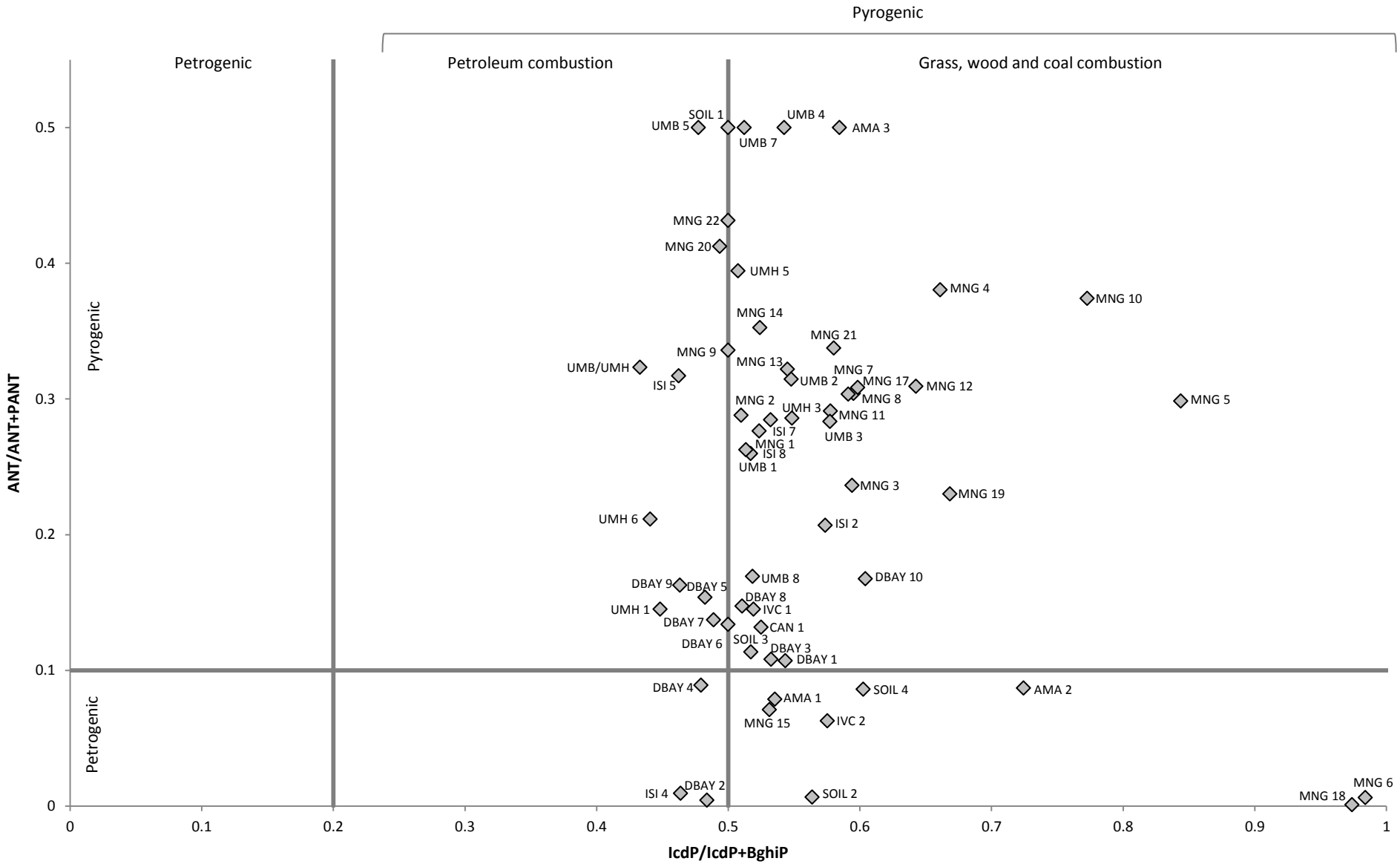


Figure 4.10. Source determination of PAHs using ANT/(ANT+PHE) and IcdP/(IcdP+BghiP) ratios as indicators of petrogenicity and pyrogenicity.

4.5 Component analysis

Another approach to investigating the sources of PAHs and/or PCBs or whether there are underlying factors influencing isomer or congener ratios is to perform principal component analysis (PCA). Since PCA cannot handle zero values, PAH concentrations < MDL were substituted with a value of one half the MDL before the data was transformed. A PCA could not be performed for the PCBs because there were too many < MDL concentrations. PCA was performed using Canoco 4.5 Statistical software. In PCA, the highest variance is explained by the first component or factor and the remaining variance by other factors. The component loadings of the isomers contributing to a particular component are represented together with the component scores of the sites on the same biplots.

The first factor accounted for 28.8% of the variance, factor 2 for 23.7%, factor 3 for 17.8%, and factor 4 for 8.8%. Between the first three factors, two-thirds (67.1%) of the variance was explained (Table 4.4 andTable 4.5) and, therefore, only the first 3 factors are discussed further.

Factor 1 had high loadings for IcdP, DahA, and PER—isomers containing 5 benzene rings—and ACY, with 2 benzene rings, on the negative side. The positive side had DMN, 2MNP and 1MNP—isomers containing 2 benzene rings (Table 4.5, Figure 4.11). Site MNG 18 had a high factor score on the positive side of factor one, and ISI 5 and MNG 4 on the negative side (Table 4.5, Figure 4.11). This implies that site MNG 18 had a greater proportion of the LMW PAHs, DMN, 2MNP and 1MNP, which drove factor 1. Sites on the positive side of factor one, namely ISI 5 and MNG 4, had higher concentrations of IcdP, DahA, and PER and ACY (Appendix A.1).

Factor two was driven by the contrast between NP, 1MNP and DBT—isomers containing 2 benzene rings—on the negative side and BbF, BeP, BaA, BkF, CHY, which are 4 and 5 benzene-ring PAHs, on the positive side (Figure 4.11 and Figure 4.13, Table 4.5). The sites SOIL 1 and MNG10, 15 and 22—on the negative side—contained a higher concentration of NP, 1MNP and DBT than BbF, BeP, BaA, BkF, CHY. Sites UMB 7, AMA 3, UMB 5 and UMB 4 were on the positive side and contained high concentrations of BbF, BeP, BaA, BkF, CHY than NP, 1MNP and DBT (Figure 4.11 and Figure 4.13,

Table 4.6).

The sediment characteristics %mud and %TOC contributed the most to the positive side of factor 3. These characteristics only became important at factor 3, while factor 1 and 2 were mostly influenced by the PAH isomers. There was only one site that had a large loading for factor 3—SOIL 1 (

Table 4.6, Figure 4.12 and Figure 4.13). SOIL 1 was also one of the sites with little pollution and explains why it related to the TOC and mud on the graph rather than to factor 1 or 2, which were created by the PAH isomer patterns.
When compared to the factor loadings (Table 4.5), isomer size seemed to drive the factors, with HMW isomers (4–6 rings) at opposite ends to LMW (2–3 rings). However, LMW (2 ring) isomers did occasionally appear amongst the HMW isomers.

The distribution of sites in the statistical space on all three biplots did not show any clear clustering based on the isomer composition of the PAH concentration. The factor scores (

Table 4.6) had to be consulted to see which sites were the most influenced by which factor. It is clear from the sites at the top and bottom of the scores columns that sites sharing similar fingerprint patterns did not occur close to each other in the real world. This situation is possibly due to pollution degrading conditions that varied greatly between the sites, even those within the same system. It could also be due to the PAHs having had the same source.

Table 4.5. Factor loadings for PAH isomers contributing to the first 3 factors for PCA. Factors are arranged in decreasing order and only factor loading values ≥ │0.5│are shown.

Factor 1		Factor 2		Factor 3	
Isomer	Factor loading	Isomer	Factor loading	Isomer	Factor loading
DMN	0.97	BbF	1.20	Mud	1.56
2MNP	0.83	BeP	1.19	%TOC	1.26
1MNP	0.79	BaA	1.18	PER	
BiP	0.58	BkF	1.18	BiP	
TMN	0.56	CHY	1.14	BghiP	
1MP	0.54	FLA	1.05	BaP	
PANT	0.52	BaP	1.05	1MNP	
PYR		PYR	1.04	DahA	
CHY		PER		NP	
NP		BghiP		2MNP	
FLA		DahA		BeP	
BaA		IcdP		ACY	
%TOC		%TOC		BbF	
BbF		ACE		IcdP	
BeP		Mud		BkF	
BkF		DMN		DMN	
BaP		TMN		CHY	
Mud		FL		BaA	
ACE		ACY		1MP	
FL	-0.50	BiP		DBT	
BghiP	-0.66	ANT		TMN	
DBT	-0.70	PANT	-0.52	PYR	
ANT	-0.86	1MP	-0.60	FL	
PER	-0.90	2MNP	-0.64	FLA	
ACY	-0.93	DBT	-0.75	ACE	
DahA	-1.00	1MNP	-0.77	PANT	
IcdP	-1.00	NP	-0.86	ANT	-0.55
% Total variance	28.6		23.7		14.8
Cumulative %	28.6		52.3		67.1

Table 4.6. PCA factor scores for 3 factors, including %TOC and mud fraction, arranged in decreasing order. Only factor score values ≥ │0.5│ are shown.

Factor 1		Factor 2		Factor 3	
Site	Factor score	Site	Factor score	Site	Factor score
MNG 18	2.47	UMB 4	1.34	SOIL 1	1.23
MNG 6	1.17	UMB 5	1.05	ISI 4	0.57
SOIL 2	1.06	AMA 3	1.04	MNG 20	0.51
IVC 2	0.98	UMB 7	0.99	SOIL 2	0.50
AMA 1	0.73	DBAY 9	0.93	DBAY 9	
ISI 4	0.68	MNG 6	0.71	UMB 4	
AMA 2	0.67	DBAY 3	0.65	MNG 12	
UMB 8	0.56	DBAY 5	0.58	MNG 6	
DBAY 2	0.54	ISI 5	0.55	MNG 21	
SOIL 4	0.50	UMB 8		MNG 7	
MNG 15		DBAY 7		MNG 4	
DBAY 1		DBAY 4		MNG 19z	
CAN 1		DBAY 8		MNG 2	
IVC 1		DBAY 6		DBAY10	
DBAY10		IVC 1		SOIL 3	
SOIL 3		SOIL 3		DBAY 2	
DBAY 5		ISI 7		MNG 1	
AMA 3		DBAY10		DBAY 8	
DBAY 4		SOIL 2		MNG 18z	
UMB 1		CAN 1		MNG 13	
DBAY 6		MNG 19		DBAY 6	
SOIL 1		MNG 18		ISI 2	
DBAY 7		MNG 4		UMB 5	
UMH 1		ISI 8		UMB 3	
MNG 22		MNG 11		DBAY 1	
ISI 2		ISI 4		IVC 2	
DBAY 8		MNG 2		UMH 5	
UMB 3		MNG 14		ISI 8	
UMH 5		MNG 3		MNG 11	
ISI 8		UMB 2		MNG 17	
MNG 5		DBAY 2		UMH 6	
DBAY 3		MNG 1		MNG10	
UMH 6		MNG 8		AMA 3	
UMB 7		MNG 12		UMB 2	
UMB 5		MNG 7		MNG 5	
UMB/UMH		DBAY 1		SOIL 4	
MNG 17		UMB/UMH		UMB 7	
MNG 1		UMH 1		MNG 8	
MNG10		SOIL 4		MNG 14	
ISI 7		MNG 21		UMH 3	
MNG 13		UMH 3		UMB/UMH	
MNG 20		ISI 2		MNG 3	
UMB 4		AMA 1		DBAY 3	
MNG 8		UMH 5		ISI 5	
MNG 3		MNG 13		MNG 15	
MNG 11		MNG 20		UMH 1	
MNG 2		MNG 9		MNG 22	
UMH 3		MNG 5		DBAY 7	
UMB 2		UMB 3		ISI 7	
MNG 9		IVC 2		UMB 8	
MNG 14		AMA 2		AMA 2	
MNG 21		UMB 1	-0.51	DBAY 4	
MNG 12		MNG 17	-0.53	AMA 1	-0.53
DBAY 9		UMH 6	-0.58	UMB 1	-0.53
MNG 19		MNG10	-0.70	CAN 1	-0.61
MNG 7		MNG 15	-0.73	MNG 9	-0.82
MNG 4	-0.56	MNG 22	-0.75	DBAY 5	-0.86
ISI 5	-0.63	SOIL 1	-0.84	IVC 1	-0.86

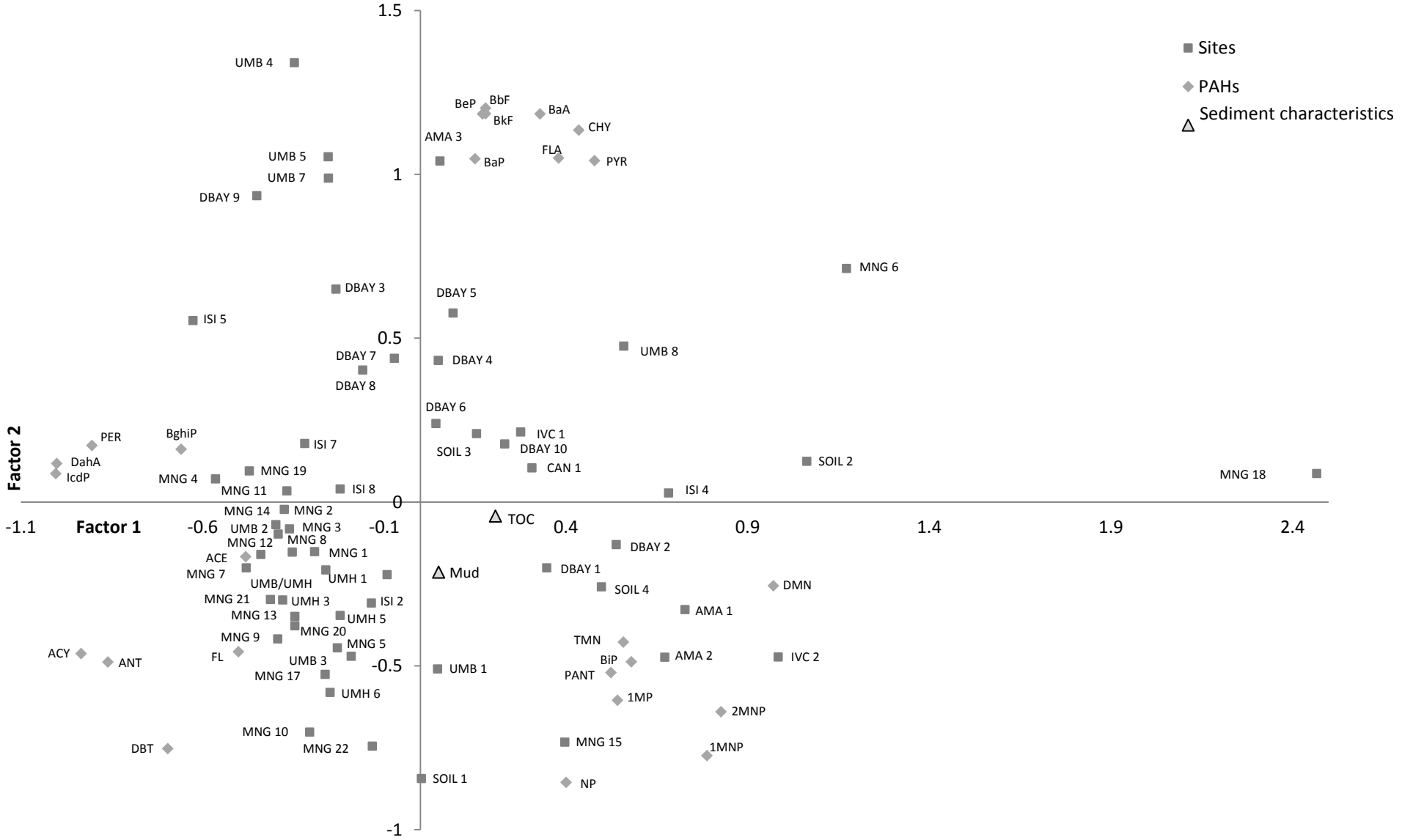


Figure 4.11. PCA comparing factors 1 (28.8% variation) and 2 (23.7% variation), indicating sites, PAH concentrations (ng.g^{-1}), total organic carbon and the mud fraction (sediment characteristics).

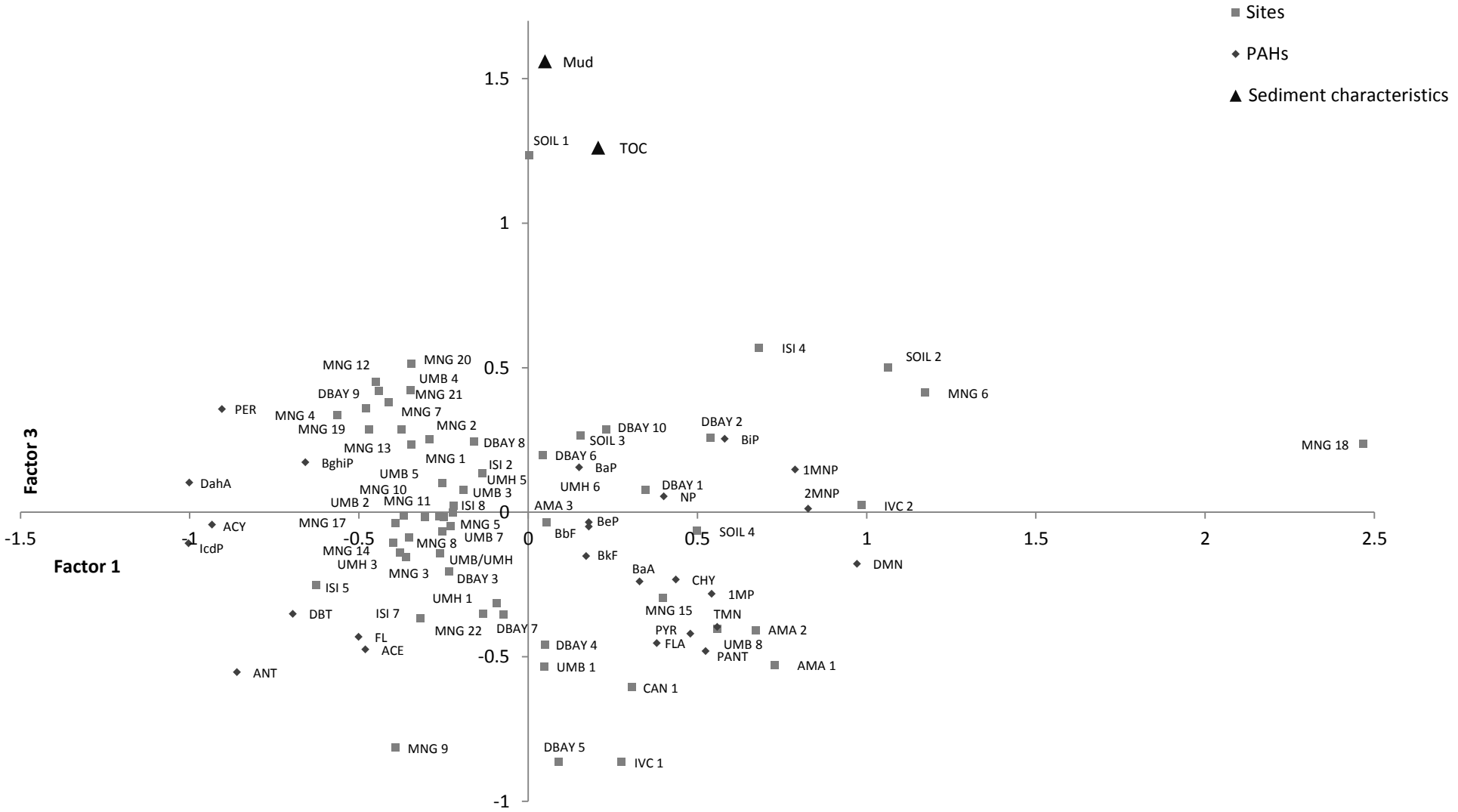


Figure 4.12. PCA comparing factors 1 (28.8% variation) and 3 (17.8% variation), indicating sites, PAH concentrations (ng.g^{-1}), total organic carbon and the mud fraction (sediment characteristics).

Figure 4.13. PCA comparing factors 2 (23.7% variation) and 3 (17.8% variation), indicating sites, PAH concentrations (ng.g^{-1}), total organic carbon and the mud fraction (sediment characteristics).

4.6 Biological analysis

In this section, bio-luminescence results of the H4IIE-luc assay are presented, discussed and compared to PAH and PCB results. Before this is done a short explanation is provided on which variation of the viability assay was selected to determine the effect of the sediment extracts on the viability of the H4IIE cells.

4.6.1 Validation of viability methods

Two methods were used to measure viability, namely the MTT-assay and cell index (CI) as provided by the xCELLigence apparatus. However, after completion of exposures on cells in the xCELLigence apparatus, the same cells were used in another MTT-assay. This way it was possible to compare the CI to MTT-assay results within the same population of cells. The cell viability percentages are given in Table 4.7. The data was normalised using Box-Cox transformations in the Statistica software package. Because the data was heterogeneous, Welch's F test was used to perform ANOVA ($F = 8.78$. $p < 0.05$). When a statistically significant difference was evident, a Tukey-b post-hoc test was performed to identify the data that differed from one another. There was a statistically significant difference between viability determined using the xCELLigence and MTT, and MTT and the MTT on the xCELLigence ($p < 0.05$). However, the means of the viabilities determined by the MTT after the CI determination on the xCELLigence did not differ statistically significantly from the viability determined with the CI of the xCELLigence ($p > 0.05$). Any one of the latter viability assays was deemed suitable for viability estimation, but the xCELLigence data was chosen for further use because the results are less prone to human error than the MTT-assay.

Table 4.7. Percentage viability of cells after treatment with the various extracts of sediment and soil.

	PCB (acid washed extracts)			PAH (non-acid washed extracts)		
	xCELLigence	MTT		xCELLigence	MTT	
Site	cell index	xCELLigence	MTT	cell index	xCELLigence	MTT
AMA 1	119	118	97	123	102	69
AMA 2	131	120	97	109	82	110
AMA 3	127	132	122	120	98	101
Can 1	130	103	90	97	84	115
DBAY 1	95	102	87	108	101	95
DBAY 2	84	96	93	102	91	94
DBAY 3	101	106	93	110	97	101
DBAY 4	102	111	89	124	98	97
DBAY 5	92	90	88	121	101	100
DBAY 6	70	90	95	140	108	91
DBAY 7	71	94	63	93	107	87
DBAY 8	92	107	63	89	107	96
DBAY 9	93	91	61	83	110	125
DBAY10	73	89	96	98	78	107
ISI 2	68	91	90	101	102	116
ISI 4	101	88	63	73	70	109
ISI 5	87	105	97	71	102	112

	PCB (acid washed extracts)			PAH (non-acid washed extracts)		
Site	xCELLigence cell index	MTT xCELLigence	MTT	xCELLigence cell index	MTT xCELLigence	MTT
ISI 7	65	89	69	82	115	115
ISI 8	72	94	73	8	55	39
IVC 1	101	105	92	108	110	72
IVC 2	92	105	96	65	84	122
SOIL 1	72	99	97	80	114	114
SOIL 2	82	99	105	99	82	97
SOIL 3	105	87	73	87	84	95
SOIL 4	100	103	62	82	73	96
UMB 1	111	104	102	88	114	76
UMB 2	128	94	78	91	78	90
UMB 3	125	120	72	68	82	88
UMB 4	136	110	119	72	62	113
UMB 5	137	103	87	105	110	112
UMB 7	142	107	105	79	91	128
UMB 8	140	125	99	73	90	96
UMB/UMH	117	107	98	71	92	106
UMH 1	117	95	97	95	106	101
UMH 3	107	103	97	91	104	95
UMH 5	119	106	89	101	108	101
UMH 6	106	89	69	99	112	94

4.6.2 Bioluminescence results

The luminescence bioassays were based on the ability of compounds in non-acid washed (PAHs) and acid washed (PCB) extracts to bind to the AhR of the genetically modified rat hepatoma cells (H4IIE-*luc*). This binding elicits a light response that is quantified in terms of the percentage response created by a known concentration of the reference compound TCDD, and referred to as the %TCDD-max. Dose-response curves were created for both the reference compound and sample extracts, with %TCDD-max on the y-axis. The effect concentration (EC) at which both the reference compound and samples elicited a 20, 50 and 80% response were calculated from the dose-response curves. The relative potency (REP) for a sample extract, at 20, 50, 80%, was determined by dividing the EC of the reference compound by the EC of the sample, creating a REP 20, 50 and 80. The REP values were back-calculated to the mass sediment/soil initially extracted. Not all samples elicited such a high response that an EC 50 or 80 was reached and these are thus mostly extrapolated values (Nieuwoudt et al., 2009, Villeneuve et al., 1999). In some instances the response elicited was too low and a REP value could not be quantified (Table 4.8). However, the EC 20 and consequent REP 20 were mostly measurable at each site (Table 4.8). Because this was the most accurate measurement, the REP 20 was used in the remainder of this discussion and has been termed the bioassay equivalent (BEQ).

Table 4.8. The %TCDD-max and relative potencies (REP) of the PCB and PAH fractions obtained from the luminescence bioassay. REP values are represented as the mean ± standard deviation. – = a response could not be quantified, values highlighted in bold are BEQs that have been extrapolated.

	PCB (acid washed extracts)				PAH (non-acid washed extracts)			
Site	%TCDD-max	REP 20 (pgTCDDeq.g^{-1})	REP 50 (pgTCDDeq.g^{-1})	REP 80 (pgTCDDeq.g^{-1})	%TCDD-max	REP 20 (pgTCDDeq.g^{-1})	REP 50 (pgTCDDeq.g^{-1})	REP 80 (pgTCDDeq.g^{-1})
AMA 1	31.44	6.22 ± 1.62	**0.05 ± 0.05**	**1.99 ± 2.07**	150.56	217.76 ± 3.23	481.6 ± 54.98	1106. ± 79.71
AMA 2	28.73	5.76 ± 1.46	**7.32 ± 5.68**	**1.53 ± 1.55**	84.38	171.15 ± 10.3	259.7 ± 17.56	396.1 ± 58.14
AMA 3	20.64	10.89 ± 3.08	**0.51 ± 0.63**	**0.04 ± 0.05**	70.7	269.28 ± 4.34	136.1 ± 10.48	**35.0 ± 49.15**
CAN 1	17.49	4.82 ± 1.19	-	**-**	126.68	304.68 ± 0.69	574.9 ± 152.3	1219. ± 159.72
DBAY 1	1.65	-	-	**-**	95.12	165.24 ± 0.68	282.6 ± 48.25	490.8 ± 167.1
DBAY 2	1.65	-	-	**-**	73.92	85.04 ± 6.12	121.6 ± 21.02	**4.3 ± 0.11**
DBAY 3	25.21	18.57 ± 5.98	**1.30 ± 1.67**	**0.09 ± 0.16**	139.2	528.49 ± 8.64	1023. ± 201.26	1985. ± 395.72
DBAY 4	11.26	0.52 ± 0.29	**-**	**-**	127.94	165.79 ± 6.95	387.2 ± 40.12	952.4 ± 79.47
DBAY 5	22.15	21.25 ± 7.63	**0.93 ± 1.03**	**0.01 ± 0.01**	149.19	634.21 ± 8.17	1104. ± 325.06	2105. ± 521.44
DBAY 6	13.01	1.57 ± 1	**-**	**-**	110.09	230.79 ± 15.7	376.5 ± 68.99	597.5 ± 258.14
DBAY 7	18.85	9.69 ± 2.76	**0.12 ± 0.06**	**0.06 ± 0.01**	90.49	766.08 ± 3.47	461.1 ± 99.6	321.9 ± 164.77
DBAY 8	13.34	0.22 ± 1.09	**-**	**-**	118.99	184.53 ± 1.12	386.4 ± 43.57	813.8 ± 177.56
DBAY 9	15.12	1.58 ± 0.48	**-**	**-**	87.53	123.37 ± 5.66	185.9 ± 29.13	286.0 ± 100.94
DBAY10	23.03	10.56 ± 7.49	**-**	**-**	98.52	536.61 ± 2.86	884.1 ± 37.72	1483. ± 136.61
ISI 2	19.26	1.84 ± 0.74	**0.93 ± 0.97**	**0.02 ± 0.02**	103.28	85.39 ± 3.66	173.6 ± 24.33	322.0 ± 111.25
ISI 4	26.42	6.54 ± 0.5	**-**	**-**	129.62	160.7 ± 7.66	330.0 ± 59.91	541.2 ± 340.23
ISI 5	38.76	35.26 ± 10.9	**20.41 ± 11.20**	**9.83 ± 7.55**	127.78	162.73 ± 7.99	382.8 ± 83.23	902.0 ± 237.36
ISI 7	17.7	1.26 ± 0.36	**0.2 ± 0.01**	**-**	72.57	108.48 ± 3.76	171.8 ± 43.02	**260.9 ± 117.66**
ISI 8	30.37	5.85 ± 1.35	**2.19 ± 1.18**	**0.11 ± 0.10**	1.68	-	-	-
IVC 1	22.84	55.12 ± 9.77	**15.90 ± 9.89**	**3.82 ± 3.71**	4.27	69.01 ± 6.65	**411.9 - 92.52**	**282.4 120.89**
IVC 2	13.73	12.68 ± 17.3	**0.94 ± 1.62**	**0.08 ± 0.14**	84.56	154.57 ± 5.84	232.6 ± 36.51	354.1 ± 104.08
SOIL 1	19.1	13.26 ± 9.89	**0.23 ± 0.38**	**-**	14.71	1.04 ± 1.62	**-**	**-**
SOIL 2	26.4	31.45 ± 2.17	**5.44 ± 5.45**	**1.59 ± 2.01**	31.53	38.44 ± 3.85	**7.2 ± 1.57**	**1.3 ± 0.64**
SOIL 3	1.53	-	**-**	**-**	69.54	99.99 ± 0.63	109.4 ± 29.78	**185.6 - 21.10**
SOIL 4	26.77	7.67 ± 6.73	**-**	**-**	116.31	422.38 ± 0.03	723.3 ± 87.34	1308. ± 69.13
UMB 1	24.18	34.14 ± 31.35	**9.96 ± 17.25**	**4.28 ± 6.06**	58.19	67.72 ± 6.23	64.5 ± 28.13	**50.7 ± 35.29**
UMB 2	11.63	0.41 ± 0.39	**-**	**-**	37.64	45.07 ± 2.2	**16.5 ± 1.65**	**5.0 ± 1.46**
UMB 3	9.87	0.02 ± 0.03	**-**	**-**	36.54	33.55 ± 1.87	**10.1 ± 9.42**	**1.0 ± 1.44**
UMB 4	54.69	93.54 ± 17.5	66.60 ± 8.32	**76.50 ± 4.24**	15.54	7.73 ± 8.04	**0.0 ± 0.05**	**-**
UMB 5	11.51	0.12 ± 0.11	**-**	**-**	39.12	55.16 ± 6.11	**27.6 ± 15.83**	**16.6 ± 18.54**
UMB 7	11.05	0.29 ± 0.21		**-**	29.99	29.46 ± 3.39	**6.4 ± 4.52**	**1.5 ± 1.53**
UMB 8	17.57	4.93 ± 1.69	**0.03 ± 0.04**	**-**	46.82	55.55 ± 5.96	**41.3 ± 8.74**	**29.1 ± 13.15**
UMB/UMH	15.09	0.41 ± 0.15	**-**	**-**	39	5.53 ± 0.95	**2.5 ± 0.15**	**1.0 ± 0.3**
UMH 1	3.23	-	**-**	**-**	19.88	9.07 ± 2.01	**0.2 ± 0.23**	**0.0 ± 0.01**
UMH 3	9.87	-	**-**	**-**	26.95	25.43 ± 2.16	**2.6 ± 0.8**	**0.2 ± 0.16**
UMH 5	5.99	-	**-**	**-**	42.36	53.92 ± 2.13	**31.8 ± 9.01**	**13.0 ± 6.34**
UMH 6	10.07	0.33 ± 0.47	**-**	**-**	17.25	4.26 ± 3.18	**0.0 ± 0.03**	**-**

PCB-exposed responses were low, providing a REP 50 in only one instance (site UMB 4), where multiple non-acid washed extracts elicited a REP 50 and in some cases even a REP 80 could be determined. Many acid washed extracts had a very low response. This indicates a low congener concentration available to elicit a response, but would need to be confirmed by the viability assay as this could be a result of cytotoxicity. PAH-exposed responses were higher, with many of the sites having a calculated REP 80. %TCDD-max indicates the percentage response elicited by the sample in terms of the maximum luminescence elicited by the most concentrated TCDD—the reference compound. As with the REP values, the %TCDD-max values for the PCBs (1.53–54.69 %TCDD-max) were much lower than those for the PAH-exposed responses (1.68–150.56 %TCDD-max). The PAH %TCDD-max was significantly greater ($p < 0.05$) than the %TCDD-max generated from the PCB extract. The PAH BEQ was also significantly larger than that of the PCB BEQs ($p < 0.05$). This indicates there are significantly more AhR agonists within the non-acid washed (PAH) extract compared to the acid washed (PCB) extract. This was expected considering the non-acid washed extract also contained the persistent compounds (PCBs and PCDD/Fs), which were the only compounds that remained in the acid washed extract, in addition to all other AhR agonists that may have been present. The clean-up methods, however, aimed to target only the compounds of interest.

Super-induction is the ability of extracts to elicit a %TCDD-max that exceeds the maximum induction caused by TCDD—which is the most potent AhR agonist (Larsson *et al.*, 2012). This phenomenon was observed in 11 of the non-acid washed samples (Table 4.8). A possible explanation is that there were other chemicals in the extract capable of vastly enhancing induction of the AhR response. However, these chemicals were not analysed. It has been speculated that proteolysis of the AhR is inhibited and hence intracellular levels of ligand bound AhR is increased, which in turn increases the magnitude of the AhR dependent gene expression. Additionally, an unstable repressor protein has been suspected. When this repressor protein was inhibited of expression or it was degraded, it would be unable to cause repression of the AhR, which would result in an enhance functionality of the AhR and enhanced transcription. But, it is thought a more likely explanation is that the agonists present may co-activate the AhR and the extracts may contain chemicals that influence other cellular signal transduction pathways, amplifying the induction response (Baston and Denison, 2011).

4.6.3 Viability

The analysis of real-time cell growth obtained from the xCELLigence data showed the same general trend throughout almost all samples. For samples that had normal cell viability, the cell index (CI), which is dependent on the number of cells in each well as well as their morphology (Limame *et al.*, 2012), increased exponentially during the growth phase after seeding into the plate (time = 0–24 h; Figure 4.14). Upon dosing, at 24 hours, there was an initial increase of the CI,

possibly due to the change in ion concentration, followed by a decrease in CI to roughly the same CI as before the addition of the extract, as indicated by the black rectangle in Figure 4.14 (time ≈ 24–28 h). The CI increased, from approximately 28–42 hours, and thereafter gradually decreased (Figure 4.14). There were exceptions for some sites, where the CI decreased steadily after dosing the extract. These were sites that had decreased cell viability or increased cytotoxicity and are marked in bold in Table 4.9. However, the decrease in CI for the PAH extract from site ISI 8 was more rapid and began to decrease at the 30th hour, resulting in a very low CI (Table 4.9). Therefore, the non-acid washed (PAH) extract from site ISI 8 was the most toxic.

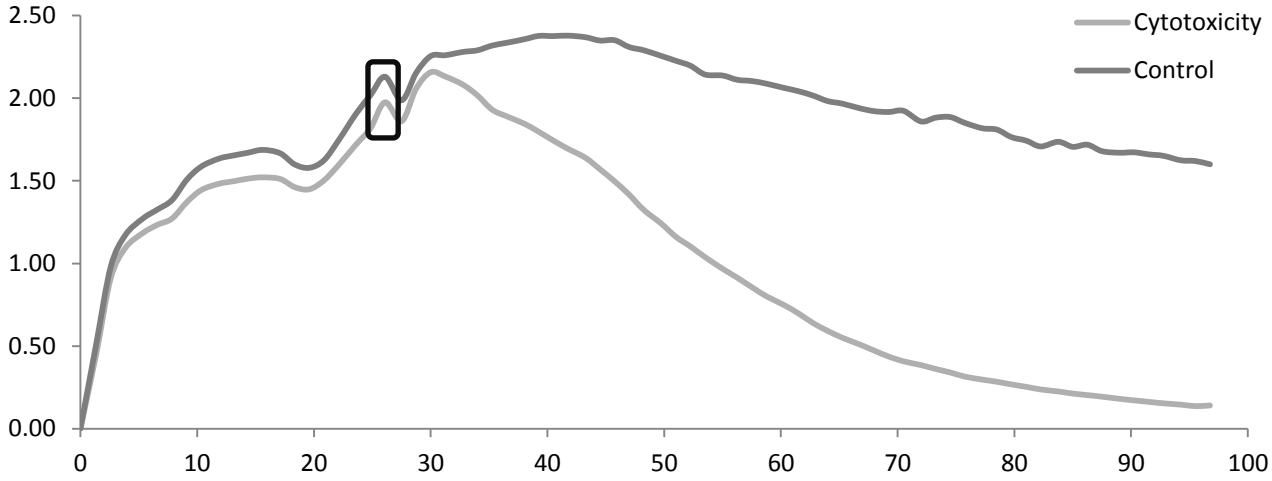


Figure 4.14. Examples of the xCELLigence graphs representing the change of the CI over time from the moment the cells were put into the wells at time = 0 h to almost time = 100 h. The time of dosing is indicated by the black rectangle.

Table 4.9. xCELLigence viability data and %TCDD-max values. Values highlighted in bold indicate that cell viability was reduced to below 75%.

	PCB (acid washed)		PAH (non-acid washed)	
Site	xCELLigence viability %	%TCDD-max	xCELLigence viability %	%TCDD-max
AMA 1	119	31.44	123	150.56
AMA 2	131	28.73	109	84.38
AMA 3	127	20.64	120	70.70
CAN 1	130	17.49	97	126.68
DBAY 1	95	1.65	108	95.12
DBAY 2	84	1.65	102	73.92
DBAY 3	101	25.21	110	139.20
DBAY 4	102	11.26	124	127.94
DBAY 5	92	22.15	121	149.19
DBAY 6	**70**	**13.01**	140	110.09
DBAY 7	**71**	**18.85**	93	90.49
DBAY 8	92	13.34	89	118.99
DBAY 9	93	15.12	83	87.53
DBAY10	**73**	**23.03**	98	98.52
ISI 2	**68**	**19.26**	101	103.28
ISI 4	101	26.42	**73**	**129.62**
ISI 5	87	38.76	**71**	**127.78**
ISI 7	**65**	**17.70**	82	72.57
ISI 8	**72**	**30.37**	**8**	**1.68**
IVC 1	101	22.84	108	4.27

	PCB (acid washed)		PAH (non-acid washed)	
Site	xCELLigence viability %	%TCDD-max	xCELLigence viability %	%TCDD-max
IVC 2	92	13.73	**65**	**84.56**
SOIL 1	**72**	**19.10**	80	14.71
SOIL 2	82	26.40	99	31.53
SOIL 3	105	1.53	87	69.54
SOIL 4	100	26.77	82	116.31
UMB 1	111	24.18	88	58.19
UMB 3	125	9.87	**68**	**36.54**
UMB 4	136	54.69	**72**	**15.54**
UMB 5	137	11.51	105	39.12
UMB 7	142	11.05	79	29.99
UMB 8	140	17.57	**73**	**46.82**
UMB/UMH	117	15.09	**71**	**39.00**
UMH 1	117	3.23	95	19.88
UMH 3	107	9.87	91	26.95
UMH 5	119	11.51	101	42.36
UMH 6	106	10.07	99	17.25

The implication of those extracts causing cytotoxicity was that they had components that inhibited cell growth and health. It is evident from Table 4.9 that where the sample caused reduced viability the resulting BEQ was generally lower in comparison to the BEQs for cells that had normal viability. There were some exceptions, where the cells experienced reduced viability and yet the BEQ was high, and similar to BEQs for cells that did not have increased cytotoxicity—such as IVC 2 PAH or ISI 8 PCB. This indicated there were many AhR ligands present and although these cells experienced reduced health they were still able to elicit a high response from the ligands, or it could be that the ligands present caused super induction of the few cells that survived. Therefore, the BEQ results reported for sites with cytotoxicity are likely an underestimation of the quantity of ligands available to bind to the AhR, and ultimately the BEQ reported might have been higher had the cells not suffered from reduced viability.

4.7 Toxicity testing

In this section a comparison is drawn between the toxicity predicted by the chemical analysis in the form of TEQ and the corresponding bioassay toxicity, or BEQ. This was done to determine if the methods used to determine toxicity provided similar results, because BEQs are directly related to TEQs (Jaikanlaya *et al.*, 2009). The TEQ was calculated by the summation of the isomer/congener concentrations multiplied by their relative TEF value, at each site. The PCB TEQ was calculated using TEF values for fish (Van den Berg *et al.*, 1998) (Table 4.11). This was because of the three animal categories for which TEFs have been developed, fish are the group most likely to be exposed to contaminants in sediment at all sites investigated in this study. PAHs were expressed as having both a TEQ_{TCDD} (Villeneuve *et al.*, 2000) and TEQ_{BaP} (Nisbet and LaGoy, 1992) (Table 4.10). The TEQ_{TCDD}—which relates the isomers in terms of TCDD—were compared to the BEQs generated from the bioassays. TEQ_{BaP}—which relates PAH concentrations to the toxicity expected

due to BaP—was used to determine whether the PAH loads, once the isomers had been converted to their equivalent BaP toxicity, would be in excess of the estuarine and marine, and freshwater SQGs for PAHs (Long *et al.*, 1995, MacDonald *et al.*, 2000).

When the PAH TEQ_{BaP} was compared to the guidelines (Long *et al.*, 1995, MacDonald *et al.*, 2000), only the lower guidelines (*i.e.* ERL and TEC) were exceeded (Figure 4.15). Sites DBAY 3, 5, 7 and 9, ISI 5 and 8, and IVC 1 exceeded the TEC of the freshwater sediment quality guidelines. Site CAN 1 was the only site where the ERL for estuarine and marine sediment quality guidelines was exceeded. Therefore, PAHs at these sites were expected to pose occasional toxicological risks to sediment-dwelling organisms. Of these sites, DBAY 9 was the only site that did not exceed the ΣPAH SQGs (Figure 4.6). However, even though site MNG 18 and SOIL 4 were in excess of the ΣPAH SQG (Figure 4.6), the BaP translated PAH levels did not exceed the levels of the BaP guideline (Figure 4.15).

Table 4.10. PAH TEQ values calculated based on TEF values of TCDD and BaP as the reference compound.

Site	PAH TEQ_{TCDD} $ng.g^{-1}$	PAH TEQ_{BaP} $ng.g^{-1}$
AMA 1	3.56×10^{-3}	43.21
AMA 2	1.78×10^{-3}	26.86
AMA 3	8.26×10^{-4}	12.99
CAN 1	3.26×10^{-2}	612.55
DBAY 1	2.63×10^{-3}	40.69
DBAY 2	1.39×10^{-3}	21.62
DBAY 3	2.04×10^{-2}	311.39
DBAY 4	5.63×10^{-3}	83.55
DBAY 5	1.49×10^{-2}	214.28
DBAY 6	6.64×10^{-3}	90.96
DBAY 7	1.70×10^{-2}	254.71
DBAY 8	5.15×10^{-3}	78.58
DBAY 9	1.09×10^{-2}	153.09
DBAY10	3.31×10^{-3}	52.83
ISI 2	1.24×10^{-3}	50.53
ISI 4	1.29×10^{-3}	20.86
ISI 5	1.13×10^{-2}	358.39
ISI 7	4.22×10^{-3}	105.06
ISI 8	8.52×10^{-3}	221.25
IVC 1	2.08×10^{-2}	321.88
IVC 2	1.20×10^{-3}	17.49
MNG 1	5.50×10^{-4}	51.83
MNG 2	9.56×10^{-4}	27.79
MNG 3	4.44×10^{-4}	18.25
MNG 4	8.36×10^{-4}	36.61
MNG 5	1.43×10^{-4}	6.71
MNG 6	2.54×10^{-3}	50.29
MNG 7	2.09×10^{-3}	80.21
MNG 8	3.71×10^{-4}	11.00

Site	PAH TEQ_{TCDD} $ng.g^{-1}$	PAH TEQ_{BaP} $ng.g^{-1}$
MNG 9	1.83×10^{-4}	6.81
MNG10	1.36×10^{-4}	6.50
MNG 11	2.65×10^{-3}	100.68
MNG 12	1.07×10^{-3}	27.61
MNG 13	5.48×10^{-4}	20.04
MNG 14	6.69×10^{-4}	24.40
MNG 15	2.57×10^{-4}	7.23
MNG 17	3.63×10^{-4}	10.89
MNG 18	3.32×10^{-3}	56.86
MNG 19	1.72×10^{-3}	64.78
MNG 20	2.40×10^{-4}	8.82
MNG 21	3.42×10^{-4}	10.57
MNG 22	1.35×10^{-4}	6.51
SOIL 1	1.35×10^{-4}	6.45
SOIL 2	1.59×10^{-3}	19.33
SOIL 3	2.02×10^{-3}	31.07
SOIL 4	9.34×10^{-3}	116.96
UMB 1	6.01×10^{-4}	21.67
UMB 2	7.08×10^{-4}	25.71
UMB 3	2.22×10^{-4}	7.33
UMB 4	1.44×10^{-3}	23.04
UMB 5	6.55×10^{-4}	11.91
UMB 7	6.98×10^{-4}	9.82
UMB 8	1.18×10^{-3}	14.66
UMB/UMH	5.67×10^{-4}	10.41
UMH 1	3.85×10^{-4}	7.87
UMH 3	2.12×10^{-4}	6.99
UMH 5	3.98×10^{-4}	13.14
UMH 6	1.72×10^{-4}	6.88

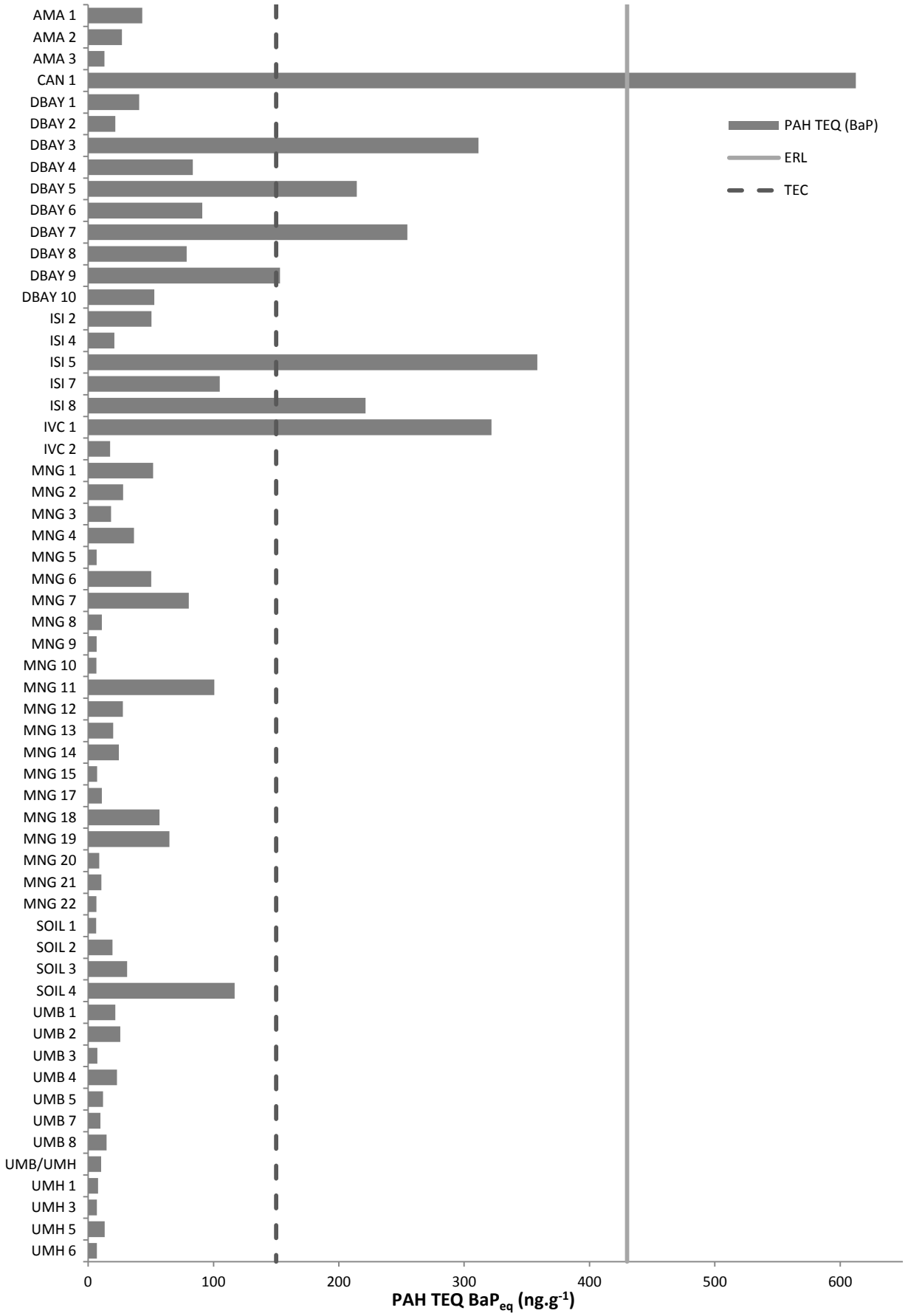


Figure 4.15. BaP TEQ values per site, with the SQG for BaP from the freshwater guidelines of (MacDonald *et al.*, 2000), indicating the TEC and the marine SQG (Long *et al.*, 1995) indicating the ERL.

Table 4.11. PCB and PAH TEQ_{TCDD} and BEQs at each site. The data have also been normalised to 1% TOC to be able to compare to the Canadian SGGs. Values highlighted in bold indicate sites where low viability was recorded. - = sites where the bioassay was not conducted.

.						Normalised to 1% TOC			
Site	TOC (%)	PAH BEQ ($pg.g^{-1}$)	PAH TEQ ($pg.g^{-1}$)	PCB BEQ ($pg.g^{-1}$)	PCB TEQ ($pg.g^{-1}$)	PAH BEQ ($pg.g^{-1}$)	PAH TEQ ($ng.g^{-1}$)	PCB BEQ ($pg.g^{-1}$)	PCB TEQ ($pg.g^{-1}$)
AMA 1	1.45	217.76	3.56	6.22	5.85×10^{-6}	1.50	2.46×10^{-2}	4.29×10^{-2}	4.03×10^{-8}
AMA 2	2.34	171.15	1.78	5.76	0.00	7.31×10^{-1}	7.60×10^{-3}	2.46×10^{-2}	0.00
AMA 3	0.6	269.28	0.83	10.89	0.00	4.49	1.38×10^{-2}	1.82×10^{-1}	0.00
CAN 1	6.84	304.68	32.64	4.82	6.65×10^{-6}	4.45×10^{-1}	4.77×10^{-2}	7.05×10^{-3}	9.72×10^{-9}
DBAY 1	1.97	165.24	2.63	0.00	1.00×10^{-5}	8.39×10^{-1}	1.34×10^{-2}	0.00	5.08×10^{-8}
DBAY 2	0.96	528.49	1.39	0.00	0.00	5.51	1.45×10^{-2}	0.00	0.00
DBAY 3	5.33	165.79	20.40	18.57	1.76×10^{-4}	3.11×10^{-1}	3.83×10^{-2}	3.48×10^{-2}	3.31×10^{-7}
DBAY 4	1.35	634.21	5.63	0.22	8.40×10^{-6}	4.70	4.17×10^{-2}	1.63×10^{-3}	6.22×10^{-8}
DBAY 5	1.22	230.79	14.87	14.18	1.00×10^{-5}	1.89	1.22×10^{-1}	1.16×10^{-1}	8.20×10^{-8}
DBAY 6	2.45	766.08	6.64	**13.01**	1.00×10^{-5}	3.13	2.71×10^{-2}	5.31×10^{-2}	4.08×10^{-8}
DBAY 7	3.17	184.53	16.97	**9.69**	6.70×10^{-5}	5.82×10^{-1}	5.35×10^{-2}	3.06×10^{-2}	2.11×10^{-7}
DBAY 8	2.46	123.37	5.15	1.35	2.18×10^{-5}	5.02×10^{-1}	2.10×10^{-2}	5.49×10^{-3}	8.86×10^{-8}
DBAY 9	2.97	536.61	10.86	1.58	0.00	1.81	3.66×10^{-2}	5.32×10^{-3}	0.00
DBAY 10	3.11	85.04	3.31	**10.56**	2.80×10^{-5}	2.73×10^{-1}	1.06×10^{-2}	3.40×10^{-2}	8.99×10^{-8}
ISI 2	2.5	85.39	1.24	**1.84**	0.00	3.42×10^{-1}	4.96×10^{-3}	7.36×10^{-3}	0.00
ISI 4	6.36	**160.70**	1.29	31.75	7.60×10^{-5}	2.53×10^{-1}	2.03×10^{-3}	4.99×10^{-2}	1.19×10^{-7}
ISI 5	2.36	**162.73**	11.32	35.26	7.20×10^{-6}	6.90×10^{-1}	4.80×10^{-2}	1.49×10^{-1}	3.05×10^{-8}
ISI 7	1.29	108.48	4.22	**17.70**	0.00	8.41×10^{-1}	3.27×10^{-2}	1.37×10^{-1}	0.00
ISI 8	4.07	**0.00**	8.52	5.85	3.12×10^{-5}	0.00	2.09×10^{-2}	1.44×10^{-2}	7.65×10^{-8}
IVC 1	7.07	61.72	20.81	36.74	1.13×10^{-5}	8.73×10^{-2}	2.94×10^{-2}	5.20×10^{-2}	1.60×10^{-8}
IVC 2	2.03	**154.57**	1.20	8.70	0.00	7.61×10^{-1}	5.92×10^{-3}	4.29×10^{-2}	0.00
MNG 1	1.95	-	0.55	-	0.00	0.00	2.82×10^{-3}	0.00	0.00
MNG 2	1.13	-	0.96	-	0.00	0.00	8.46×10^{-3}	0.00	0.00
MNG 3	0.4	-	0.44	-	0.00	0.00	1.11×10^{-2}	0.00	0.00
MNG 4	1.71	-	0.84	-	0.00	0.00	4.89×10^{-3}	0.00	0.00
MNG 5	0.25	-	0.14	-	0.00	0.00	5.72×10^{-3}	0.00	0.00
MNG 6	2.75	-	2.54	-	0.00	0.00	9.23×10^{-3}	0.00	0.00
MNG 7	2.99	-	2.09	-	0.00	0.00	6.98×10^{-3}	0.00	0.00
MNG 8	0.32	-	0.37	-	0.00	0.00	1.16×10^{-2}	0.00	0.00
MNG 9	0.16	-	0.18	-	0.00	0.00	1.14×10^{-2}	0.00	0.00

.						Normalised to 1% TOC			
Site	TOC (%)	PAH BEQ ($pg.g^{-1}$)	PAH TEQ ($pg.g^{-1}$)	PCB BEQ ($pg.g^{-1}$)	PCB TEQ ($pg.g^{-1}$)	PAH BEQ ($pg.g^{-1}$)	PAH TEQ ($ng.g^{-1}$)	PCB BEQ ($pg.g^{-1}$)	PCB TEQ ($pg.g^{-1}$)
MNG 10	0.19	-	0.14	-	0.00	0.00	7.14×10^{-3}	0.00	0.00
MNG 11	2.6	-	2.65	-	0.00	0.00	1.02×10^{-2}	0.00	0.00
MNG 12	1.84	-	1.07	-	0.00	0.00	5.82×10^{-3}	0.00	0.00
MNG 13	0.91	-	0.55	-	0.00	0.00	6.03×10^{-3}	0.00	0.00
MNG 14	0.38	-	0.67	-	0.00	0.00	1.76×10^{-2}	0.00	0.00
MNG 15	0.37	-	0.26	-	0.00	0.00	6.94×10^{-3}	0.00	0.00
MNG 17	0.4	-	0.36	-	0.00	0.00	9.08×10^{-3}	0.00	0.00
MNG 18	5.89	-	3.32	-	3.00×10^{-4}	0.00	5.63×10^{-3}	0.00	5.09×10^{-7}
MNG 19	3.76	-	1.72	-	0.00	0.00	4.58×10^{-3}	0.00	0.00
MNG 20	0.76	-	0.24	-	0.00	0.00	3.15×10^{-3}	0.00	0.00
MNG 21	1.01	-	0.34	-	0.00	0.00	3.39×10^{-3}	0.00	0.00
MNG 22	0.11	-	0.14	-	0.00	0.00	1.23×10^{-2}	0.00	0.00
SOIL 1	2.08	0.88	0.14	**13.26**	0.00	$4.25x\ 10^{-3}$	6.50×10^{-4}	6.38×10^{-2}	0.00
SOIL 2	6.75	38.44	1.59	31.45	0.00	$5.70x\ 10^{-2}$	2.36×10^{-3}	4.66×10^{-2}	0.00
SOIL 3	2.62	99.99	2.02	1.53	0.00	3.82×10^{-1}	7.71×10^{-3}	5.84×10^{-3}	0.00
SOIL 4	9.46	422.38	9.34	7.67	1.96×10^{-4}	4.46×10^{-1}	9.87×10^{-3}	8.11×10^{-3}	2.07×10^{-7}
UMB 1	0.34	67.72	0.60	34.14	0.00	1.99	1.77×10^{-2}	1.00	0.00
UMB 2	0.67	45.07	0.71	0.16	0.00	6.73×10^{-1}	1.06×10^{-2}	2.39×10^{-3}	0.00
UMB 3	0.61	**33.55**	0.22	9.87	0.00	5.50×10^{-1}	3.64×10^{-3}	1.62×10^{-1}	0.00
UMB 4	0.79	**6.84**	1.44	93.54	0.00	8.66×10^{-2}	1.82×10^{-2}	1.18	0.00
UMB 5	0.33	55.16	0.65	11.51	0.00	1.67	1.98×10^{-2}	3.49×10^{-1}	0.00
UMB 7	0.18	29.46	0.70	11.05	0.00	1.64	3.88×10^{-2}	6.14×10^{-1}	0.00
UMB 8	1.41	**55.55**	1.18	4.93	0.00	3.94×10^{-1}	8.38×10^{-3}	3.50×10^{-2}	0.00
UMB/UMH	0.54	**5.53**	0.57	15.09	0.00	1.02×10^{-1}	1.05×10^{-2}	2.79×10^{-1}	0.00
UMH 1	0.22	9.07	0.38	3.27	0.00	4.12×10^{-1}	1.75×10^{-2}	1.49×10^{-1}	0.00
UMH 3	0.28	25.43	0.21	9.87	0.00	9.08×10^{-1}	7.58×10^{-3}	3.53×10^{-1}	0.00
UMH 5	1.03	53.92	0.40	11.51	0.00	5.23×10^{-1}	3.86×10^{-3}	1.12×10^{-1}	0.00
UMH 6	0.27	3.76	0.17	10.07	0.00	1.39×10^{-1}	6.38×10^{-3}	3.73×10^{-1}	0.00

When the TEQ_{TCDD} was compared to the BEQs (Table 4.11) for PAHs, the BEQ values were at least an order of magnitude larger than the TEQ values. The PCB TEQ values were 2 to 3 magnitudes smaller than the BEQs (Table 4.11). Even when the extract caused reduced viability of the cells—thus reducing their ability to elicit a response—the BEQ values were still greater than the TEQ_{TCDD} values.

PCB BEQs may be slightly over estimated because the acid-wash clean-up step does not remove other persistent organic pollutants (*e.g.* PCDD/Fs) that are also AhR agonists and might have eluted with the same fraction during the GPC clean-up. If PCDD/Fs were present in the final extract they would have contributed to the BEQ. This situation might explain the higher BEQ than the calculated TEQ. BEQs of PAHs were also greater than the PAH TEQs, and were due to not having had the acid clean-up. Hence, persistent compounds and any other AhR agonists could bind to the AhR, although this would be a limited portion due to the targeted fraction collection during the GPC clean-up. PCB TEQs are an under-calculation of what is actually possible within the environment because not all the PBCs with TEF values were analysed for and there were multiple instances of congeners present at < MDL and which were treated as zero, whereas for PAHs a half of the MDL was used for the few instances of a < MDL. However, cell bioassays commonly yield BEQ values that are significantly higher than the calculated TEQs (Behnisch *et al.*, 2001, Denison *et al.*, 2004). It is suggested this difference is a result of inequalities in the TEF values used and the bioassay based relative potency, along with the additional agonists present in the extract that have not been analysed for chemically.

The PCB TEQ and BEQ values generally seemed to follow a similar trend—indicating similar increases and decreases in concentration from site to site (Figure 4.16a). Many of the sites did not have measurable TEQ data. However, the BEQ at these sites had a similarly low value. This was displayed in the UMB and UMH sites, except UMB 1, 4 and 8 where the BEQs were higher than the TEQs. Five other sites also had a similar deviation from the trend—increased BEQ and a decreased TEQ. The higher BEQ values at sites where the TEQ was low could be due to other AhR ligands (PCDD/Fs) eliciting an effect. Sites DBAY 1 and ISI 8 showed the opposite tendencies, where BEQs decreased and the TEQs increased. Site ISI 8 had reduced cell viability (Table 4.8) and may explain the deviation.

SQGs, based on TEQ_{TCDD} levels, have been used to compare the BEQ and TEQ results of the PCBs and PAHs (Figure 4.17–Figure 4.19). These guidelines were developed for use with dioxins, but are used here because the mode of action for both PCBs and PAHs is via the AhR, which is shared with the mode of toxicity by the dioxins. Both of the sample extracts used in the bioassay had these persistent compounds present. Therefore, if the BEQ exceeds the guideline there is a high probability the sample mixture will have detrimental effects on the organisms in the

environment. Because South Africa does not have guidelines for monitoring these compounds, guidelines have been used from other countries, namely Canada (CCME, 2008), Japan (Japan EPA, 2002), Netherland (Health Council of the Netherlands, 1996) and Australia (Birch et al., 2007), to assess possible contamination of sites—the SQGs used previously do not have TEQ_{TCDD} limits.

The PCB TEQ values were so low they are not visible on the graph (Figure 4.17) with the BEQ values. Because of this and the fact that MNG sites had no BEQ values, the MNG sites have been excluded from the graph. None of the TEQ values exceeded any of the guideline values. However, it should be noted that the TEQ values could be higher than those reported because not all of the compounds with TEF values were chemically quantified. There were 11 sites, DBAY 3, 5 and 6, ISI 4, 5 and 7, IVC 1, SOIL 1 and 2, UMB 1 and 4, and UMB/UMH, where the TEQ was in excess of the allowable level of the Netherlands for the protection of birds and mammals. This is a cause for concern because these compounds bioaccumulate and biomagnify, so if the levels in the sediment are in excess of the bird and mammal guidelines it is expected that the food sources would contain higher levels than what is found in the sediment-therefore exposing the birds and mammals.

For the PAH data (Figure 4.18), the TEQ values were much lower than the BEQ but unlike the PCB data the TEQ values could mostly be viewed on the graph. Sites CAN 1, DBAY 3, 5 and 7, and IVC 1 had concentrations in excess of the allowable level of the Netherlands for the protection of birds and mammals.

All the PAH BEQs (Figure 4.18) exceeded the Netherlands guideline for bird and mammal protection with the exception of UMB 4, UMB/UMH, UMH 1 and 6. Of these, UMH 6 was the only extract that did not cause reduced viability of the cells (Table 4.8). Thus, in spite of decreased viability, the BEQs were still high. If the viability had not been compromised it is likely the BEQ values would be much greater. All AMA, CAN, DBAY (except 8 and 10) sites, and ISI 4 and 5, IVC 2, and SOIL 4 exceeded Japan's allowable limits. AMA 1 and 3, CAN 1, DBAY 2, 4, 5, 6 and 9, and SOIL 4 were in excess of Netherlands allowable limits for aquatic health. None of the sites exceeded Australia's high level of 1 000 $pgTEQ.g^{-1}$.

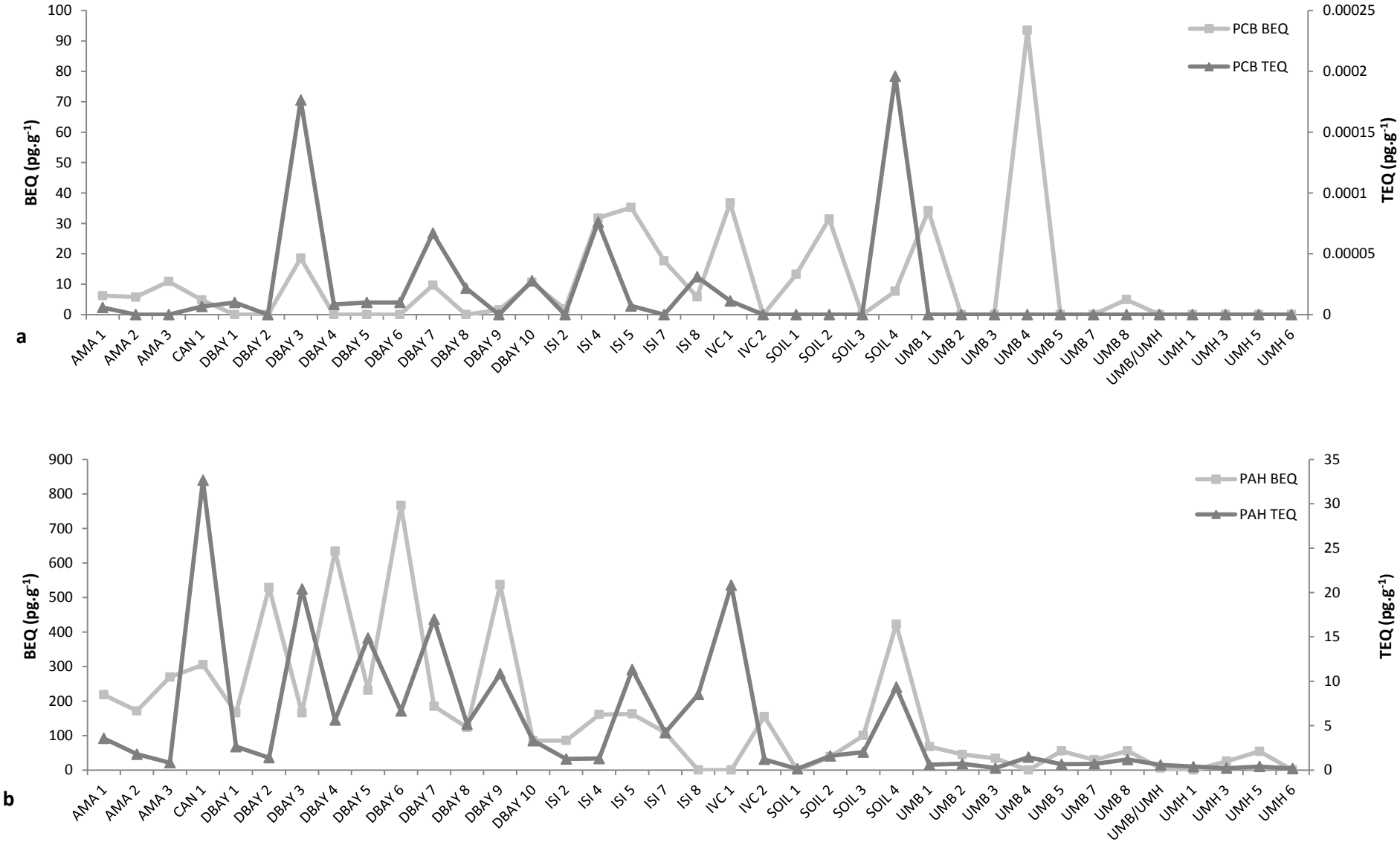


Figure 4.16. Comparisons of BEQs to TEQ_{TCDD} of PCBs (a) and PAHs (b) for sediment and soil samples.

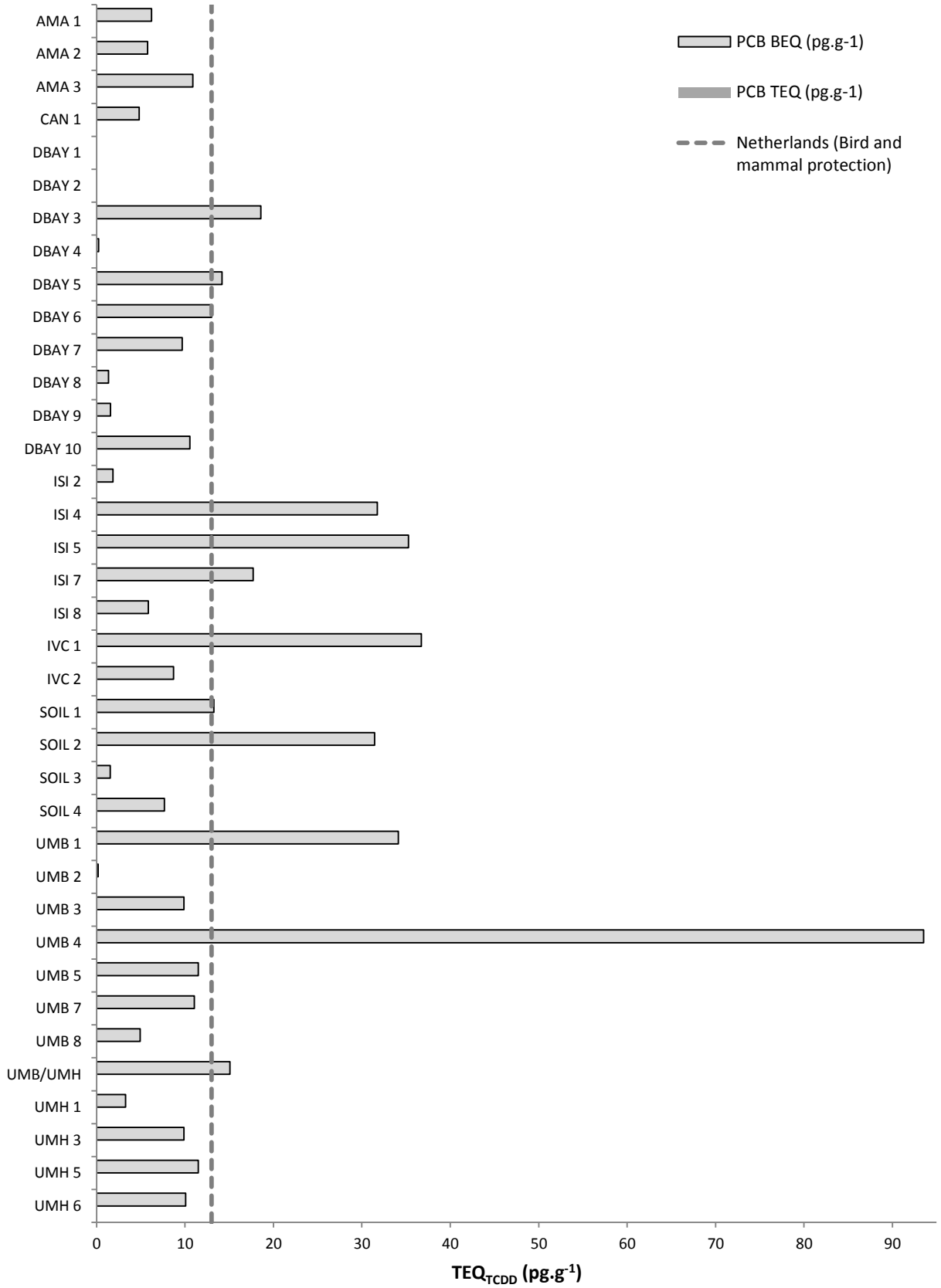


Figure 4.17. PCB BEQs and TEQs compared to TEQ_{TCDD} SQGs. The TEQs had very low concentrations and were not visible on the graph.

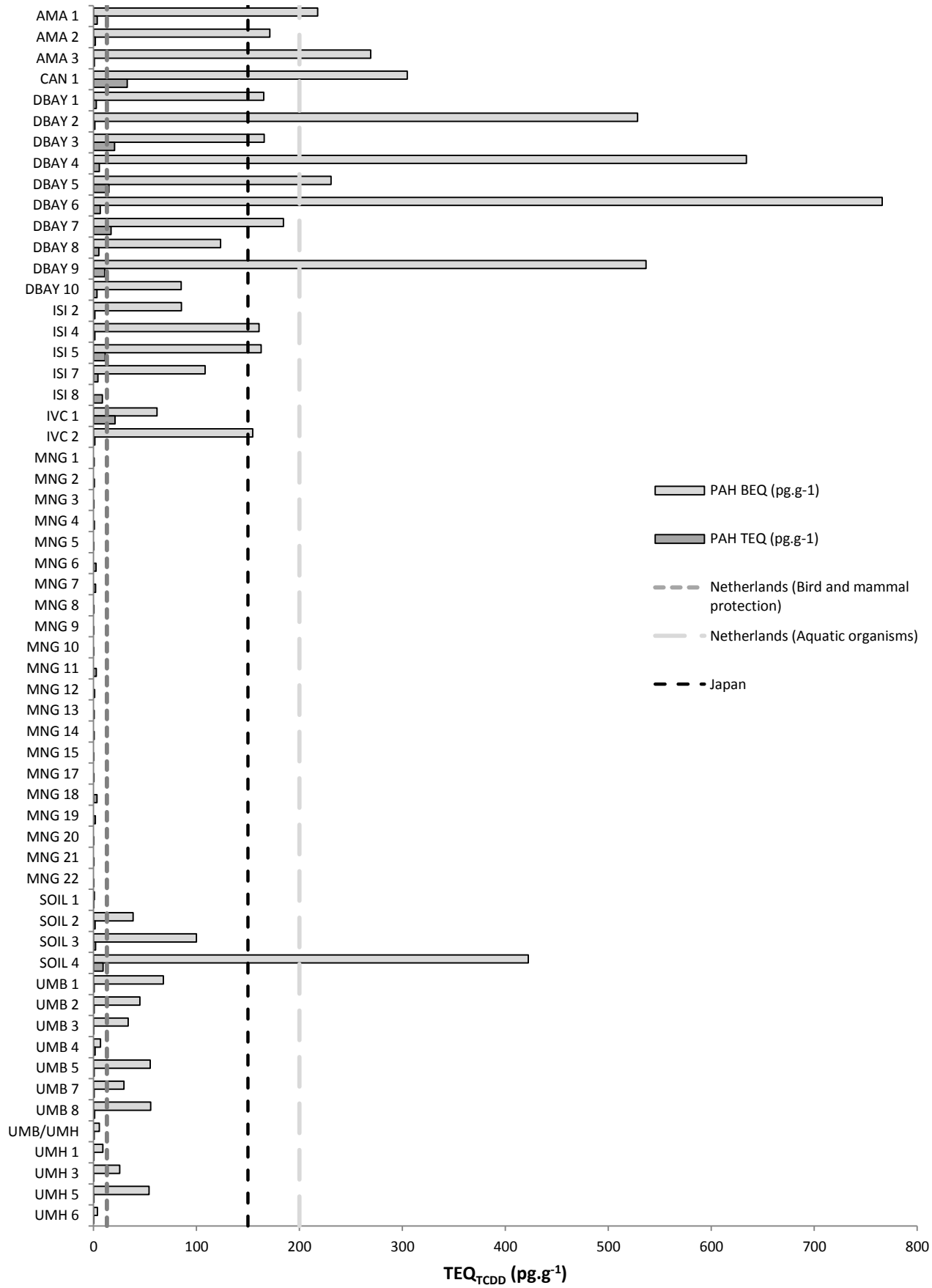


Figure 4.18. PAH BEQs and TEQs compared to TEQ_{TCDD} SQGs of various countries.

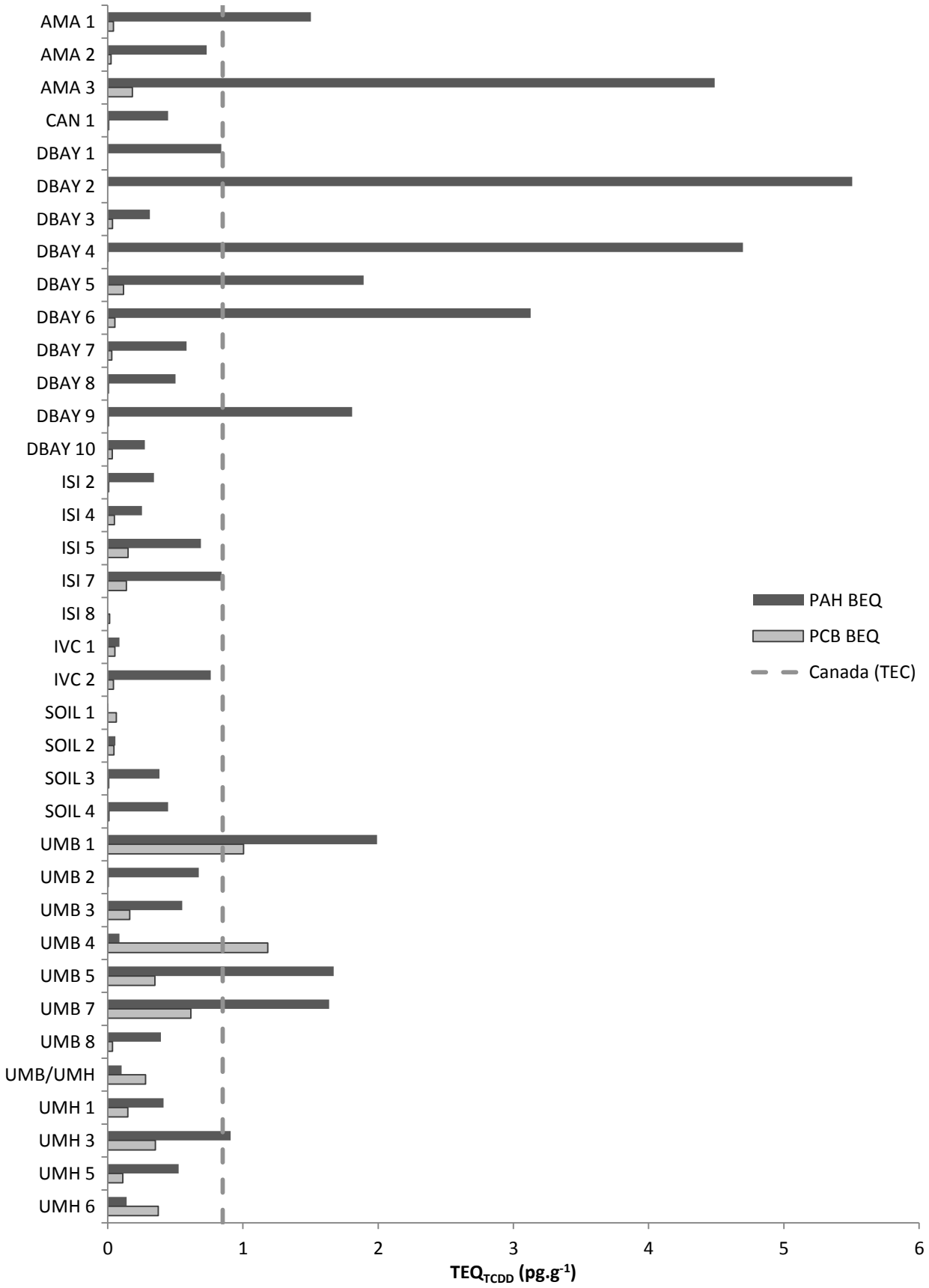


Figure 4.19. PAH and PCB BEQs (expressed as 1% TOC) compared to Canadian TEQ_{TCDD} SQGs.

The BEQ and TEQ data had to be normalised to 1% TOC (Table 4.11) to allow for comparison to the Canadian SQGs (Figure 4.19). Neither the PAH nor PCB TEQ had values high enough to be visualised on the graph and were thus excluded. The BEQ results for both the acid-washed (PCBs) and non-acid washed (PAHs) extracts only exceeded the lower guideline (TEC). Because the graph only consisted of BEQs, the MNG sites were excluded from the graph since no assays were conducted for these sites. Sites AMA 1 and 3, DBAY 2, 4, 5, 7, and 9, UMB 1, 5, 7, and UMH 3 had PAH BEQs in excess of the Canadian TEC. PCB BEQs were in excess of the TEC at sites UMB 1 and 4. This indicates that organisms at these sites may have experienced adverse health effects due to chemical exposure.

On the whole, the study region has levels of pollutants that were in excess of allowable limits in other countries and reduced ecosystem health is thus expected. To identify the sites where adverse effects are expected based on SQGs, the sites which exceeded the ΣPAH and ΣPCB SQGs (Long *et al.*, 1995, MacDonald *et al.*, 2000) have been compared to the sites which had TEQ and/BEQ concentrations that exceeded the Canadian SQGs. The Canadian SQGs have been used because they are the most sensitive.

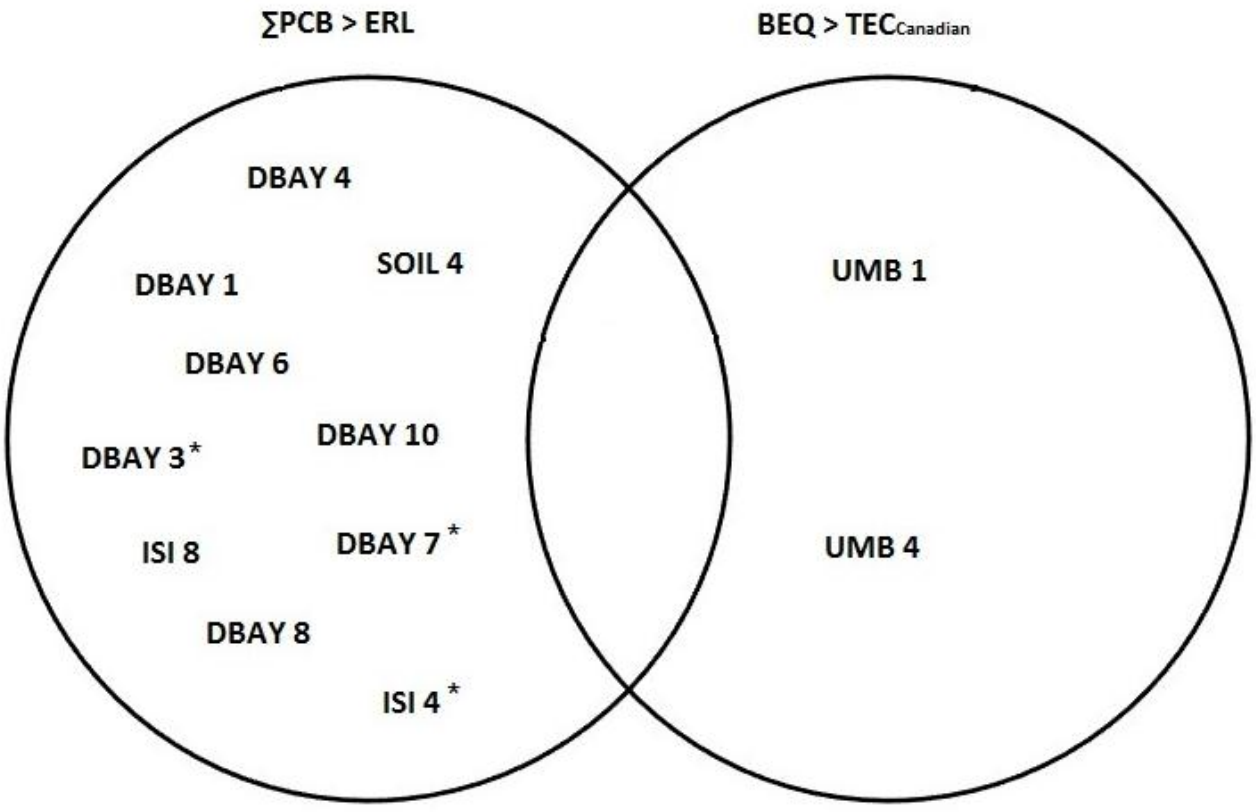


Figure 4.20. Comparison of the sites where the ΣPCBs exceeded the TEC and the ERL (marked with *), and sites where the BEQ exceeded the TEC set out by Canada.

For the comparison of PCBs, there were no calculated TEQs above the Canadian TEC and these were therefore not compared (Figure 4.20). Because there were no bioassays conducted on MNG sites and hence no BEQs for these sites they were excluded from the comparisons. Additionally, none of these sites were in excess of the ΣPCB SQGs used. At 27% of the sites the ΣPCB concentrations exceeded the ΣPCB guidelines (ERL and/or TEC), while at only 5% of sites was a BEQ in excess of the Canadian TEC. When ΣPCB exceedances were compared to BEQ exceedances, there were no sites which exceeded both SQGs. A possible reason for there being no sites in common is that four of the extracts caused cytotoxicity within the assay (Table 4.11) (DBAY 6, 6, 7 and 10, and ISI 8). The two sites that did have BEQ concentrations in excess of the Canadian TEC guideline may have had AhR ligands other than PCBs, such as PCDD/Fs, which elicited the response. It could also be that the ΣPCBs contained a large proportion of ndl-PCBs, which are not able to bind to the AhR and therefore cannot elicit a response.

In the comparisons of PAH SQG exeedances (Figure 4.21), there were again no TEQs that were in excess of the Canadian SQGs. Because there were no bioassays and therefore no BEQs at the MNG sites these were not included in the comparisons. MNG 18 was however, the only MNG site that had ΣPAHs in excess of the guidelines (Figure 4.6). In this comparison the BEQ, ΣPAH and BaP_{eq} are dealt with. At 30% of the sites BEQ concentrations were in excess of the TEC of the Canadian SQG. When the PAHs were converted to a BaP equivalent, 19% of the sites had concentrations in excess of the TEC, and CAN 1 was also in excess of the ERL. At 27% of the sites had concentrations that were in excess of the ΣPAH TEC, and concentrations at 5 of these sites were also in excess of the ERL. There were sites where concentrations exceeded more than one of the SQGs. Concentrations at sites DBAY 3 and 7, CAN 1, IVC 1 and ISI 5 and 8 were in excess of the TEC of the Canadian SQGs and the TEC guideline for BaP. This is expected because the BaP_{eq} converted all PAH isomers into a ratio of the potency of BaP, the most toxic PAH. At site AMA 1, concentrations exceeded the BEQ and ΣPAH guidelines. It is likely this site had potent concentrations of isomers chemically analysed. DBAY 5 was the only site which exceeded all the Canadian TEC, the ΣPAH TEC and the BaP TEC.

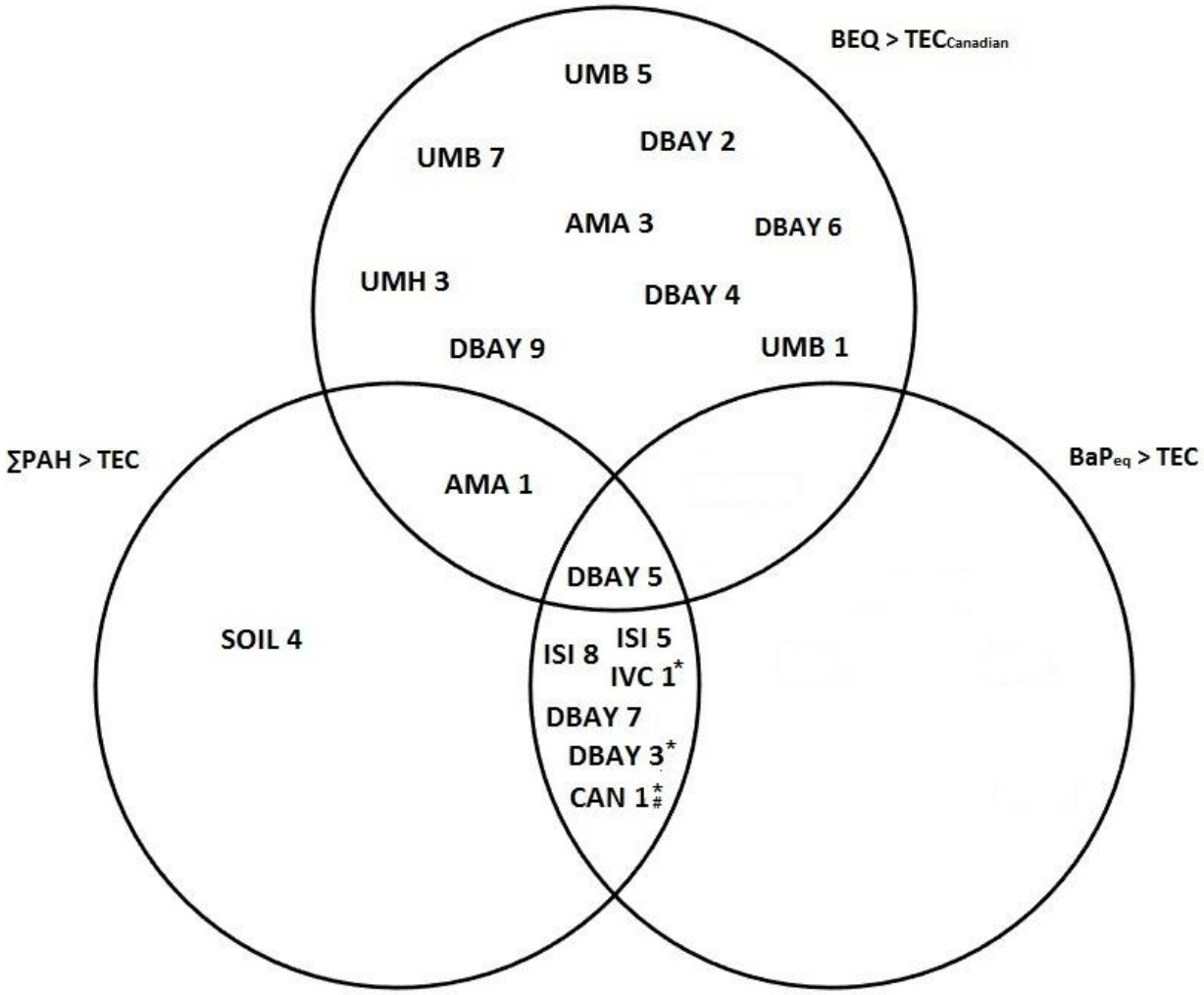


Figure 4.21. Comparison of sites where the ΣPAHs exceeded the TEC and the ERL (marked with *), where concentrations exceeded the TEC set out by Canada, and sites where concentrations exceeded the TEC and ERL (marked with #) for BaP.

4.8 System contamination

The contaminants under consideration (PAHs and PCBs) are known to accumulate in sediment, and estuaries are the ultimate sink for contaminants because they are of a depositional nature. In light of this the PAH and PCB concentrations and the relative toxicities (TEQs and BEQs) were mapped to identify trends in concentrations. The maps were generated by myself using ArcGIS10.1.

The SOIL sites (Figure 4.22–Figure 4.24) are within subsistence farming regions. SOIL 1–3 had much lower concentrations of ∑PAH, while SOIL 4 had a much higher concentrations in comparison to all the other SOIL sites (Figure 4.22) (Table 4.1). SOIL 2 and 3 had similar PAH concentrations and SOIL 1 had almost negligible concentrations. SOIL 1 and 3 had pyrogenic PAH emission sources (Figure 4.8 and Figure 4.10), indicative of combustion processes and HMW PAHs were also dominant (Table 4.1). SOIL 2 and 4 had emission sources that were petrogenic and pyrogenic in nature and were dominated by LMW PAHs (Table 4.1). It is likely the SOIL sites

received PAH loadings mainly from atmospheric deposition from the many factories in the area—possibly from the industrial area alongside SOIL 4—and were transported in a south westerly direction. This would explain the declining concentrations at consecutive sites. The PAH loadings could possibly also have been from agricultural practices, such as the burning of plant waste.

Of the SOIL sites (Figure 4.23), the highest PCB concentrations were at SOIL 4 followed by SOIL 3, while no PCBs were detected for SOIL 1 and 2. SOIL 3 had only dl-PCBs, whereas SOIL 4 has similar levels of dl-PCBs and ndl-PCBs. The source of PCBs at SOIL sites was probably PCBs directly from industries in the vicinity rather than irrigation water.

The SOIL sites show different toxicity when compared to what was indicated with the chemical data–high BEQs due to persistent compounds (acid washed extract), especially in SOIL 1 and 2, where the chemical analysis resulted in no detection. These BEQ results may be due to other AhR ligands in the extracts, such as PCDD/Fs. The other toxicity that was recorded for SOIL 1 and 2 was negligible. SOIL 3 had measurable relative potencies. However, the levels were below any guideline. None of the sites had BEQ concentrations in excess of the Canadian SQGs, however, the PAH and PCB loadings exceeded the ERL (Figure 4.6 and Figure 4.7).

The ISI sampling sites did not have multiple downstream sites, except ISI 8 which was followed by ISI 5, and ISI 7 followed by ISI 4 (Figure 4.22). ISI 5 had higher ΣPAHs than ISI 8, and both these sites had a high ratio of HMW to LMW PAHs (Figure 4.22, Table 4.1). ISI 4 did not show an increase in the levels of PAHs in comparison to ISI 5. ISI 4 had a lower than average PAH load when correlated with %TOC, indicating the sediment retention was not a reason for having low PAH concentrations (Table 4.1). ISI 2 had a low load of PAHs in regards to what was upstream, although it is not directly downstream of these sites. The ISI sites were dominated by HMW PAHs (Table 4.1), which are formed by high temperature combustion and these are more resistant to degradation. The source determination (Figure 4.8–Figure 4.10) supports this, as the PAHs at these sites were characterised as having a pyrogenic source formed mainly from the combustion of grass, wood and coal. Site ISI 4 was characterised by a combination of both petrogenic and pyrogenic derived PAHs.

There was not a definite trend for PCBs at ISI sites (Figure 4.23). Sites ISI 4 and 8 had high concentrations of PCBs, and ISI 5 with lower concentrations, all of which were dominated by ndl-PCBs (Table 4.2). Site ISI 2 had very low concentrations of ndl-PCBs and ISI 7 was below the detection limit (Table 4.2). None of these sites deviated from the relationships between PCBs and the %mud or TOC (Figure 4.3), which indicates these sediment characteristics did not influence the concentrations detected. There may have been some other factor causing the reduction in the PCB

levels, or these sites experienced point source emissions from the nearby industries or electrical capacitors.

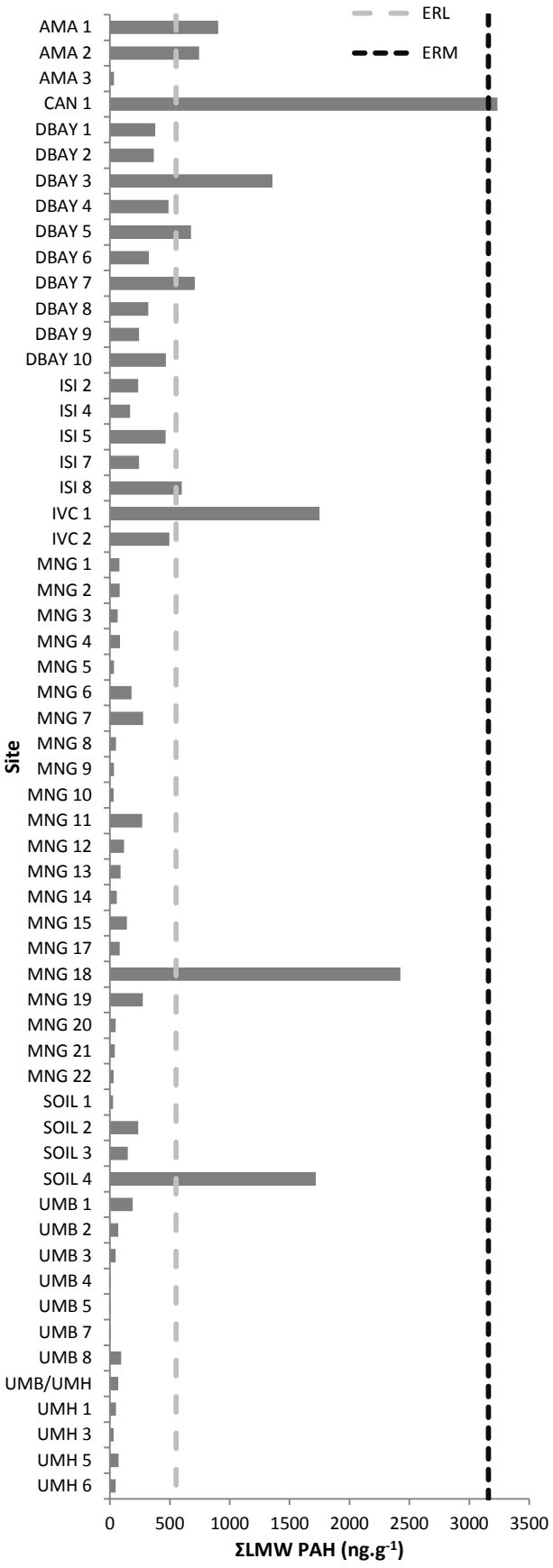
The ISI sites showed a downstream increase in the PAH and PCB BEQ results, where ISI 5 and 4 had higher concentrations than upstream sites ISI 8 and 7 (Figure 4.24, Table 4.11). The persistent extract (PCB) BEQ elicited a greater response than the toxicity calculated by the PCB TEQ (these values were adjusted to be visible on the map). This effect may have been due to other persistent AhR agonists in the extracts. But neither the PAH (non-acid washed) nor PCB (acid washed) BEQs exceeded the Canadian TEC guidelines (Table 4.11, Figure 4.18). Site ISI 8, which displayed serious cytotoxicity—8% viable cells (Table 4.9)—therefore, could have had more AhR ligands than what was indicated. None of the TEQs for PCBs or PAHs exceeded the Canadian SQGs either. ΣPAHs at sites ISI 5 and 8 exceeded the ERL for marine sediment (Figure 4.6). The concentrations of ΣPCBs at site ISI 8 exceeded the ERL, and ISI 4 exceeded both the ERL and the TEC guidelines (Figure 4.7). Concentrations at all ISI sites exceeded at least one of the SQGs and adverse effects to sediment-dwelling organisms were thus possible.

Few PAH isomers were present at quantifiable concentrations at MNG sites (Appendix A.1, Figure 4.25). The highest concentrations were at MNG 18, which was dominated by LMW_{23} PAHs (Table 4.1). The PAHs at this site had petrogenic and pyrogenic sources (Figure 4.8–Figure 4.10). Sites MNG 6, 7, 11 and 19 showed higher levels of PAHs in comparison to the remaining sites. These sites were dominated by HMW PAHs (Table 4.1). PAHs at site MNG 6 had petrogenic and pyrogenic sources, while at other sites the PAHs were pyrogenic in nature (Figure 4.8–Figure 4.10). There was no clear downstream increase in PAH concentrations. It is possible that the PAHs at site MNG 18 were derived from the industrial area immediately alongside this site.

The PCB concentrations at MNG sites were generally low, with concentrations at most sites (14 of the 21) < MDL (Table 4.2, Figure 4.26). Sites MNG 7, 11 and 18 had the highest PCB concentrations within this system. Site MNG 18 was dominated by dl-PCBs, whereas all the other sites were dominated by ndl-PCBs (Table 4.2). These sites were probably contaminated very near to the sample site because there were no clear increases in concentrations at downstream sites.

No bioassays were conducted for MNG sites, therefore no BEQ values were reported (Figure 4.27). MNG 18 had a clear TEQ due to PCBs, and this was due to the high dl-PCB concentration (Table 4.11). However, even with the high concentration neither site MNG 18 nor any other MNG site had PCB TEQ levels that exceeded the Canadian TEC guideline. None of the PAH TEQs exceeded the Canadian TEC (Figure 4.19). ΣPAHs exceeded the freshwater TEC at sites MNG 18 (Figure 4.6). This indicated that there was low level contamination across this system, and

sediment-dwelling organisms at site MNG 18 were likely to experience occasional adverse effects due to exposure to PAHs.



Within the UMB catchment there was fairly low PAH contamination in comparison with other systems (Figure 4.28, Table 4.1). Site UMB 1 was dominated by ΣLMW_{23} PAHs (Table 4.1) and had the highest PAH concentration. Since site UMB 1 and was in the lower reach of the system, it was anticipated that high PAH concentrations might also be evident at sites upstream—UMB 2, 4 and 8. However, sites upstream were dominated by HMW PAHs and not LMW PAHs, like at site UMB 1. Therefore, the fact that site UMB 1 had the highest PAH concentration did not indicate a progressive increase in PAH concentrations at sites situated downstream. The source determination indicated that PAHs in the system originated from the combustion of grass, wood and coal. Even site UMB 1, which had a higher ratio of LMW PAHs, the PAH source was also diagnosed as pyrogenic (Figure 4.8–Figure 4.10). There are few industrial areas along the UMB system, yet where there are industrial areas the PAH concentrations at adjacent or nearby sites were elevated and suggest the contamination may have originated from industries (Figure 4.28).

Sites in the UMH system had low PAH concentrations in comparison to other systems and did not show a distinct downstream pattern. Rather, concentrations varied erratically throughout the system (Figure 4.28). Sites UMH 1 and 5 had the highest concentrations, and all the sites were contaminated by similar proportions of HMW and LMW PAHs, but in all cases the HMW concentration was higher (Table 4.1). The sites all had emission sources diagnosed as pyrogenic (Figure 4.8–Figure 4.10). The UMB/UMH site, situated below the point where the Umhlatuzana and the Umbilo Rivers merge, did show an elevated PAH concentration in comparison to sites upstream in the UMB and UMH systems (Table 4.1).

AMA sites showed a definite increase in PAH concentrations in the lower reach (Figure 4.28). Site AMA 3 was dominated by ΣHMW_{23} PAHs, while sites AMA 1 and 2 had similar concentrations of ΣLMW_{23} and ΣHMW_{23}. PAHs at sites AMA 1 and 2 were categorised as having a mixed source while PAHs at site AMA 3 were categorised as having a pyrogenic source (Figure 4.8–Figure 4.10).

There was only one instance of PCB concentrations in the UMB and UMH system which exceeded the MDL at site UMB 4 in an industrial area and at a very low concentration (Figure 4.29, Table 4.2). Within the AMA system, sites AMA 1 and 3 had PCB concentrations that exceeded the MDL mainly of a ndl-PCB type, while at site AMA 2 the concentration was < MDL (Figure 4.29, Table 4.2).

The PAH and PCB TEQs were so low that they were negligible for the UMB and UMH sites, and none exceeded the TEC of the Canadian SQGs (Figure 4.30). Sites UMB 1, 5 and 7, and UMH 3 had a PAH BEQ level that was in excess of the Canadian TEC (Figure 4.18). Sites UMB 1 and UMH 3 had PCB BEQs that exceeded the Canadian TEC, and were the only sites from the whole

study that had levels in excess of the Canadian TEC (Figure 4.17). None of the chemical concentrations at these sites exceeded the marine or freshwater SQGs (Figure 4.5Figure 4.6). These systems are expected to occasionally experience adverse effects. The toxicological data does not indicate a clear downstream trend.

LMW PAHs at sites AMA 1 and 2 exceeded the ERL of the marine SQGs (Figure 4.5a), yet the ΣPAHs were below the marine and freshwater guideline levels. The PAH BEQs at AMA 1 and 3 exceeded the Canadian TEC guideline (Figure 4.19). The BEQ of acid washed (PCBs) extract and the PCB and PAH TEQs did not exceeded the Canadian SQGs (Figure 4.19). There was no clear trend in toxicity among the sites into the downstream regions. According to the non-acid washed (PAH) BEQs these sites are cause for concern.

DBAY and the surrounding canals (IVC and CAN) had the highest levels of PAHs for any system studied (Figure 4.31). The sites were dominated by ΣHMW_{23} PAHs except for sites DBAY 1, 2 and IVC 2, which were dominated by ΣLMW_{23} PAHs (Table 4.1). A similar trend was seen in that sites with the highest LMW PAH concentrations were identified as having a petrogenic and pyrogenic source, while sites dominated by HMW PAHs were diagnosed as pyrogenic in nature (Figure 4.8–Figure 4.10). Sites CAN 1, DBAY 3 and 5 were outliers in the relationship between the PAH concentration and %TOC present (Figure 4.1), and sites DBAY 3, CAN 1 and IVC 1 were also outliers in the relationship between the PAH concentrations and %mud (Figure 4.2). This indicated that these sites were highly contaminated by PAHs. Site DBAY 10, which was situated downstream of the AMA sites, showed no continuation of the trend seen in PAH concentrations within the AMA system.

High concentrations of PCBs were measured in the DBAY and surrounding canals (IVC and CAN) (Figure 4.32). All of the sites except IVC 2 had detectable concentrations of PCBs, and the dl-PCBs were dominant. The relationship between PCB concentrations and TOC and %mud indicated that DBAY 3 and 7 were outliers and were highly contaminated (Figure 4.3 and Figure 4.4).

The DBAY region and surrounding canals showed some form of toxicity at each site (Figure 4.33). The LMW PAH concentration at sites DBAY 3, 5, 7 and IVC 1 exceeded the marine ERL, while the LMW PAH concentration at site CAN 1 exceeded the marine ERL and ERM (Figure 4.5a). A similar trend was seen with the HMW PAHs, where the concentrations at sites CAN 1, DBAY 3, 5 and 7, and IVC 1 exceeded the marine ERL (Figure 4.5b). Overall, the sites in this region seemed to have high concentrations of ΣPAHs as well, with the concentrations at DBAY 5 and 7 exceeding the freshwater TEC and sites CAN 1, DBAY 3 and IVC 1 having concentrations that exceeded the marine ERL (Figure 4.6). Sites DBAY 2, 4, 5, 6 and 9 had non-acid washed (PAH) BEQ results that

were in excess of the Canadian TEC (Figure 4.19). DBAY 1, 4, 6, 8, and 10 had levels of ΣPCBs that exceeded the marine ERL, and the concentrations at DBAY 3 and 7 also exceeded the PEC (Figure 4.7). TEQ for PAHs and PCBs and BEQs for the acid washed extract did not exceed the Canadian TEC guideline. In general, sites DBAY 3, 5, 7, CAN 1 and IVC 1 show highly concentrations for PAHs and PCBs alike.

The range of PAH_{16} concentrations found in this study (14.1–5 186.3 $ng.g^{-1}$) only Durban Bay and surrounding the canals had concentrations of PAHs that were comparable, or higher than other areas. In a study in El Paso, Texas, soil samples taken from industrial areas had ΣPAH_{16} concentrations in the range of 0.1–2 225.5 $ng.g^{-1}$ (De La Torre-Roche *et al.*, 2009). PAHs found in sediments of from Xiamen Bay, China, in a region known for having PAH contamination, concentrations ranged from 203.7–1 590.5 $ng.g^{-1}$ (Li *et al.*, 2010). Levels of PAH_{16} in Sydney Harbour, Australia, varied from 100 to as much as 380 000 $ng.g^{-1}$ (Birch *et al.*, 2008). In a study on PAH concentrations in soil and sediment collected in the industrialised Vaal Triangle in central South Africa, the ΣPAH_{16} concentration ranged from 44–39 000 $ng.g^{-1}$ (Nieuwoudt *et al.*, 2009)

In a study by Batterman *et al.* (2009), overlapping with the area between Durban Bay (DBAY) and Isipingo (ISI), soil had a maximum ΣPCB_{38} of 109.64 $ng.g^{-1}$**,** which was in the range reported in this study (0–113.83 $ng.g^{-1}$). The concentrations for 12 PCBs in soil in the industrialised Vaal Triangle were higher, ranging between 120 and 4 700 $ng.g^{-1}$ (Nieuwoudt *et al.*, 2009). A study based in Michigan, USA, in the Saginaw Bay region, showed higher concentrations with a maximum ΣPCB concentration of 1 520 $ng.g^{-1}$ (Kannan *et al.*, 2008).

From the results it can be deduced that to fully gauge toxicity utilising one method is not sufficient. Using any one method it can be seen that some sites were not deemed to be cause for concern, whereas another method showed the opposite. Estimating toxicity with bioassays does have limitations—a non-acid-washed sample may elicit responses from all possible AhRs, while the acid-washed extract may elicit a response from all persistent compounds (PCDD/Fs). This can be remedied by not focussing solely on PAHs or PCBs, but all compounds which could bind to the AhR. Additionally, the cells may experience cytotoxicity and the true response cannot be determined. It is usually impossible, due mainly to financial constraints, to perform chemical analysis on a full suite of compounds, whether it be PAHs, PCBs or dioxins (PCDD/Fs). It would be more efficient to analyse for the priority PAHs and dioxin-like compounds (dl-PCBs, PCDD/Fs), which are more likely to have adverse health effects and also have TEF values. This should be done in conjunction with bioassays, which can estimate a concentration of any additional components that may have been present in the sample. A drawback of using any of these methods is that when the sediment samples were extracted the compounds were forced into solution by the

various extraction methods, and may not all have been easily available in the collection environment.

Figure 4.22. Map of the Isipingo River and surrounds, showing the ΣPAHs, ΣLMW and ΣHMW PAH concentrations (ng.g^{-1} dw).

Figure 4.23. Map of the Isipingo River and surrounds, showing ΣPCBs, Σdl PCBs and Σndl PCB concentrations (ng.g^{-1} dw).

Figure 4.24. Map of the Isipingo River and surrounds, showing the relative toxicity caused by PAHs and PCBs as determined using TCDD TEF values (TEQ) and the BEQs. To make all values visible on the same bar graph the values have been adjusted: PCB TEQ x10^{5} pg.g^{-1}, PAH BEQ x10^{-1} pg.g^{-1}, and the PAH TEQ and PCB BEQ have been recorded as pg.g^{-1}.

Figure 4.25. Map of the Mngeni River and surrounds, showing the ΣPAH, ΣLMW and ΣHMW PAH concentrations (ng.g^{-1} dw).

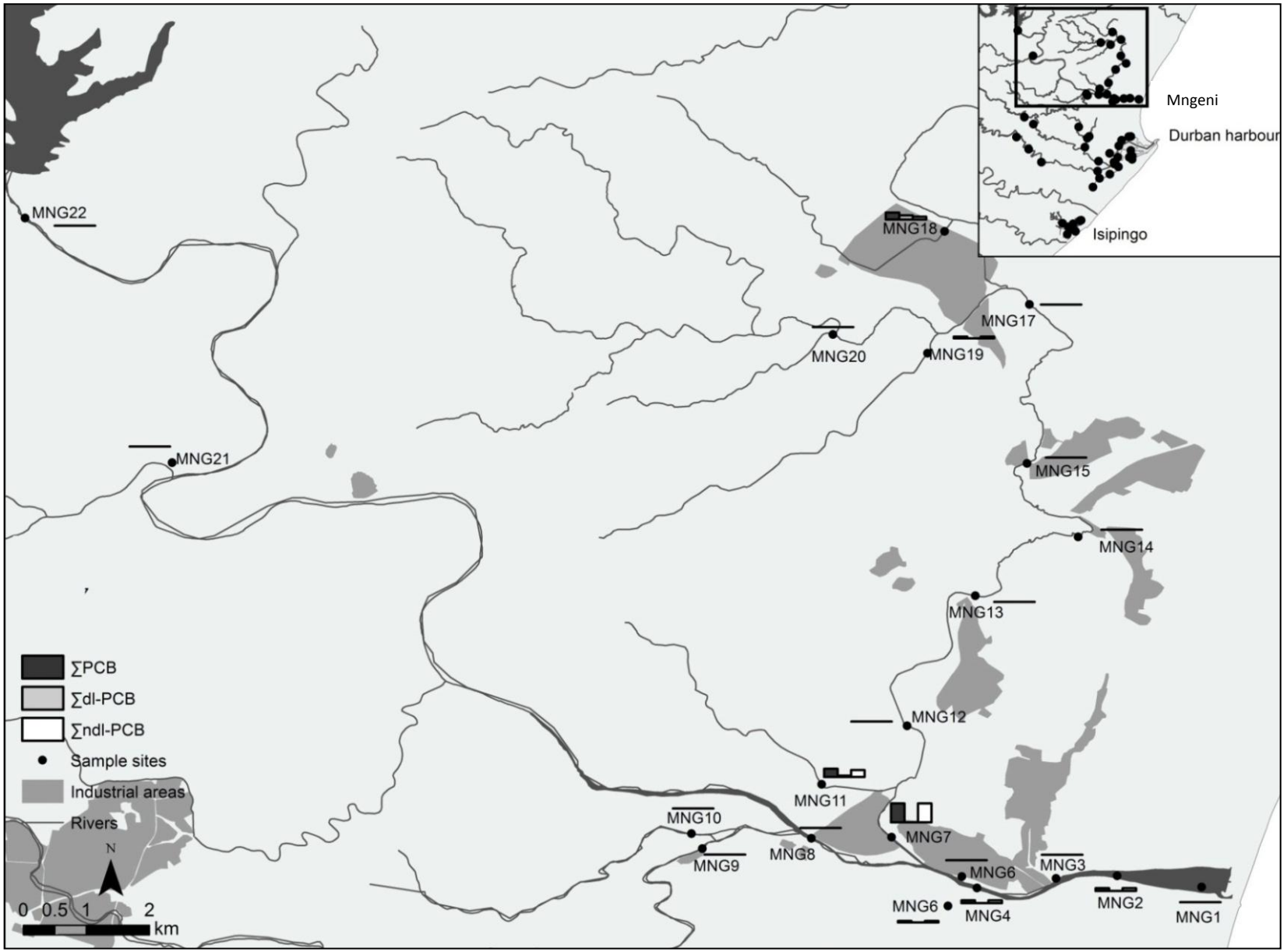


Figure 4.26. Map of the Mngeni River and surrounds, showing the ΣPCBs, Σdl PCBs and Σ non-dl PCBs concentrations (ng.g^{-1} dw).

Figure 4.27. Map of the Mngeni River and surrounds, showing the relative toxicity caused by PAHs and PCBs as determined using TCDD TEF values (TEQ) and the BEQs. To make all values visible on the same bar graph the values have been adjusted, PCB TEQ x10^{5} pg.g^{-1}, PAH BEQ x10^{-1} pg.g^{-1}, and the PAH TEQ and PCB BEQ have been recorded as pg.g^{-1}.

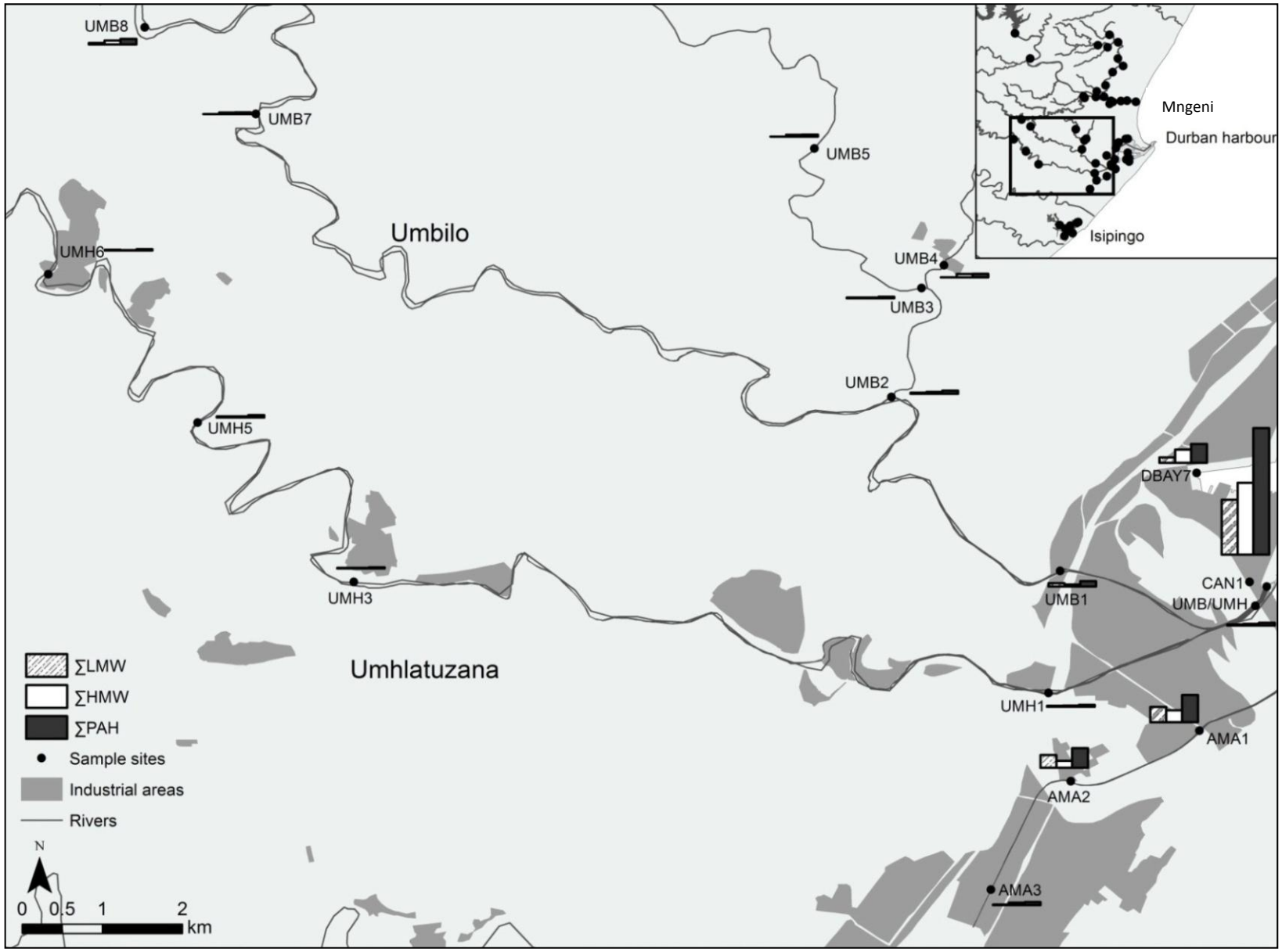


Figure 4.28. Map of the Umbilo and Umhlatuzana Rivers, showing the ΣPAHs, ΣLMW and ΣHMW PAH concentrations (ng.g^{-1} dw).

Figure 4.29. Map of the Umbilo and Umhlatuzana Rivers, showing the ΣPCBs, Σdl PCBs and Σndl PCB concentrations (ng.g^{-1} dw).

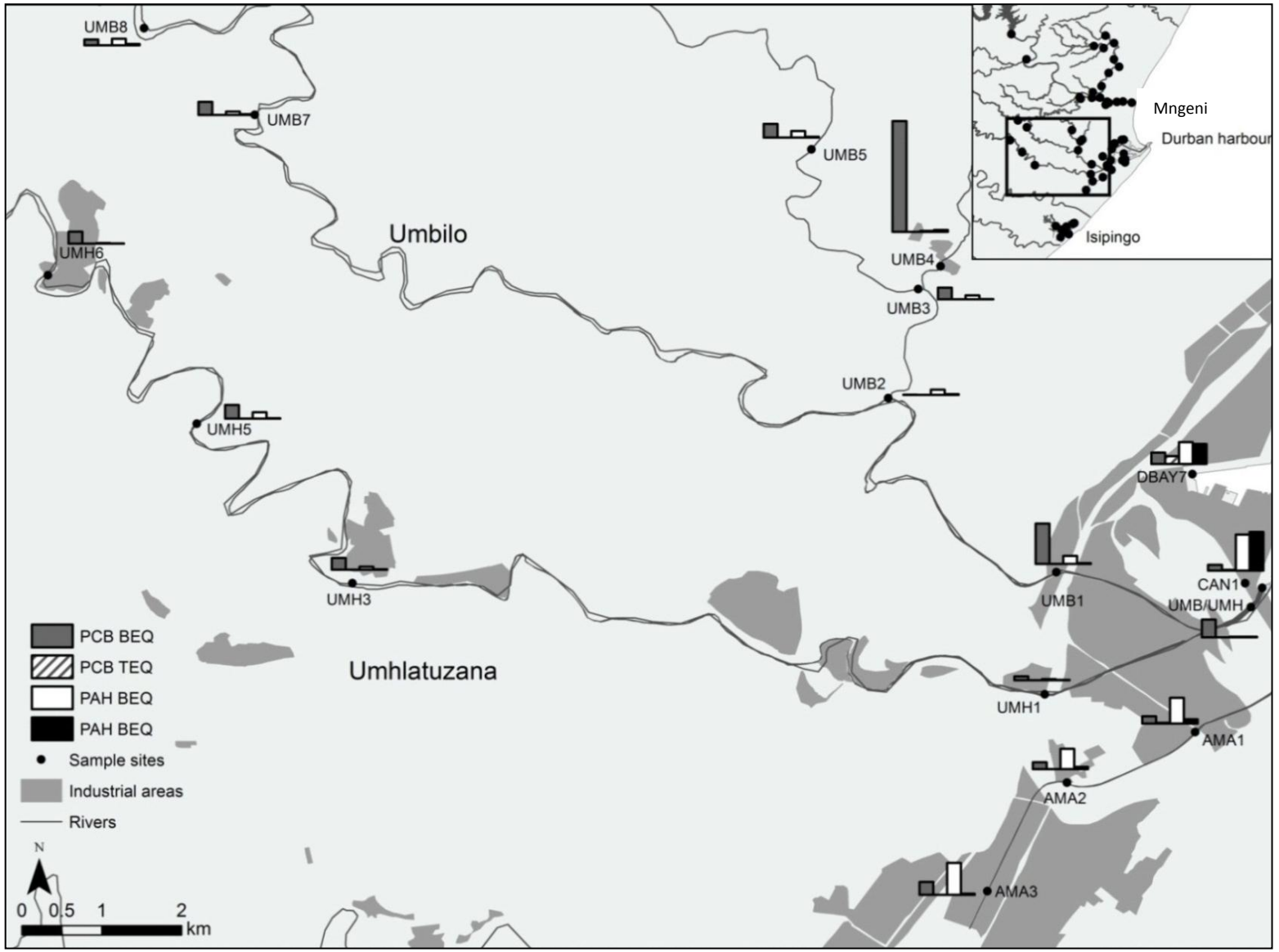


Figure 4.30. Map of the Umbilo and Umhlatuzana Rivers, showing the relative toxicity caused by PAHs and PCBs as determined using TCDD TEF values (TEQ) and the BEQs. To make all values visible on the same bar graph the values have been adjusted, PCB TEQ x10^{5} pg.g^{-1}, PAH BEQ x10^{-1} pg.g^{-1}, and the PAH TEQ and PCB BEQ have been recorded as pg.g^{-1}.

Figure 4.31. Map of Durban Bay showing the ΣPAHs, ΣLMW and ΣHMW PAH concentrations (ng.g^{-1} dw).

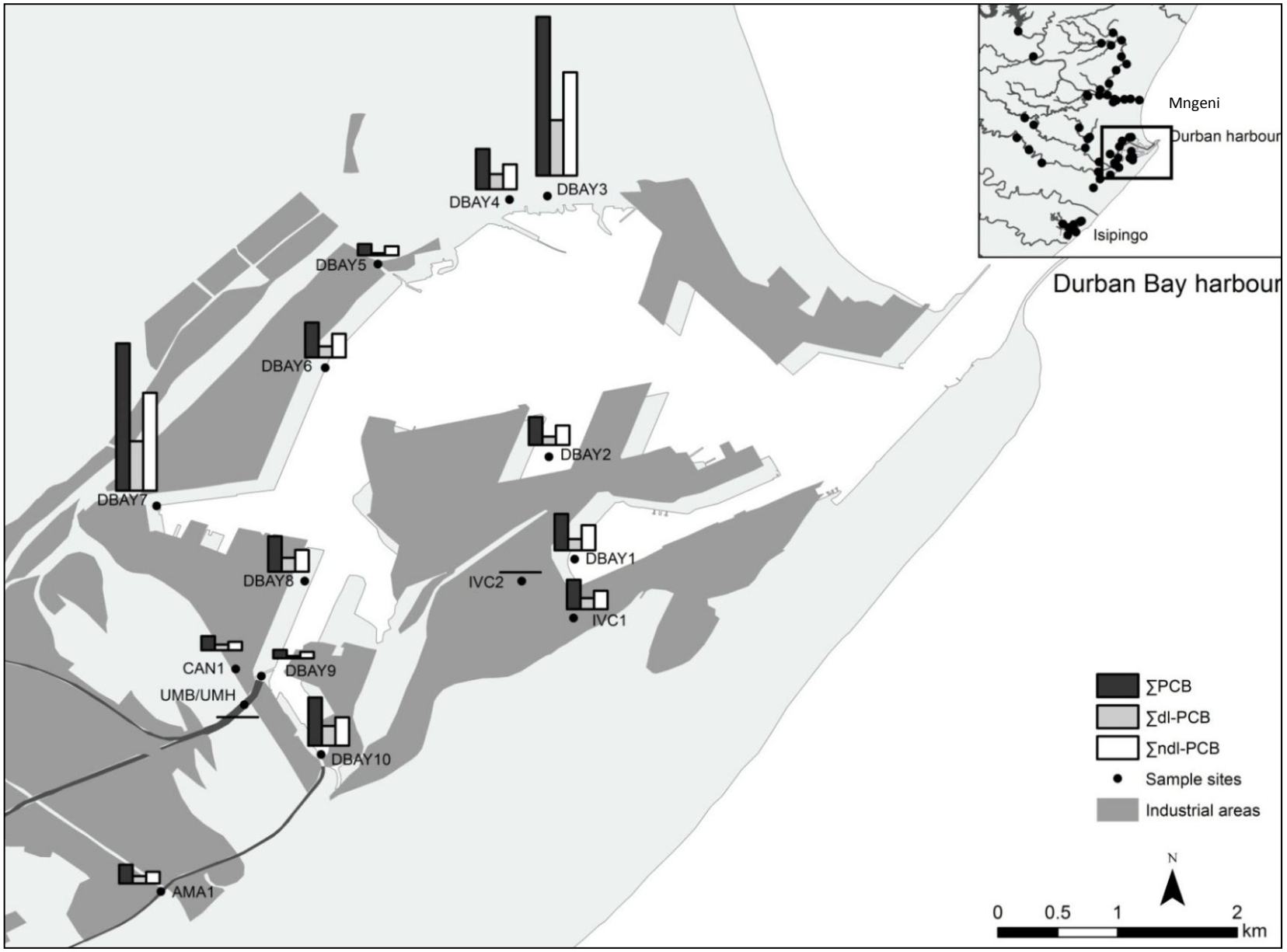


Figure 4.32. Map of Durban Bay showing the ΣPCBs, Σdl PCBs and Σndl PCB concentrations (ng.g^{-1} dw).

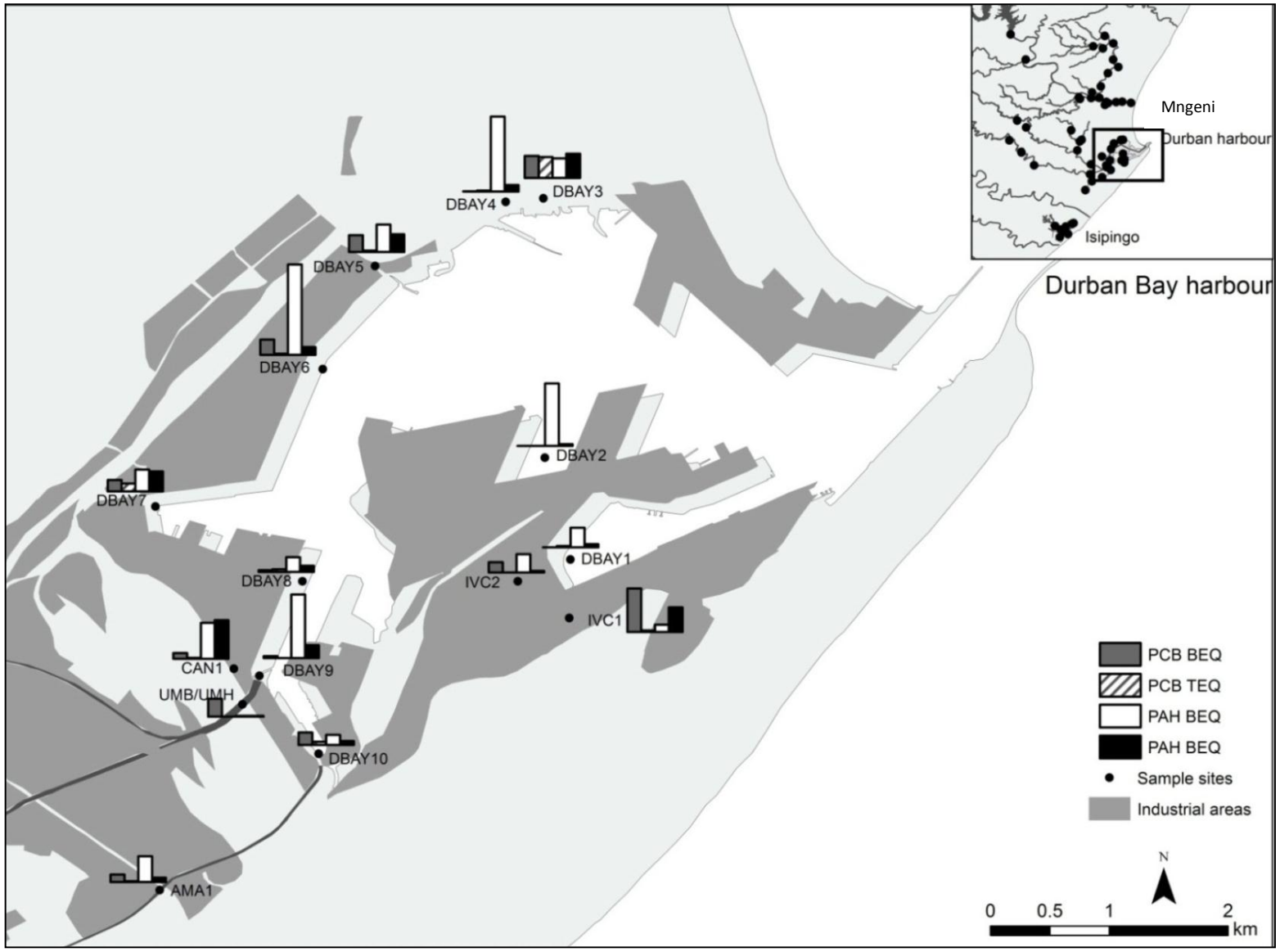


Figure 4.33. Map of Durban Bay showing the relative toxicity caused by PAHs and PCBs as determined using TCDD TEF values (TEQ) and the BEQs. In order to make all values visible on the same bar graph the values have been adjusted, PCB TEQ x10^{5} pg.g^{-1}, PAH BEQ x10^{-1} pg.g^{-1}, and the PAH TEQ and PCB BEQ have been recorded as pg.g^{-1}.

5 Conclusion and recommendations

This study has shown that PAHs are ubiquitous contaminants of sediment in Durban Bay, Isipingo River and Mngeni River catchments in area of KwaZulu-Natal. Although PCBs were not ubiquitous contaminants, they were nevertheless relatively widespread contaminants. These compounds were found to partition to mud and more selectively to the total organic content of the sediment. Because of this binding, sediment-dwelling organisms are likely to experience adverse health effects.

The concentrations of the PAHs and PCBs were often in excess of the SQGs. However, there is uncertainty on which sites are a cause for concern since this depends on which set of SQGs were used for the assessment. This is because the PAH and PCB guidelines have different concentrations prescribed by the guidelines. This motivates the use of toxicity testing.

The TEF scheme was utilised to estimate the toxicity that fish would experience from exposure to the PCBs and PAHs. The TEQs were compared to the Canadian $TCDD_{eq}$ SQGs. This revealed that none of the sites had toxicity levels that were in excess of the SQGs and these sites did not present a toxicity concern.

The H4IIE assay was additionally used for determination of toxicity, via the binding of AhR agonists to the AhR. The responses elicited from the non-acid washed (PAH) extract were higher than those elicited from the acid washed (PCB) extract. The concentrations of the BEQs and TEQs generally showed similar trends between sites. When the BEQs were compared to Canadian $TCDD_{eq}$ there were again deviations from the sites that had been in excess of the SQGs used for the ΣPCB and ΣPAHs.

The area that was deemed to be the most affected by PAHs and PCBs was Durban Bay—including the Island view and Bayhead canals (Figure 5.1). Here, every site exceeded one or more of the SQGs that were used in the investigation and many sites were highly contaminated by both the PAHs and PCBs. The Isipingo, Amanzimnyama, Umbilo and Umhlatuzana Rivers had contamination evident at sample sites along the systems. The Mngeni system only had one instance of a guideline exceedance and therefore this system seems to be the least affected by contamination of PAHs and PCBs.

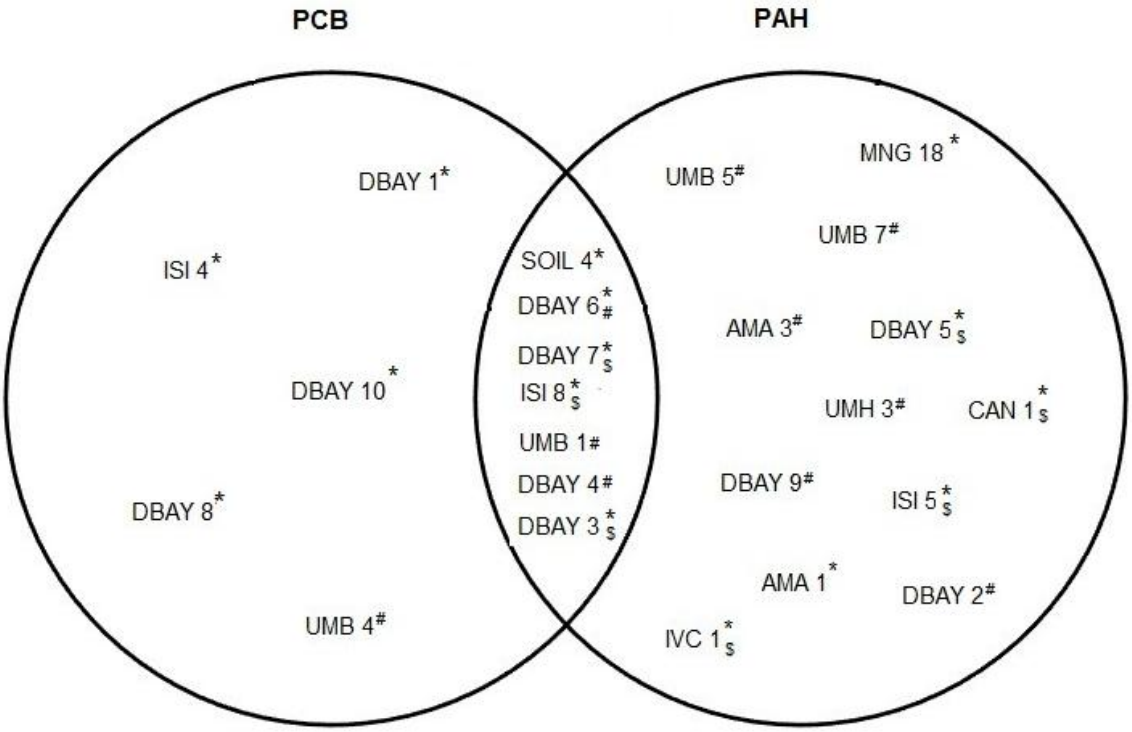


Figure 5.1. Comparison of the sites that had concentrations of PCBs or PAHs that were in excess of SQGs. * = site where ΣPCB or ΣPAH was in excess of the ERL, # = sites where PCB or PAH BEQ was in excess of Canadian TEC, $ = sites where BaP_{eq} was in excess of ERL.

Using any one method to assess areas where contamination due to PAHs and PCBs is not sufficient to fully grasp the affects that these contaminants are causing. It would be beneficial to screen the environmental samples for AhR ligands, including dl-PCBs, PAHs and PCDD/Fs. These are common environmental contaminants and have toxicological importance. These responses can be quantified then by chemical analysis, here the focus compounds should be the dl-PCBs, PCDD/Fs and only the priority PAHs. This will allow for the most similarity between the chemical data and assay responses. It may be beneficial to also investigate the actual loadings of these compounds in fish tissue and compare to the BEQ and TEQ results, because the bioassay could produce false positives or negatives, and the compounds may not be bioavailable in their current state. Additionally determine if there is bioaccumulation of these compounds in the environment.

6 References

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7 Appendices

Appendix A.1. PAH concentrations in soil and sediment samples.

Site	NP	BiP	ACY	ACE	FL	ANT	1MNP	2MNP	PANT	1MP	TMN	DMN	FLA	PYR	BaA	CHY	BbF	BkF	BeP	BaP	PER	BghiP	IcdP	DahA	DBT
AMA 1	50.7	71.1	2.2	12.2	40	19.9	57.8	98.9	233.1	112.3	59.6	123.7	220.9	209.7	47.7	97.7	33	28	23.9	15.7	7.6	8.3	7.2	2.8	23.3
AMA 2	132.9	105	4.1	4.9	35.6	15	42.3	77.7	157.5	60.4	30.6	66.7	106.5	124.9	28.7	53.7	19.9	13.5	23.1	10.8	4.5	13.4	5.1	1.6	12.6
AMA 3	<1	18.9	<1	<1	<1	<1	<1	<1	<1	<1	<1	8.5	39.3	34.6	12	22.6	8.7	6.3	7.3	4.7	2.3	3.8	2.7	<1	<1
CAN 1	308.2	195.2	20.8	27.3	112.4	121.1	309.1	626.4	797.8	199.5	102.6	324.5	789.2	777.1	355.9	583.4	449.5	245.9	370.7	252.4	74.5	157.2	142.2	45.9	90.7
DBAY 1	32.8	28.7	2.5	3.2	19.4	12.9	23.3	54.8	107.7	26.9	9	37.3	89.9	91.8	37.1	49.9	24.6	20.8	16.3	13.8	12.3	7.5	6.3	3.4	19.1
DBAY 2	11.4	33.7	<1	2.2	26	<1	21.4	53.4	115.4	18.7	<1	64.3	51	54.4	18.8	28.5	13.8	10.8	9.8	6.7	11.6	4.5	4.8	1.9	19.1
DBAY 3	121.2	52.6	16.6	62.8	109.7	75	56	101.3	618.5	<1	25.8	61.4	1266.4	1100.3	315.4	406.4	162.5	160.1	126.4	132.2	35	69.4	60.9	20.3	55.1
DBAY 4	56.5	25.1	2.6	18	48.3	17.5	24	40.4	179.2	<1	24.8	37.2	318.4	264.4	81.5	117.8	46.5	44.1	34.5	29.9	8	16.2	17.6	6.5	15.6
DBAY 5	21.5	13	5.4	9.2	30.1	60.2	20.2	27.2	331.3	74.1	28.6	30.2	737.8	561.2	269.7	314	125.6	116.6	93.9	91	22.9	35.7	38.3	12.6	25.1
DBAY 6	24.9	17.2	3.1	4.5	16.1	17.2	18.5	36.1	111.3	24.7	9.6	26.2	159.1	150.1	66.5	96	61.1	53	45.6	34.8	77.4	19.2	19.2	6.9	15.1
DBAY 7	40.4	36.8	4.7	30.8	59.6	42.4	37.5	70.1	266.8	41.8	16.4	36	472.2	477.4	167.3	199.5	153.7	134.4	110.2	108	116.4	59.4	62.1	18.2	25.9
DBAY 8	23.9	28.3	3.2	3.6	28.6	15.4	22.5	52.8	89.1	<1	13.6	27.2	139.8	154.1	51.1	70	48.8	40.9	39.8	31.5	98.3	17	16.3	6	11.9
DBAY 9	11	10.3	4.8	<1	14.2	22.3	11.5	22.7	114.8	<1	<1	15.7	247.7	216.2	105.3	155.3	82.7	86	74.5	57.4	45.9	36.5	42.3	12.3	13.7
DBAY 10	29	132.9	1.9	4.4	20.9	19.7	21.9	51.9	98	<1	17.4	49.4	152.9	190.2	55.2	75.1	32.5	25.8	25.9	18.8	7.2	11.6	7.6	4.1	18.4
ISI 2	19.2	15.7	4.3	2.9	6.5	14.5	23.7	35.9	55.6	9.8	7.2	27.6	40.2	36.9	14.3	21.8	16.9	6.9	13.4	6.9	18.1	33.1	24.6	7.3	13.8
ISI 4	10.2	10.2	<1	1.1	5.8	<1	13.2	27	52.6	17	6.3	19.4	46.7	41.5	18	26.3	19	9.4	16.6	10	7.6	6.4	7.4	<1	5.5
ISI 5	22.8	9.6	29	9.6	17.1	82.6	14.9	25.2	178	21.5	7.1	19.1	420	309	171	210	129	72.7	94.3	81.2	125	129	150	44	28.3
ISI 7	20	8	8.2	4.5	10.2	27.6	13.5	20.3	69.4	20.3	7.7	14.4	119	103	37	76.2	46	27.9	33	18.6	17	62.1	54.6	13.6	18.2
ISI 8	53.9	27.3	17.2	12.5	26.7	52.6	43.8	70	150	40.8	21.3	49.2	197	191	79.5	132	91.9	56.3	78.9	46.6	44	121	113	27.4	34.3
IVC 1	153.4	118.9	15	34.2	78	87.5	115.7	259.2	515.5	120	43.9	141.4	766.3	768.9	391.3	452.7	207.6	161	154	128.8	39.1	62.1	57.5	20.7	67.7
IVC 2	21.1	17.3	<1	<1	10.5	8.8	36.9	52.9	131.4	104.7	33.9	49.8	63.3	100.6	19.8	57.4	15.3	8.8	15.3	6.8	2.2	4.2	3.1	<1	27.6
MNG 1	6.8	5.4	2.5	1.3	3.3	7.1	8	8	18.6	4.8	<1	5.4	15.1	12	4.4	6.8	4.8	3.3	4.5	36.6	6.5	7.8	7.1	2.6	6.8
MNG 2	8.3	4.7	3.3	<1	3.2	9.1	4.8	6.8	22.5	5	2.5	4.7	26.3	24.1	10.9	14.1	11.1	5.7	9.1	4.8	20.9	18.1	17.4	3.6	5.1
MNG 3	<1	4.4	1.6	<1	2.7	8.2	2.1	2.7	26.5	4.7	3.3	3.6	17.5	16.4	4.4	9.2	5.5	2.4	4.1	2.2	4.4	13.9	9.5	2.7	4.6

Site	NP	BiP	ACY	ACE	FL	ANT	1MNP	2MNP	PANT	1MP	TMN	DMN	FLA	PYR	BaA	CHY	BbF	BkF	BeP	BaP	PER	BghiP	IcdP	DahA	DBT
MNG 4	6.4	8.7	3.1	<1	4.6	12.4	5.2	5.5	20.2	<1	3.7	5.9	23.1	24.4	9.7	15.2	11.2	4.7	9.3	4.8	19.9	31.2	16	5.4	7.5
MNG 5	3.6	1.6	1.6	<1	1.5	3.7	2.3	2.8	8.7	2.2	<1	1.8	7.2	6.3	1.9	2.6	2	<1	2	<1	4.2	2.7	<1	<1	2.9
MNG 6	15.4	8.6	<1	<1	<1	<1	12.8	20.8	79.3	22.4	<1	18.2	102.9	107.1	40.5	67.4	41.8	19.5	42.7	33.8	1	30.7	<1	<1	<1
MNG 7	27.4	29.1	12.5	4.8	15.1	26.2	15.1	22.7	60	17.3	8.1	19.4	69.6	66.2	22.5	36.6	29.8	12.4	20.3	11.9	253	51.3	34.9	11.4	19.1
MNG 8	5.6	2.3	1.9	<1	1.4	6.6	2.7	4.1	14.8	4.3	<1	2.2	13.8	11.3	4.8	6.7	4.4	2.4	3.3	1.6	5.6	7.3	4.9	1.5	3.6
MNG 9	3	1.5	1.8	<1	1.2	4.4	1.2	2.5	8.7	2.6	<1	1.5	3.7	3.6	1.5	1.9	1.7	1.2	1.3	<1	1.4	2.6	2.6	<1	3.6
MNG 10	3.1	2.3	1.3	<1	<1	5.2	1.7	1.9	8.7	2.8	<1	<1	4.1	3.8	1	1.2	<1	<1	<1	<1	2.2	1.7	<1	<1	3
MNG 11	21.9	17.2	8.8	9.9	11.9	34.7	11.6	19.7	84.5	17.6	5.6	11.4	110	85.9	36.4	55.2	36.8	15	27.6	21.6	17.6	67.3	49.2	12.7	15.5
MNG 12	11.1	9	7.7	2.2	5.1	13.3	7	7.9	29.7	7.4	1.5	7.2	32.4	27.1	10.4	16.6	12.8	7	9.8	5.5	26.5	25.9	14.4	3.4	8.3
MNG 13	9	7.8	4.1	1.9	3.8	9.4	4.7	7.9	19.8	5.7	3.5	6.4	17.1	17	5.6	9.7	6.7	3.2	5.7	2.6	14.8	12.1	10.1	2.9	5.9
MNG 14	5.3	1.9	2.3	<1	2.1	8.6	2.4	4	15.8	4.7	1.7	2.8	14.9	11.8	7.2	8.5	7.4	4.5	5.6	2.4	5.5	8.7	7.9	3.8	4.4
MNG 15	8.1	3.3	1.6	<1	2.6	2	5	9.2	26.2	5.4	60.7	10.4	11.1	7.8	2.9	5.8	3	1.7	2.7	<1	<1	3.4	3	<1	7.9
MNG 17	7.3	6.2	2.6	1.4	3.5	7.8	3.8	5.7	17.9	8.3	4.1	5	10.8	12.3	3.7	6	3.6	2.3	3.3	1.6	8.9	8.1	5.6	1.5	7.8
MNG 18	49.9	107.8	<1	<1	<1	<1	145.6	207.1	545.5	398.3	343.2	625.7	442.8	435.5	92.8	196.5	60.3	23.4	39.3	30.7	<1	18.7	<1	<1	<1
MNG 19	27.5	17.8	4.8	7.7	16.3	29.3	16.6	19.8	98.1	<1	2.7	14.5	92.3	67.3	28.6	39.1	22.4	11.2	20.6	10.8	19.7	38.7	19.2	8.9	18
MNG 20	4.7	4.8	2.3	<1	1.9	7.3	3.6	3.1	10.4	3.8	<1	2.4	11.4	9.9	3.2	5	3.6	1.4	2.2	1.4	6.7	3.9	4	1.2	3.5
MNG 21	4.4	1.8	2.8	1	1.8	5.4	2.3	2.6	10.6	2.5	<1	1.9	8.9	6.4	1.9	3.7	4.3	2.4	3	1.7	2.5	4.7	3.4	1.5	3.3
MNG 22	2.5	1.7	<1	<1	1.3	6.3	2	1.9	8.3	2.2	<1	1.2	7.6	3.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	2.3
SOIL 1	6.6	6.2	<1	<1	<1	<1	3.2	5.1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
SOIL 2	29.5	13.4	<1	<1	<1	<1	25.3	46.1	76.5	18.1	8.2	17	67.9	51.9	21.1	30.2	15.6	12.6	12.6	5.4	2.9	5.3	4.1	1.6	<1
SOIL 3	8.8	6.3	1.5	3	5.2	7.2	11.8	17	56.2	12.3	<1	12.3	79.2	64.8	27.4	39.8	20.9	15.8	16.7	10.9	5.1	6	5.6	2.5	5.9
SOIL 4	159.8	125.6	20.9	20.3	75.1	39.5	155.5	294.3	419.4	97.5	53.5	199	408.4	458.7	115.6	230.2	92.1	72.9	74.7	48.9	19	38.2	25.2	6.6	58.7
UMB 1	12.2	12.9	3.8	2	6.9	13.2	17.6	27.4	37.1	11.6	10.8	26.7	26.3	25.1	7.9	14.1	6.5	3.5	6	2.3	3.6	11.4	10.8	3.2	7.2
UMB 2	7.5	3	3.9	<1	3	8.9	4.4	5.1	19.4	4.4	1.9	3.3	23.7	19.9	7	11.4	5.8	4.4	6	2.9	6.4	14.3	11.8	3.9	4.4
UMB 3	4.8	3.2	1.2	<1	2.1	5.3	3.8	3.9	13.4	3.4	1	2.4	8.9	6.7	2.9	3.1	2.1	1.2	1.9	1	1.9	7.1	5.2	<1	3.2
UMB 4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	50.4	41.5	16.2	28.2	16.7	11.2	12.5	8.3	3.8	5.1	4.3	1.9	<1
UMB 5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	34	24.6	10.3	13.1	7.5	5	6.1	4.2	2.6	2.1	2.3	<1	<1
UMB 7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	23.5	19	9.2	13.2	6.6	5.5	6.4	2.3	1.9	2.1	2	<1	<1
UMB 8	5.7	8.9	<1	<1	<1	8	3.9	11.1	39.3	8.2	<1	6.3	81	59.6	22.4	29.9	13.3	9.3	10.4	4.5	2.8	1.4	1.3	<1	<1
UMB/UM	5	2.9	2.1	1.1	3.2	8.5	7	6.8	17.8	4.2	2	4	18.9	16.1	5.8	8.9	5.1	3.2	4.4	2.4	7.9	9.7	12.7	<1	4.9

Site	NP	BiP	ACY	ACE	FL	ANT	1MNP	2MNP	PANT	1MP	TMN	DMN	FLA	PYR	BaA	CHY	BbF	BkF	BeP	BaP	PER	BghiP	IcdP	DahA	DBT
H																									
UMH 1	4.3	2.4	1.2	1.7	1.8	2.9	2.9	4.2	17.1	3.7	<1	2.6	18.7	13.2	3.4	5.3	4.7	2.4	4.1	<1	<1	5.2	6.4	<1	4.4
UMH 3	2.4	1.9	1.2	<1	1.4	3.8	1.6	2.1	9.5	2.3	<1	1.3	6.3	6.2	2.3	2.9	2.2	1.4	2.4	<1	3.9	3.4	2.8	<1	3.6
UMH 5	6.4	4.8	2.4	<1	3.7	9.7	4.4	6.2	14.9	4.7	3	4.9	13.7	16.6	4.5	7.5	5	2.4	4.9	2	4.2	6.8	6.6	1.8	6.5
UMH 6	3.8	2.5	1.2	1.3	14.5	2.6	2	2.5	9.7	2.9	<1	1.6	6.6	5.5	1.2	2.2	2.2	1	1.5	<1	<1	2.6	3.3	<1	2.6

Appendix A 2. PCB concentrations in soil and sediment samples.

Site	001	008	018	028	044	052	066	077	101	105	118	126	128	138	153	169	170	180	187	195	206	209
AMA 1	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	1.17	<1	<1	4.25	2.58	<1	1.59	2.40	1.42	<1	<1	<1
AMA 2	<5	<5	<5	<10	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
AMA 3	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	2.00	<1	<1	<1	<1	<1	<1	<1	<1
CAN 1	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	1.33	<1	<1	2.88	2.12	<1	1.02	1.68	1.05	<1	<1	<1
DBAY 1	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	2.00	<1	<1	9.00	6.00	<1	2.00	4.00	2.00	<1	1.00	<1
DBAY 2	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	7.00	5.00	<1	2.00	4.00	2.00	<1	<1	<1
DBAY 3	<5	<5	<5	<5	<5	<5	<5	1.26	8.79	5.36	4.73	<1	2.82	29.40	17.80	<1	9.94	18.50	9.24	1.69	2.86	1.44
DBAY 4	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	1.68	<1	<1	8.89	5.84	<1	3.31	6.00	3.21	<1	<1	<1
DBAY 5	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	2.00	<1	<1	3.00	2.00	<1	<1	1.00	<1	<1	<1	<1
DBAY 6	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	2.00	<1	<1	9.00	5.00	<1	2.00	4.00	2.00	<1	1.00	<1
DBAY 7	<5	<5	<5	<5	<5	<5	<5	<1	9.09	5.70	7.69	<1	2.26	26.70	20.00	<1	7.81	14.34	8.19	1.33	2.56	<1
DBAY 8	<5	<5	<5	<5	<5	<5	<5	<1	<5	2.17	2.19	<1	1.44	7.68	4.31	<1	2.18	3.31	2.10	<1	<1	<1
DBAY 9	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	3.00	2.00	<1	<1	1.00	<1	<1	<1	<1
DBAY 10	<5	<5	<5	<5	<5	<5	<5	<1	<5	2.52	3.07	<1	<1	10.70	6.95	<1	3.26	5.36	2.70	<1	<1	<1
ISI 2	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	1.00	<1	<1	<1	<1	<1	<1	<1	<1
ISI 4	<5	<5	<5	<5	<5	<5	<5	<1	<1	6.11	9.08	<1	2.55	23.80	13.80	<1	4.99	7.57	3.37	<1	<1	<1
ISI 5	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	1.44	<1	<1	2.21	2.14	<1	<1	1.17	<1	<1	<1	<1
ISI 7	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ISI 8	<5	<5	<5	<5	<5	<5	<5	<1	6.10	3.14	3.09	<1	1.27	15.17	10.00	<1	4.11	9.12	5.31	<1	<1	<1
IVC 1	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	2.26	<1	1.10	7.37	4.74	<1	2.25	3.47	<1	<1	<1	<1
IVC 2	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MNG 1	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MNG 2	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	2.00	<1	<1	<1	<1	<1	<1	<1	<1
MNG 3	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MNG 4	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	2.00	<1	<1	<1	<1	<1	<1	<1	<1
MNG 5	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MNG 6	<5	<5	<5	<5	<5	<5	<5	<5	<5	<2	<1	<1	<1	1.08	<1	<1	<1	<1	<1	<1	<1	<1
MNG 7	<5	<5	<5	<5	<5	<5	<5	<1	11.90	<2	<1	<1	<1	1.61	<1	<1	<1	<1	<1	<1	<1	<1
MNG 8	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
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UMB 7	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
UMB 8	<5	<5	<5	<5	<5	<5	<5	<1	<5	<2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
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Table indicating the PAH abbreviations and number of rings. PAHs highlighted in bold are 16 USEPA priority PAHs.

PAH	Abbreviation	Number of rings
Naphthalene	**NP**	**2**
1-Methylnaphthalene	1MNP	2
2-Methylnaphthalene	2MNP	2
2,6-Dimethylnaphthalene	DMN	2
2,3,5-Trimethylnaphthalene	TMN	2
Acenaphthylene	**ACY**	**2**
Acenaphthene	**ACE**	**2**
Biphenyl	BiP	2
Fluorene	**FL**	**2**
Dibenzothiophene	DBT	2
Anthracene	**ANT**	**3**
Phenanthrene	**PANT**	**3**
1-Methylphenanthrene	1MP	3
Fluoranthene	**FLA**	**3**
Pyrene	**PYR**	**4**
Benz[*a*]anthracene	**BaA**	**4**
Chrysene	**CHR**	**4**
Benzo[*b*]fluoranthene	**BbF**	**4**
Benzo[*k*]fluoranthene	**BkF**	**4**
Benzo[*a*]pyrene	**BaP**	**5**
Dibenz[*a,h*]anthracene	**DahA**	**5**
Indeno[*1,2,3-c,d*]pyrene	**IcdP**	**5**
Benzo[e]pyrene	BeP	5
Perylene	PER	5
Benzo[*g,h,i*]perylene	**BghiP**	**6**