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# 22 Abstract

23 Mercury (Hg) speciation and bioavailability were studied in surface water, surface sediment and freshwater fish 24 samples collected upstream and downstream of the Medupi (currently under construction) and Matimba Power 25 Stations in the Waterberg Area, Limpopo Province. The initial survey was conducted in May 2010 and 26 continued periodically/seasonally until October 2014. This study was designed to provide an overall description 27 of the levels of Hg in areas potentially impacted by emissions from the coal-fired power station, and provide the 28 necessary information to enhance the understanding of the factors regulating the fate and transport of Hg in the 29 environment. Percent Loss on Ignition (LOI) and ancillary water quality measurements were also carried out. In 30 this study, the TotHg concentrations ranged between 0.92 and 29.13 ng/L, and 0.13 and 8.00 ng/L for MeHg in 31 water. Total Hg concentrations ranged between 0.50 and 28.60 ng/g, while the MeHg concentration ranged 32 between 0.08 and 2.22 ng/g in sediments. Mercury concentrations in fish ranged between 40 ng/g and 1 200 33 ng/g for TotHg, and 13.42 ng/g and 600 ng/g for MeHg. Methylmercury concentrations in freshwater fish 34 sampled exceeded the US EPA criteria (300 ng/g) in 10% of the total fish sampled and 5% exceeded the WHO 35 guideline (500 ng/g).

### 1 Keywords

- 2 Total mercury, methylmercury, water, sediments, freshwater fish coal fired power station emissions,
- 3 bioavailability, bioaccumulation, environmental impact

#### 4 **1. Introduction**

5 There has been growing concern over the release of mercury (Hg) into the atmosphere and its potential 6 hazardous impact on human health as well as the receiving environment (Hylander and Meili, 2003). Mercury is 7 regarded as a neurotoxin with demonstrated adverse human health impacts. Anthropogenic sources are estimated to account for 50-75 % of the annual input of Hg to the global atmosphere (Meili 1991; USEPA 8 9 1997; Seigneur et al. 2004). Coal combustion represents the largest industrial contributor to global Hg emissions 10 (Pacyna and Pacyna 2001), and although it is present in trace amounts in coal, it can still contribute significantly 11 to the Hg load. South Africa is considered as one of the world's largest coal producers, and relies on coal for 12 more than 90 % of its electricity generation (DME, 2005). Coal production in South Africa has traditionally 13 been concentrated in the Highveld region of Mpumalanga Province. Historical estimates of mercury have 14 reported that during 2010, 19.8 tons of mercury was emitted by Eskom's coal-fired power stations, estimated at 15 77% of total mercury emissions in the country (Roos 2011). Mercury emissions from coal-fired power stations 16 are estimated to account for approximately 75% of anthropogenic sources in South Africa (Scott 2011).

17 Eskom currently operates thirteen coal-fired power stations (including Matimba), most of which are located in 18 the Mpumalanga and Limpopo provinces, with two new coal fired power stations (Medupi and Kusile) currently 19 under construction. Emissions from coal-fired power stations are regarded as the major anthropogenic mercury 20 (Hg) source, contributing in excess of 50% of the overall anthropogenic Hg emissions. Previous studies have 21 stated that Hg emissions from sources in South Africa, mostly coal combustion, contribute more than 10% to 22 global Hg emissions (Pacyna et al., 2006). However, in a later study, Pirrone et al. (2010) reported much lower 23 values (< 4 %). The Matimba power station is fuelled by the open cast Grootegeluk coal mine on the Waterberg 24 Coalfield and is also contracted to supply the Medupi power station. The Waterberg and watershed areas 25 represent significant spatial components to conduct surface water, sediment and biological monitoring. The 26 historic and economic relevance of the areas supports the need to monitor Hg in these water resources. The 27 bioaccumulation potential of Hg within these areas has previously been poorly quantified.

28 Several processes affect the fate, transport and speciation of Hg in aquatic ecosystems. These processes have 29 previously been highlighted in similar areas in South Africa (Somerset et al. 2011). In aquatic ecosystems, 30 MeHg is found in elevated concentrations in top predators. The process by which Hg is accumulated in the 31 lower trophic levels of aquatic food webs is still unclear (Wiener et al. 2003). Although diet has been 32 demonstrated to be the most dominant mechanism of MeHg uptake in fish (Hall et al. 1997), other factors such 33 as size, age, and feeding habits are also important in the ultimate MeHg sequestration in fish-tissue The 34 biomagnification and concomitant toxicity of MeHg through the aquatic food chain is thus of particular concern. 35 Wet deposition is thought to be the primary mechanism by which Hg emitted to the atmosphere is transported to 36 surface waters and land, although dry deposition may also contribute substantially (Braune et al., 2005).

Concerns for human and ecosystem health have provided a basis for the increased attention on studying mercury
(Hg) in the environment. Mercury emissions and guideline values for Hg levels in the environment are regulated
by several agencies worldwide. These include the World Health Organization (WHO), United States

guidelines for the presence of Hg in aquatic resources (40 ng/g). However, there is no legislation governing Hg emissions. Possible future control limits for atmospheric emission of mercury in South Africa, may result in a need to design and implement a programme to monitor ecosystem responses to changes. This project was initiated by Eskom to establish a baseline of mercury levels in the vicinity of these stations and to implement a long term monitoring plan, as part of a sustainable approach, to establish any changes that could be attributed to the increase in combustion of coal in the area when Medupi comes online. The results obtained from this report could aid in the development of policies on a regional and national scale.

Environmental Protection Agency (US EPA) (Table 1). In South Africa, current legislation has set quality

### 9 **2.** Methodology

## 10 **2.1. Study area**

The Waterberg area is located in the Limpopo Water Management Area (WMA) of the Limpopo Province 11 12 (Figure 1; Table 1). It contains four main drainage rivers, namely the Lephalala, Matlabas, Mogalakwena and 13 Mokolo Rivers. The Limpopo River serves as a border between South Africa, Botswana, Zimbabwe and 14 Mozambique, and flows in an easterly direction exiting into the Indian Ocean. The Mokolo River runs through 15 the Mokolo Dam and confluences in the north with the Limpopo River. The region is characterised by dry 16 winters and wet summers, and has an average annual rainfall of approximately 485 mm, of which 420 mm falls 17 between October and March. The area between one of its major cities Lephalale and the Limpopo River 18 contains South Africa's richest remaining coalfield. Two of the 13 coal-fired power stations are found in the 19 Waterberg Area. These are the Medupi (currently under construction) and Matimba power stations.

20



Fig 1 Map of the geographical location of the sampling sites in the Waterberg (Limpopo WMA) in South Africa 3 and location of the Medupi and Matimba power stations (PS).

4 5

1 2

Table 1 List of sampling site descriptions and their locations

Site Name	Site ID	Water Resource Name	Source Location
Mokolo Dam	MD	Mokolo Dam	Upstream of power stations
Moord Mokolo	MM	Mokolo River	Directly downstream of power stations
Croc Mokolo	СМ	Mokolo River	Downstream of power stations
Beauty	BT	Lephalalae River	Downstream of power stations

6

7

# 2.2. Sample collection and processing

8 Standard protocols for collecting samples for TotHg and MeHg analysis were employed throughout sample 9 collection (US EPA 1996; Mason and Sullivan 1998). The collection protocol utilizes acid-cleaned Teflon® 10 bottles and preservation with HCl as per US EPA recommendations. Acid cleaning consists of an initial soaking 11 in 25% HNO3 for two weeks, triple rinsing with a MilliQ water, and a further two week soak in 10% HCl 12 followed by a triple rinse in MilliQ water before drying completely in a laminar flow hood. A triple rinse in 13 MilliQ water is also performed when transferring bottles between acid baths. Teflon® bottles are then double-14 bagged in ziplock bags until needed. Unfiltered water samples were collected by submerging a 500 mL acid-15 cleaned Teflon bottle approximately 0.5 m below the water surface (without making contact with the sediment bed). Sample bottles were firmly sealed following acidification with a 0.5 % v/v HCl solution. The samples 16 17 were then labelled, double-bagged and transported on ice to the analytical laboratory for analysis. Surface

- 1 sediment cores (up to 6 cm in depth) were extruded using an acid-cleaned polypropylene cylindrical corer and 2 sectioned at 2-cm intervals, after which each section was individually transferred into clean 50-mL centrifuge 3 tubes, labelled, double-bagged and transported on dry ice to the analytical laboratory. Surface water and 4 sediment samples for TotHg and MeHg determination were collected at selected sites in May 2010 (wet season), 5 August 2010 (dry season), March 2011 (wet season), May 2011 (dry season), August 2011 (dry season), January 6 2012 (wet season), April 2012 (wet season), July 2012 (dry season), October 2012 (wet season), January 2013 7 (wet season), April 2013 (wet season), June 2013 (dry season), September 2013 (wet season), January 2014 (wet 8 season), April 2014 (wet season), July 2014 (dry season) and October 2014 (wet season) (Table 1; Figure 1). 9 Not all sites were sampled during each sampling period. For example, Site MM was not included during the 10 initial sampling phase (May 2010). From the April 2013 sampling phase, freshwater fish samples were also 11 collected to be included in the monitoring process. Sample collection was undertaken over a period of 17 12 sampling phases which allowed for both spatial and seasonal comparisons to be made.
- 13

# 14 **2.3. Field analysis**

Field measurements of surface water temperature (°C), pH, TDS (g/L), SP conductivity (mS/cm) and dissolved oxygen (DO; mg/L), were collected at each sampling site during each sampling event. All measurements were conducted using a YSI Model 556 Multi-parameter instrument. At each site, water samples were also collected for chemical analysis at Eskom's Research Testing and Development (RT&D) laboratories using accredited methods. Analyses included ammonia, chlorides, magnesium, nitrates, dissolved oxygen, phosphates and sulphates.

- 21
- 22

# 2.4. Sediment Loss on Ignition (LOI)

Loss on ignition (LOI) was determined in each sediment layer by drying wet sediment at 105°C and heating
 dried sediment samples at 550 °C.

25

# 26 **2.5. Mercury analysis**

### 27 **2.5.1.** Total mercury (TotHg)

Total Hg in surface water followed the US EPA Method 1631 (US EPA 2002). This protocol included the oxidation of Hg with bromine monochloride (BrCl), pre-reduction with hydroxylamine hydrochloride (NH<sub>2</sub>OH·HCl) and further reduction with stannous chloride (SnCl<sub>2</sub>). The TotHg analysis for water samples was performed by Cold Vapour Atomic Fluorescence Spectrometry (CVAFS) using a Tekran Model 2500 CVAFS Mercury Detector and HP 3396 Integrator.

33 Total Hg (TotHg) concentrations in surface sediment and freshwater fish samples were determined following

34 USEPA protocols (Method 7473; USEPA 2007). The TotHg in solid samples, i.e. sediment and freeze-dried

35 fish, were measured using a DMA-80 Solid Phase Direct Mercury Analyzer (Milestone Inc., Monroe, CT,

36 USA).

### 1 2.5.2. Methylmercury (MeHg)

Methylmercury concentrations in surface water, surface sediment and freshwater fish samples were determined following US Environmental Protection Agency (USEPA) protocols (Method 1630; USEPA 2001a). For surface water and surface sediment, samples were distilled in 20% KCl and 50% H<sub>2</sub>SO<sub>4</sub>. For freshwater fish, samples were freeze-dried and digested in 25% KOH in methanol. The MeHg analysis for all samples was performed by CVAFS using a Tekran Model 2500 CVAFS Mercury Detector and HP 3396 Integrator.

7

8

#### 2.6. Statistical analysis

9 Statistical analysis was performed using Microsoft Excel XLSTAT. The mean standard deviation was obtained 10 for all replicate samples, and was statistically compared. A one-way analysis of variance (ANOVA) was 11 undertaken to determine any significant differences. The criterion for significance was set at <0.05 (p < 0.05). 12

### 13 **2.7. Quality assurance**

14 Reported values (unless otherwise stated) are averages of concentrations of replicates. A standard calibration curve with a regression coefficient ( $R^2$ ) of at least 0.998 was obtained daily during analysis. Routine quality 15 16 assurance and quality control (QA/QC) was carried out on each group of 10 samples, which included matrix 17 spikes and matrix spike duplicates, matrix blanks, replicate samples and certified reference material (CRM) of known Hg content (TORT-2, Lobster Hepatopancreas; 270.0 ng/g  $\pm$  60 for TotHg and 152.0 ng/g  $\pm$  13 for 18 19 MeHg). The limit of detection (LOD), expressed as three standard deviations of the blanks, was 0.05 ng/L for 20 TotHg and 0.07 ng/L for MeHg. The instrument LOD for the Tekran 2500 is 0.02 ng/L, and for the DMA-80 it 21 is 0.003 ng Hg. Replicate samples yielded no significant differences, while all sample reference materials 22 (SRMs) analysed fell within the specified certified ranges.

23

# 24 **3. Results and discussion**

25

### 3.1. Physico-chemical field measurements

Table 2 presents the average physico-chemical data for surface water collected on-site at each sampling site and for all sampling trips. In general, the four sampling stations were similar regarding environmental characterization.

29

# **30 3.2. Mercury in the aqueous phase**

The concentrations of TotHg in natural unpolluted waters are generally between 0.2 - 15 ng/L and most often below 5 ng/L (Stein et al., 1996), although the US EPA dictates a maximum guideline of 12 ng/g which may result in chronic effects to aquatic life (USEPA 1992). The TotHg and MeHg concentrations in surface water in this study are shown in Figure 2. The lowest average TotHg and MeHg concentrations were 1.87 ng/L at CM (2012 wet season) and 0.25 ng/L at MD (2013 wet season) and the highest were 29.13 ng/L at MM (2013 dry season) and 8.01 ng/L at CM (2014 wet season). Approximately 70% of TotHg concentrations exceeded the global average of 5ng/L (Mason et al. 1994), while 35% of TotHg exceeded the 12 ng/L concentration indicated by the US EPA (USEPA 1992). The aqueous TotHg concentrations reported in this study are similar to previous studies conducted at sampling sites with impacts from coal-fired power stations (Williams et al. 2010; Walters et al. 2011), while generally higher MeHg concentrations are reported in the current study. Although MeHg concentrations in aqueous samples were overall low, chronic exposure to MeHg can become a concern for biota, if exposed over extended durations (Williams et al. 2010). The average percentage of MeHg in relation to TotHg was 25.38%. This is above the proportion (1 - 5 %) observed by Mason et al. (1993), for water resources in the

- 7 USA. It is likely that a significant amount of methylation occurs in sediment, suggesting that significant fluxes
- 8 of MeHg from sediment to the overlying water column occurs (Hall et al., 2008).
- 9

10 Spatial distributions were observed. Aqueous Hg (i.e. TotHg and MeHg) was generally higher at the 11 downstream sites (i.e. MM and CM), while sediment Hg (TotHg and MeHg) was highest at the most 12 downstream site (i.e. BT) (see below). This suggests that most of the Hg is suspended and transported 13 downstream from the power stations, and the local hydrological regimes and dynamic of sediment transport can 14 explain the lower aqueous and higher sediment concentrations downstream site (i.e. BT). This could also serve 15 as a potential storage basin for Hg. Because water is transient, any MeHg present upstream of the power stations 16 will be transported downstream (or deposited in sediment), similar to what has been reported previously 17 (Williams et al. 2010; Walters et al. 2011). Understanding the atmospheric sources of Hg deposited to 18 ecosystems is important for determining the local and regional impacts of anthropogenic Hg sources. Wet and 19 dry deposition represents an important source of Hg to both terrestrial and aquatic ecosystems. Stack emissions 20 from coal fired power stations includes both vapour and particle-bound phases. Matimba has a stack height of 21 approximately 250m. The wind rose using hourly data between 2010 - 2014 in Figure 3 provides a means of 22 predicting the potential regional sources of Hg by plotting the percentages of time when the wind blew from 23 each of the 16 directions. The prevailing winds for the study period were largely between east north east and 24 east. As evidenced by the wind rose, the overall major potential source locations lie to the west and west north 25 west, thereby suggesting other potential regional sources. 26 Evidence of seasonal variation was observed in the Hg concentrations of water samples. Higher TotHg 27 concentrations were generally reported during the wet season. An increase in aqueous Hg concentrations during

the wet season suggests wet deposition of Hg to the aquatic environment during storm events. In addition,

- 29 periods of high rainfall promote the physical remobilization of fine-grained sediments and resuspension of Hg
- 30 from sediment to the water column, similar to what has been reported by others (Churchill et al. 2004; Williams
- et al. 2010). In terms of aqueous MeHg, concentrations were negatively correlated to sediment MeHg during the
- 32 wet season at all sites with the exception of MM, while during the dry season aqueous MeHg was positively
- 33 correlated to sediment MeHg at all sites.
- 34











Croc Mokolo - MeHg





**Fig. 2** Average total mercury (TotHg) and methylmercury (MeHg) concentrations in surface water collected from study sites seasonally between 2010 – 2014.



Fig. 3 Direction (wind from) and intensity of wind (wind rose) using hourly data (2010 – 2014).

# 4 **3.3.** Mercury in sediments

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5 In the aquatic environment Hg accumulates in sediments where methylation and demethylation processes occur. 6 Sediments are also regarded as a potential source of metal contamination (which includes Hg) for the 7 surrounding water column. As such, sediments are regarded as a major repository of anthropogenic pollutants 8 such as Hg. It is estimated that > 90% of trace metals present in the aquatic environment are associated with 9 suspended particles and sediments (Calmano et al. 1993; Wang et al., 2009). The TotHg and MeHg 10 concentrations in surface sediments are shown in Figure 4. In this study, the lowest average TotHg and MeHg concentrations were 0.50 ng/g (BT) and 0.08 ng/g (MD), respectively; while the highest TotHg and MeHg were 11 12 28.60 ng/g (BT) and 2.22 ng/g (MM), respectively. All TotHg concentrations were below the US EPA sediment 13 quality guideline of 200 ng/g (US EPA 2000). Total Hg concentrations in sediment samples were highest at the 14 most upstream site (i.e. BT). Unlike to the distribution pattern of TotHg, the highest MeHg concentrations were 15 noted at MM (2.22 ng/g). Compared with other recent studies conducted at similar sampling sites with similar 16 impacts, the TotHg concentrations in river sediment reported in this study were generally lower than those 17 previously reported for sediments adjacent to coal-fired power stations (Williams et al., 2010). In this study, the 18 MeHg:TotHg ratio ranged between 0.71 and 92.62%. Generally, %MeHg in surface sediments can be used as a 19 proxy for the rate of methylation (Drott et al., 2008), and accounts for approximately 1 - 3% of TotHg in 20 sediment (Revis et al., 1990). The elevated % MeHg values reported, suggest either that enhanced methylation 21 has occurred (Conaway et al., 2003). Under anaerobic conditions, Hg<sup>2+</sup> has a high affinity for sulfides, resulting in the formation of insoluble HgS, 22 which is deposited in the sediment and is unavailable for methylation (Andersson et al. 1990). Similarly, Hg has 23

a high affinity for organic matter (Hintelmann et al. 1995), as such their toxicity and bioavailability in sediment
is largely dependent on their interactions within the matrix (Pinedo-Hernandez et al. 2015). The relation of Hg

- 26 concentration in sediments to the corresponding organic carbon content (i.e. loss of ignition (LOI)) was
- 27 examined. While both Hg species were not correlated to organic carbon content, site specific correlations were

1 found between TotHg and MeHg concentrations (MD:  $R^2 = 0.43$ , MM:  $R^2 = 0.90$ ). This indicates that at these

2 sites Hg is available for methylation under specific conditions.

3



<sup>5</sup> **Fig. 4** Average total mercury (TotHg) and methylmercury (MeHg) concentrations in surface sediment collected

 $<sup>6 \</sup>qquad \text{from study sites seasonally from } 2010-2014.$ 

### 3.4. Mercury in fish

1

2 Methylmercury is the most toxic form of organic Hg, produced by the conversion of metallic, inorganic, or 3 organic Hg by sulphate- reducing microorganisms present in sediments (Benoit et al. 1998). It is easily and 4 rapidly taken up by living organisms and biomagnified through the aquatic food chain and, consequently, 5 represents a hazard to higher trophic level organisms, including humans that accumulate Hg through the 6 consumption of fish (Bourdineaud et al. 2011; Nøstbakken et al. 2012). As such, fish play a significant role in 7 the distribution of Hg between biotic compartments in aquatic environments. The TotHg and MeHg 8 concentrations of freshwater fish samples which were collected seasonally from April 2013 are presented in 9 Table 3. Total Hg concentrations varied from 37.30 ng/g dry weight (d.w.) for B. spp collected at BT in April 10 2013 to 1291.95 ng/g dw for A. johnstoni collected from MD in January 2014 (average TotHg concentration = 11 267.78 ng/g dw). Methylmercury concentrations varied from 13.42 ng/g dry weight (d.w.) for B. spp collected at 12 BT in April 2013 to 656.02 ng/g dw for A. johnstoni collected from MD in January 2014 (average MeHg 13 concentration = 158.76 ng/g dw). The Hg concentrations reported in this study are higher than those reported in 14 previous studies with sampling areas having similar anthropogenic impacts (Williams et al. 2010). Interestingly, 15 the highest TotHg and MeHg concentrations are reported at MD. 16 The observed variations in fish muscle Hg (i.e. TotHg and MeHg) concentrations can be explained by several 17 factors including biological variability associated with the fish species (i.e. age, size, diet), geological influences 18 (i.e. sediment), and chemical variability (i.e. ancillary water quality), and other environmental factors. A 19 Principal Component Analysis (PCA) was used to explain any potential correlations between aqueous and 20 sedimentary Hg concentrations with fish Hg collected from their corresponding locations. As shown in Figure 5, 21 the PCA explained 73.42, 70.01, 80 and 84.32% of the total variability at MD, MM, CM and BT; respectively.

- 22 Site specific associations with Hg variables are evident in the PCA plots. These indicated that Hg concentrations
- 23 in fish were largely correlated to sediment Hg concentrations, suggesting that sediment associated Hg is readily
- 24 available for uptake by fish (Williams et al., 2010), reinforcing the role that sediment plays in being a source of
- Hg to fish (Gilmour et al. 1992). Similarly, Hg in the water column is readily absorbed into muscle tissues of aquatic organisms, particularly via digestion (US EPA 1997; NRC 2000; Leaner and Mason 2002; Gilbertson
- and Carpenter 2004; Kontas 2006; Drott et al. 2007) as evidence in the PCA diagrams. In this study, fish Hg
- 28 concentrations were largely associated with aqueous Hg (MD, CM, BT) and sediment Hg (MD, MM, CM).
- 29 Mercury in sediments can be bioaccumulated in aquatic organisms and transferred along the food chain,
- 30 resulting in a threat to humans (Díez 2009; Wang et al, 2013). Subsistence fishing forms part of the daily
- 31 livelihoods of the communities living near the water resources sampled in this study. Although less than 10% of
- 32 samples exceeded the USEPA of 300 ng/g (US EPA, 2001b), and less than 5% the WHO safety limit of 500
- ng/g and the Joint FAO-WHO Food Standards Programme CODEX Committee on Contaminants in Foods
   (CODEX Alimentarius Commission, 2011).
- 35

- 1 Table 2 Average Hg concentrations in freshwater fish species collected from study sites seasonally from 2013 –
- 2 2014.

Site ID	Date	Season	Temp	TDS	SP Cond	DO Conc	pН
			С	g/l	mS/cm	mg/l	
MD	2010	wet	18.56±0.00	0.03±0.00	0.05±0.00	8.84±0.00	6.18±0.00
	2010	dry	17.39±0.00	0.03±0.00	0.05±0.00	8.93±0.00	6.78±0.00
	2011	wet	22.85±5.88	0.03±0.00	0.05±0.00	8.74±0.55	6.43±0.25
	2011	wet	16.95±0.00	0.03±0.00	0.05±0.00	9.18±0.00	7.02±0.00
	2012	dry	25.68±1.52	0.03±0.00	0.05±0.00	7.63±1.52	7.10±0.11
	2012	wet	19.50±4.14	0.04±0.00	0.06±0.00	8.82±3.55	6.52±0.71
	2013	wet	25.64±1.96	0.03±0.00	0.05±0.01	6.21±0.63	6.67±0.33
	2013	dry	18.63±5.48	0.04±0.00	0.06±0.01	8.06±0.91	7.31±0.00
	2014	dry	27.42±5.86	0.04±0.00	0.05±0.02	6.63±1.86	7.02±0.52
	2014	wet	17.95±3.44	0.06±0.02	0.09±0.03	6.76±0.00	7.00±0.60
MM	2010	wet	-	-	-	-	-
	2010	dry	20.17±0.00	0.04±0.00	0.05±0.00	8.64±0.00	6.70±0.00
	2011	wet	22.95±8.03	0.03±0.00	0.05±0.00	8.17±2.35	6.30±0.50
	2011	wet	19.05±0.00	0.04±0.00	0.06±0.00	8.87±0.00	6.91±0.00
	2012	dry	25.41±1.90	0.05±0.02	0.07±0.03	5.98±3.42	6.50±0.13
	2012	wet	22.02±7.41	0.04±0.00	0.07±0.00	6.72±0.7	7.32±0.42
	2013	wet	27.10±2.37	0.05±0.01	0.07±0.01	4.04±3.15	6.79±0.27
	2013	dry	20.28±3.15	0.04±0.00	0.06±0.00	8.18±1.07	5.37±0.00
	2014	dry	27.16±5.01	0.04±0.02	0.06±0.03	4.67±2.43	6.33±0.00
	2014	wet	22.71±7.45	0.08±0.01	0.12±0.02	6.50±0.00	6.85±0.40
СМ	2010	wet	21.30±0.00	0.33±0.00	0.05±0.00	7.63±0.00	6.65±0.00
	2010	dry	18.87±0.00	0.04±0.00	0.06±0.00	8.03±0.00	6.91±0.00
	2011	wet	22.36±7.83	0.03±0.00	0.05±0.07	7.00±0.07	6.67±0.34
	2011	wet	16.68±0.00	0.04±0.00	0.06±0.00	7.11±0.00	6.80±0.00
	2012	dry	28.41±0.25	0.04±0.00	0.07±0.05	5.89±1.14	6.85±0.01
	2012	wet	17.71±6.75	0.05±0.01	0.08±0.00	9.16±4.46	5.94±0.81
	2013	wet	26.90±3.39	0.04±0.00	0.07±0.00	4.12±0.59	6.78±0.00
	2013	dry	18.60±6.19	0.05±0.01	0.08±0.00	7.15±0.00	5.34±0.00
	2014	dry	25.58±1.87	0.05±0.03	0.07±0.00	4.33±0.73	6.41±0.54
	2014	wet	19.94±5.54	0.09±0.01	0.14±0.01	5.65±0.00	6.66±0.11
BT	2010	wet	18.09±0.00	0.03±0.00	0.09±0.00	7.15±0.00	-
	2010	dry	17.03±0.00	0.08±0.00	0.10±0.00	3.81±0.00	6.02±0.00
	2011	wet	21.04±7.38	0.05±0.00	0.10±0.00	5.25±0.57	6.69±0.15
	2011	wet	16.26±0.00	0.13±0.00	0.13±0.00	-	6.74±0.00
	2012	dry	22.96±3.87	0.05±0.02	0.10±0.00	5.12±2.04	6.80±0.88
	2012	wet	18.11±5.54	0.13±0.03	0.05±0.02	8.76±0.00	6.45±0.00
	2013	wet	26.66±3.34	0.06±0.01	0.13±0.04	7.59±0.13	7.00±0.03
	2013	dry	18.98±5.07	0.11±0.04	0.14±0.02	8.12±0.34	7.35±0.19
	2014	dry	24.18±1.27	0.03±0.02	0.07±0.02	7.48±1.53	7.33±0.04
	2014	wet	20.42±5.56	0.15±0.02	0.20±0.00	7.08±0.78	7.42±0.47



1

Fig. 5 Biplot of the first two components of Principal Component Analysis (PCA) including all measured
 markers total mercury (TotHg) and methylmercury (MeHg) measured in water, sediment and fish

### 5 **4.** Conclusions

Mercury is a hazardous contaminant occurring naturally and anthropogenically in the aquatic environment. Its 6 7 speciation, fate and transport in the aquatic environment are controlled by severally physical, chemical and 8 biological factors, which decide its toxicity in the environment. This study investigated the distribution of Hg 9 species (TotHg and MeHg) in environmental compartments from four sampling sites located in the vicinity of a 10 coal-fired power plant in the Waterberg Area (Limpopo Province). Several factors are known to affect TotHg 11 and MeHg concentrations in aquatic environments. Point sources of Hg pollution as well as long range 12 atmospheric transport and deposition from global sources can be a large contributor of Hg to water resources 13 (Mason et al. 1994). In this study, the highest aqueous TotHg and MeHg concentrations were measured at 14 downstream sampling sites and could likely be attributed to the immediate deposition of atmospheric Hg and the 15 subsequent downstream movement of suspended Hg. Total Hg concentrations in sediment were generally 16 highest at BT, while the highest MeHg concentration was measured immediately downstream of the power 17 stations (i.e. MM). Declines in Hg concentrations were evident during the wet season indicating that Hg is 18 flushed to the downstream surface waters possible dilution effects, reinforcing the influence of subsurface 19 transport and/or production of MeHg. The results also indicated that Hg bioaccumulation and risks to 20 ecosystems is highly variable. Total Hg concentrations in freshwater fish ranged over several orders of 21 magnitude. The lowest concentration measure was less than 40 ng/g (BT), while the highest concentration 22 exceeded 1 200 ng/g (MD). For MeHg, the lowest concentration measured 13.42 ng/g (BT) and the highest

1 exceeding 600 ng/g (MD). Methylmercury concentrations in freshwater fish sampled exceeded the US EPA 2 criteria (300 ng/g) in less than 10% of the total fish sampled and less than 5% exceeded the WHO guideline 3 (500 ng/g). Consumption of elevated MeHg content in fish are the primary route of exposure to humans, and is 4 also the primary cause for fish consumption advisories worldwide. The presence of relatively high TotHg 5 concentrations (close to and exceeding 300 ng/g and 500 ng/g) are of concern due to the fact that (1) MeHg 6 often accounts for 90% of TotHg, (2) the fish caught in this study were small and likely juveniles and, (3) that 7 Hg levels in fish are known to increase with age and trophic position. In this study, fish Hg concentrations 8 frequently exceeded guideline limits for fish consumption (US EPA and WHO guidelines). Fish consumption is 9 the main exposure pathway of Hg to humans (NRC, 2000). This is especially true in rural areas where 10 populations rely on subsistence fishing.

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### 15 **6. References**

- 16 Andersson, I., Parkman, H. & Jernelov, A. (1990). The role of sediments as sink or source for environmental
- 17 contaminants: A case study of mercury and chlorinated organic compounds. *Limnologica*, 20, 347–359
- 18 Benoit, J.M., Gilmour, C.C., Mason, R.P., Riedel, G.S. & Riedel, G.F. (1998). Behavior of mercury in the
- 19 Patuxent River estuary. *Biogeochemistry*, 40(2), 249–265.
- 20 Bourdineaud, J.P., Fujimura, M., Laclau, M., Sawada, M. & Yasutake, A. (2011). Deleterious effects in mice of
- fish-associated methylmercury contained in a diet mimicking the Western populations' average fish
   consumption. *Environment International*, 37(2), 303–313.
- 23 Braune, B.M., Outridge, P.M., Fisk, A.T., Muir, D.C.G., Helm, P.A., Hobbs K, Hoekstrad, P.F., Kuzyf, Z.A.,
- 24 Kwan, M., Letcher, R.J., Lockhart, W.L., Norstrom, R.J., Stern, G.A. & Stirling, I. (2005) Persistent organic
- 25 pollutants and mercury in marine biota of the Canadian Arctic: an overview of spatial and temporal trends.
- 26 Science of the Total Environment, 351–352, 4–56.
- 27 Calmano, W., Hong, J. and Förstner, U. (1993). Binding and mobilization of heavy metals in contaminated
- sediments affected by pH and redox potential. In: Proceedings of the First International Specialized Conference,
   pp. 223–235.
- 30 Churchill, R.C., Maethrel, C.E. & Suter, P.J. (2004). A retrospective assessment of gold mining in the Reedy
- 31 Creek sub-catchment, northeast Victoria, Australia: residual mercury contamination 100 years later.
- 32 *Environmental Pollution*, 132(2), 355 363.
- 33 Conway, C.H., Squire, S., Mason, R.P. & Flegal, A.R. (2003). Mercury speciation in the San Francisco Bay
- 34 estuary. *Marine Chemistry*, 80(2-3), 199 225.
- 35 CODEX Alimentarius Commision (2011) Joint FAO/WHO Food Standards Programme CODEX Committee on
- 36 Contaminants in Foods. Fifth Session, The Hague, The Netherlands.
- 37 DME (Department of Minerals and Energy). 2005. Digest of South African Energy Statistics Department of
- 38 Minerals and Energy, Pretoria.

- 1 Díez, S. (2009). Human health effects of methylmercury exposure. Reviews of Environmental Contamination
- 2 and Toxicology, 198, 111–132.
- 3 Drott, A., Lambertsson, L., Bjorn, E. & Skyllberg, U. (2007). Effects of oxic and anoxic filtration on
- 4 determining methyl mercury concentrations in sediment pore waters. *Marine Chemistry*, 103(1-2), 76 83.
- 5 Gilbertson, M. & Carpenter, D.O. (2004). An ecosystem approach to the health effects of mercury in the Great
- 6 Lakes basin ecosystem. *Environmental Research*, 95(3), 240 246.
- 7 Gilmour, C. C., Henry, E. A. & Mitchell, R. (1992) Sulfate stimulation of mercury methylation in freshwater
- 8 sediments. *Environmental Science and Technology*, 26(11), 2281 2287.
- 9 Groundworks. 2014. The Health Impact of Coal: The responsibility that coal-fired power stations bear for
- 10 ambient air quality associated health impacts.
- 11 Hall, B.D., Bodaly, R.A., Fudge, R.J.P., Rudd, J.W.M. & Rosenberg, D.M. (1997). Food as the dominant
- 12 pathway of methylmercury uptake by fish. *Water, Air, and Soil Pollution*, 100(1), 13–24.
- 13 Hall, B.D., Aiken, G.R., Krabbenhoft, D.P., Marvin-DiPasquale, M.& Swarzenski, C.M. (2008) Wetlands as
- 14 principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region.
- 15 Environmental Pollution, 154(1), 124-134.
- 16 Hintelmann, H., Welbourn, P.M. & Evans, R.D. (1995). Binding of methylmercury compounds by humic and
- 17 fulvic acid. Water, Soil and Air Pollution, 80(1), 1031–1034.
- 18 Hylander, L.D. and Meili, M. (2003) 500 Years of mercury production: global annual inventory by region until
- 19 2000 and associated emissions. The Science of the Total Environment, 304, 13-27.
- Kontas, A. (2006). Mercury in the Izmir Basin: An assessment of contamination. *Journal of Marine Systems*,
  61(1-2), 67 78.
- 22 Leaner, J.J. & Mason, R.P. (2002). Methylmercury accumulation and fluxes across the intestine of channel
- 23 catfish, Ictalurus punctatus. Comparative Biochemistry and Physiology Part C, 132(2), 247 259.
- 24 Mason, R.P., Fitzgerald W.F. & Morel, F.M.M. (1994). The biogeochemical cycling of elemental mercury:
- 25 Anthropogenic influences. *Geochimica et Cosmochimica Acta*, 58(15), 3191 3198.
- 26 Meili, M. (1991). The coupling of mercury and organic matter in the biogeochemical cycle Towards a
- 27 mechanistic model for the boreal forest zone. *Water, Air, and Soil Pollution*, 56(1), 333 347.
- 28 National Research Council (NRC). (2000) .Toxicological Effects of Methylmercury. National Academy Press,
- 29 344pp, Washington, DC.
- 30 Nøstbakken, O.J., Martin, S.A, Cash, P., Torstensen B.E., Amlund, H. & Olsvik, P.A. (2012). Dietary
- 31 methylmercury alters the proteome in Atlantic salmon (*Salmo salar*) kidney. *Aquatic Toxicology*, 108, 70–77.
- 32 Pacyna, J.M., Pacyna, E.G. (2001). An assessment of global and regional emissions of trace metals to the
- 33 atmosphere from anthropogenic sources worldwide. *Environmental Reviews*, 9(4), 269–298.
- 34 Pacyna, E.G, Pacyna, J.M., Steenhuisen, F., Wilson, S. (2006). Global anthropogenic mercury emission
- inventory for 2000. *Atmospheric Environment*, 40(22), 4048 4063.
- 36 Pinedo-Hernandez, J., Marrugo-Negrete, J. & Dize, S. (2015) Speciation and bioavailability of mercury in
- 37 sediments impacted by gold mining in Columbia. *Chemopshere*, 119, 1289-1295.
- 38 Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R.B., Firedli, H.R., Leaner, J., Mason, R., Mukherjee, A.B.,
- 39 Stracher, G.B., Streeet, D.G., Telmer, K. (2010). Global mercury emissions to the atmosphere from
- 40 anthropogenic and natural sources. Atmospheric Chemistry and Physics, 10, 5951-5964.

- 1 Revis, N.W., Osborne, T.R., Holdsworth, G. & Hadden, C. (1990) Mercury in soil: a method for assessing
- 2 acceptable limits. Archives of Environmental Contamination and Toxicology, 19, 221–6.
- 3 Roos, B.L. (2011). Mercury emissions from coal-fired power stations in South Africa. MSc Dissertation.
- 4 University of Johannesburg, p93.
- 5 Scott, G. (2011). Emission testing at two South African power stations, using the US-EPA's mercury toolkit.
- 6 Presentation at the International Mercury Emissions from Coal Meeting (MEC), Kruger Park Hotel, 18 May
  7 2011.
- 8 Seigneur, C., Vijayaraghavan, K., Lohman, K., Karamchandani, P., Scott, C. (2004). Global source attribution
- 9 for mercury deposition in the United States. *Environmental Science and Technology*, 38(2), 555–569.
- 10 Somerset, V., Williams, C., Van der Horst, C., Silwana, B., Le Roux, S., Iwuoha, E. (2011). Survey and analysis
- 11 of the Pb, Zn, Hg, Cd, Ni, Al, Fe, Pd, Pt and Rh levels in specific river catchments of the North West and
- 12 Limpopo Provinces. CSIR-NRE Internal Report, February 2011. CSIR, Stellenbosch, Report No.
- 13 CSIR/NRE/WR/ER/2011/0005/B.
- Stein, E.D., Cohen, Y. & Winer, A.M. (1996). Environmental distribution and transformation of mercury
  compounds. Critical Reviews of Environmental Science and Technology, 26(1), 1-43.
- 16 US EPA (United States Environmental Protection Agency). (1992) Water quality standards; establishment of
- 17 numeric criteria for priority toxic pollutants; states' compliance; final rule. Federal Register, 40 CFR Part 131, v
- 18 57; 246(60) 847 860, 916.
- 19 US EPA (United States Environmental Protection Agency). (1997) Mercury study report to Congress, Vol. 3-
- 20 Fate and transport of mercury in the environment: Washington, D.C., Office of Air Quality Planning and
- 21 Standards and Office of Research and Development, EPA–452/R–97–005, 376 p, Washington DC, USA.
- 22 US EPA (United States Environmental Protection Agency). (2000) Guidance for assessing chemical
- 23 contamination data for use in fish-advisories. Vol 1: field sampling and analysis, Third Ed. EPA publication no.
- 24 EPA-823-B-00-007. Washington DC, USA.
- 25 US EPA (United States Environmental Protection Agency). (2001a) Method 1630: Methylmercury in water by
- distillation, aqueous ethylation, purge and trap, and CVAFS. EPA Publication No. 821-R-01-020. Washington
   DC, USA.
- 28 US EPA (United States Environmental Protection Agency). (2001b) Mercury update: impact on fish advisories.
- 29 EPA-823-F-01-012. US EPA, Washington DC.
- 30 US EPA (United States Environmental Protection Agency). (2002) Mercury in water by oxidation, purge and
- trap, and cold water atomic fluorescence spectrometry. EPA Publication No.: EPA-821-R-02-019. Washington
- 32 DC, USA.
- USEPA (2007) Method 7473: mercury in solids and solutions by thermal decomposition amalgamation, and
   atomic absorption spectrophotometry. EPA SW-846, Washington DC, USA.
- 35 Walters, C.R., Somerset, V.S., Leaner, J.J. and Nel, J.M. (2011). A review of mercury pollution in South Africa:
- 36 Current status. Journal of Environmental Science and Health, Part A, 46(10), 1129-1137.
- 37 Wang, T., Wang, J., Tang, Y., Shi, H. & Ladwig, K. (2009). Leaching characteristics of arsenic and selenium
- from coal fly ash: role of calcium. *Energy Fuels*, 23(6), 2959–66.

- 1 Wang, H.S., Xu, W.F., Chen, Z.J., Cheng, Z., Ge, L.C., Man, Y.B., Giesy, J.P., Du, J., Wong, C.K.C. & Wong,
- M.H., (2013). In vitro estimation of exposure of Hong Kong residents to mercury and methylmercury via
   consumption of market fishes. *Journal of Hazardous Materials*, 248-249, 387–393.
- 4 Wiener, J.G., Krabbenhoft, D.P., Heinz, G.H., and Scheuhammer, A.M. (2003). Ecotoxicology of mercury. In
- 5 Hoffman, D.J., Rattner, B.A., Burton, G.A., Jr., Cairns, J., Jr. (Eds). Handbook of ecotoxicology (2d ed.).
- 6 Lewis, New York, pp 409-463.
- 7 Williams, C.R., Leaner, J. J., Nel, J.M., Somerset, V.S.(2010). Mercury concentrations in water resources
- 8 potentially impacted by coal-fired power stations and artisanal gold mining in Mpumalanga, South Africa.
- 9 Journal of Environmental Science and Health, Part A, 45(11), 1363-1373.