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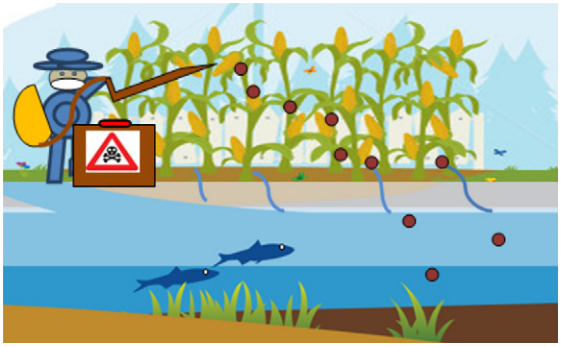
HIGHLIGHTS

- Summer contributed the highest triazine herbicide loads into the Hartbeespoort Dam.
- The Crocodile River is the major source of herbicides in the Hartbeespoort Dam.
- Atrazine groundwater concentrations in the Hartbeespoort Dam proximity are >130 ng L^{-1}.
- DET is the most abundant triazine metabolite in the Hartbeespoort Dam catchment.
- Atrazine metabolites were detected in fish muscle.

GRAPHICAL ABSTRACT



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ABSTRACT

Seasonal variation of eight chloro-s-triazine herbicides and seven major atrazine and terbuthylazine degradation products was monitored in the Hartbeespoort Dam catchment using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC-MS/MS). Lake, river and groundwater were sampled from the Hartbeespoort Dam catchment over four seasons and the downstream Jukskei River was monitored during the winter season. Triazine herbicide concentrations in the Hartbeespoort Dam were in the order atrazine > simazine > propazine > ametryn > prometryn throughout the four seasons sampled. Triazine herbicide concentrations in the Hartbeespoort Dam surface water were highest in summer and gradually decreased in successive seasons of autumn, winter and spring. Terbuthylazine was the only triazine herbicide detected at all sampling sites in the Jukskei River, though atrazine recorded much higher concentrations for the N14 and Kyalami sites, with concentrations of 923 and 210 ng L^{-1} respectively, compared to 134 and 74 ng L^{-1} for terbuthylazine. Analytical results in conjunction with river flow data indicate that the Jukskei and Crocodile Rivers contribute the greatest triazine herbicide loads into the Hartbeespoort Dam. No triazine herbicides were detected in the fish muscle tested, showing that bioaccumulation of triazine herbicides is negligible. Atrazine and terbuthylazine metabolites were detected in the fish muscle with deethylatrazine (DEA) being detected in both catfish and carp muscle at low concentrations of 0.2 and 0.3 ng g^{-1}, respectively. Desethylterbuthylazine (DET) was detected only in catfish at a concentration of 0.3 ng g^{-1}. With atrazine herbicide groundwater concentrations being >130 ng L^{-1} for all seasons and groundwater $\sum$ triazine herbicide concentrations ranging between 527 and

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367 ng L^{-1}, triazine compounds in the Hartbeespoort Dam catchment may pose a risk to humans and wildlife in light findings of endocrine and immune disrupting atrazine effects by various researchers.

1. Introduction

The presence of chloro-s-triazines in the environment is predominantly due to herbicide application, particularly from atrazine, simazine, terbuthylazine, ametryn, gesatamin, propazine and prometon (Wackett et al., 2002; Zhang et al., 2016). South Africa is historically the 9th biggest corn producer in the world and atrazine is often used as the herbicide of choice, with 88% of atrazine in the country being applied on corn, though in combination with other triazine herbicides (Dabrowski, 2015; Du Preez et al., 2005). After application, triazine herbicides can seep through soil to contaminate groundwater due to their moderately hydrophilic nature, low K_{ow} and weak soil adsorption (Du Preez et al., 2005; Graymore et al., 2001; Solomon et al., 2008). Atrazine is the most widely used chloro-s-triazine globally and has been detected in various water bodies and groundwater throughout South Africa since the 1980′s (Ansara-Ross et al., 2012; Takáts et al., 2001). Its use is banned in Europe since 2004 due to its relative persistence and risk of water contamination, though the use of the other triazine herbicides is still permitted (Ackerman, 2007; Hang et al., 2005; Herranz et al., 2008; Lin et al., 2011; Omotayo et al., 2011; Wang and Xie, 2012). Atrazine is however still used extensively in China, USA, India, South America, Africa and Australia (Ansara-Ross et al., 2012; Liu et al., 2016; Siddiqua et al., 2010). The maximum allowable limit of atrazine in drinking water in the USA and India is 3 μg L^{-1} (Singh and Cameotra, 2014) whilst the European maximum permissible level of atrazine in drinking water is 0.1 μg L^{-1} (El Sebaï et al., 2011; Herranz et al., 2008; Omotayo et al., 2011).

Triazine herbicide mode of action proceeds by inhibiting photosynthetic electron transport and blocking CO_2 fixation, causing accumulation of CO_2 in the plant, leading to plant death (Wackett et al., 2002; Wiegand et al., 2001). Atrazine is a herbicide of major significance in the Hartbeespoort Dam catchment as it is historically found in the highest concentrations in surface water (Ansara-Ross et al., 2012). In fish, atrazine has been shown to have effects ranging from inhibition of acetylcholinesterase, leading to decreased reflexes, increased respiration (Wiegand et al., 2001) and in addition, it has been discovered to cause a range of adverse effects on the reproductive and immune systems of oral and intravenously exposed rats and frogs (Ross and Filipov, 2006). The effects of ecologically relevant atrazine concentrations on biological species is highly controversial and the scientific community remains divided as there have been inconsistent conclusions drawn by various scientists in different studies, based on different endpoints including growth and gonadal abnormalities (Rohr and McCoy, 2010).

Atrazine has been proven to be an endocrine disruptor in many species (Freeman et al., 2011; Rohr and McCoy, 2010). However, numerous studies of effects of atrazine on the reproductive, nervous and immune systems on humans and animals have produced contradicting results with lack of consistency (Rohr and McCoy, 2010; Solomon et al., 2008; Wirbisky and Freeman, 2017). Atrazine has been observed to cause gene alterations that lead to endocrine disruption in zebrafish, inhibition of ovary growth in crabs, altering sex ratios in crayfish and frogs as well as demusculinising male gonads in fish, amphibians, reptiles and mammals (Hayes et al., 2011; Loughlin et al., 2016; Silveyra et al., 2017; Wirbisky and Freeman, 2017).

1.1. Atrazine and terbuthylazine degradation

Metabolism of atrazine and terbuthylazine is species-specific (Catenacci et al., 1993). In the majority of animals fed with radiolabelled atrazine, the vast majority of atrazine was eliminated unmetabolised, indicating a low bioaccumulation (Solomon et al., 2008). The degradation of triazine herbicides proceeds by microbial, photolytic or non-enzymatic chemical action such as the benzoxazinone catalysed reaction (Lin et al., 2008; Wiegand et al., 2001). This results in dechlorination, deamination or dealkylation, forming a variety of degradation products (Belfroid et al., 1998; Graymore et al., 2001; Loos and Niessner, 1999; Wang and Xie, 2012). The degradation of atrazine in the environment is mainly due to microbial action (Wackett et al., 2002). Atrazine can be metabolized by plants through N-dealkylation primarily by cytochrome P-450 followed by a phase II conjugation reaction to glutathione by glutathione-S-transferase (Ross and Filipov, 2006; Wiegand et al., 2001). Atrazine metabolites have been detected in carp liver of fish exposed to different concentrations of atrazine ranging from 4.28 to 428 μg L^{-1} (Xing et al., 2014) and *in vitro* atrazine exposure tests have shown the presence of monodealkylated deethylatrazine and desisopropylatrazine. Desisopropylatrazine can also be formed by degradation of both atrazine and simazine whilst deethylatrazine can be formed by degradation of atrazine and propazine (Jiang et al., 2005).

Terbuthylazine inhibits photosynthesis by altering chloroplast membrane proteins. Its degradation proceeds primarily either by microbial N-dealkylation of one of the s-triazine side chains to form desethylterbuthylazine (DET) or photolytic hydroxylation and dechlorination of the C2 chlorine to form hydroxyterbuthylazine and desethylhydroxyterbuthylazine (Bottoni et al., 2013; Sanlaville et al., 1996; Velisek et al., 2016). Terbuthylazine is slightly toxic to fish and can also be degraded to desisopropylatrazine and atrazine desethyldesisopropyl which are secondary degradation products arising from terbuthylazine metabolism (Du Preez et al., 2005). This study aims to assess the degree of triazine pollution in the Hartbeespoort Dam catchment and determine the points with the most significant influence triazine pollution for ecological risk profiling. The study design is only indicative of the seasonal variation of triazine herbicides as well as atrazine and terbuthylazine degradation products in the Hartbeespoort Dam catchment area. Though triazine herbicides are still used in South Africa, very little studies have looked at their fate in the environment and biota, particularly triazine metabolites.

2. Materials and methods

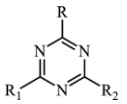
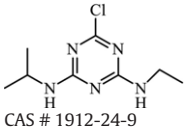
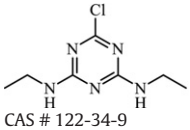
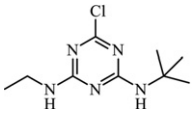
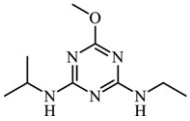
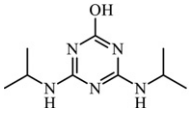
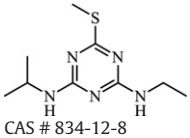
2.1. Chemicals and reagents

All analytical standards had a purity ≥96% (Tables 1 & 2) and all solvents had a purity >99.5%. Ultrapure Milli-Q water used in all preparation work was produced by a Millipore Advantage system (Merck, Johannesburg, South Africa) with a total organic carbon <3 mg L^{-1}.

2.2. Study area and sampling

Five sampling sites were located primarily around the major inlets and outlet of the Hartbeespoort Dam (Fig. 1) for determination of the point with the most influence on the Hartbeespoort Dam pollution. Another 5 points were located upstream of the Hartbeespoort Dam, in the Jukskei River where atrazine-use maps, produced by Dabrowski (2015), show significant atrazine use. The GPS coordinates of the Hartbeespoort Dam catchment sampling points are listed in supplementary information (SI) Table S1. The Jukskei River sampling points were sampled in the winter season. They are located downstream of heavily anthropogenically impacted areas further downstream in Johannesburg where the N14, Kyalami, Midrand, Buccleuch and Marlboro sites are located.

Table 1
Triazine herbicides analysed by GC–MS.

Compound		Supplier	Purity (%)	T	Q1	Q2	Q3
Triazine functional group							
Atrazine	 CAS # 1912-24-9	Accustandard	100	200	215	202	173
Simazine	 CAS # 122-34-9	Accustandard	99.6	201	186	158	173
Terbuthylazine	 CAS # 5915-41-3	Accustandard	100	214	173	216	229
Gesatamin	 CAS # 1610-17-9	Accustandard	99.8	196	211	169	58
Prometon	 CAS # 1610-18-0	Accustandard	100	210	168	225	58
Ametryne	 CAS # 834-12-8	Accustandard	100	227	212	170	98

T = Target ion.
Q = Qualifier ion.

The Marlboro, Buccleuch and Midrand sites are located primarily in areas with significant built construction. Sources of triazine herbicides in the Jukskei River catchment include golf courses located within 7 km upstream of the Marlboro site and peri-urban agricultural activities. The Magalies River point is located downstream of rich farming land further upstream where herbicides are extensively used (Dabrowski, 2015). The Harbour point is situated at the Department of Water and Sanitation offices and is also where boats are launched daily into the Hartbeespoort Dam for dam remediation projects. The groundwater site is situated in close proximity to the Dam wall point (1.1 km away) and the water is used for drinking purposes by the surrounding community and government offices. Sampling was conducted between November 2014 and September 2015, covering the four seasons of the year (Table 3). Sediment samples were sampled using a Van Veen grab sampler, filling a 500 mL glass jar. Water samples were sampled by sub-surface grab sampling using a bailer for the Hartbeespoort Dam 5 and 30 m depths, filling a 4 L amber glass bottle. All samples were taken in a random manner in a 2 m radius. The sampling plan was designed to coincide with peak herbicide application.

2.2.1. Seasons and climate

The Hartbeespoort Dam is characterized by a subtropical climate with summer rainfall and dry winters (Hely-Hutchinson and Schumann, 1998). According to the South African weather service, South African climate can be divided into 4 seasons (Table 3).

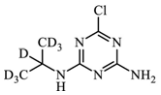
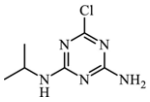
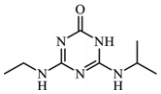
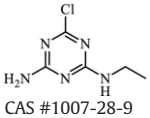
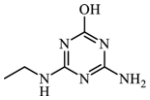
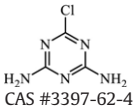
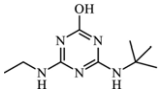
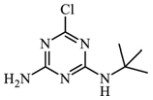
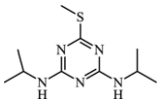
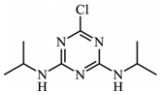
The 2014/2015 sampling period was characterized by moderately less than normal rainfall in the Hartbeespoort Dam catchment area, though the average rainfall was still within the 300–500 mm, 7- year historical level (South African Weather Service b). Of particular significance is the average annual rainfall for the Gauteng region which maintained the historical levels of 200–2000 mm, whose run-off contributes 90% of the water in the Hartbeespoort Dam from flow through the Crocodile River (Amdany et al., 2014; South African Weather Service b). Three main strategic sampling points have been identified as overtly influential to humans and wildlife. These are (i) the Crocodile River (and its upstream Jukskei sampling points) due to its significant contribution to the Hartbeespoort Dam by volume (ii) the Dam wall point, due to its offloading of pollutants out of the dam and (iii) the groundwater point due to it being a source of drinking water for humans.

2.3. Sample preparation and analysis

2.3.1. Sample preparation

2.3.1.1. Water samples. Bond Elut Plexa solid-phase extraction (SPE) cartridges (200 mg Styrene divinyl benzyl, Agilent Technologies, Chemetrix, Johannesburg, South Africa) were conditioned sequentially with 6 mL methanol (Sigma-Aldrich, Germany), 6 mL dichloromethane (DCM, (Sigma-Aldrich, Germany)) and 6 mL Milli-Q water before loading 1 L water samples onto them at a flow rate of 10 mL min^{-1}. The cartridges were dried under gentle vacuum before transporting to The

Table 2
Triazine degradation products and herbicides analysed by LC-MS/MS.

Compound		Supplier	Formation mechanism	Purity (%)	$[M+H]^+$	Product ion (Confirmation ions)
D7 deethylatrazine	 CAS # 1216649-31-8	Toronto Research Chemicals	Internal Standard	96	195.6	147.5 (68.2, 62.2)
Deethylatrazine (DEA)	 CAS # 6190-65-4	Dr Ehrenstorfer	Cytochrome P450 dealkylation (liver microsome metabolism) and microbial dehalogenation of atrazine (Graymore et al., 2001; Papadopoulos et al., 2012; Singh and Cameotra, 2014).	98	188.1	146.4 (68.2, 62.2)
Hydroxyatrazine (HA)	 CAS # 2163-68-0	Dr Ehrenstorfer	Chemical action (Graymore et al., 2001; Palm and Zetzsch, 1996) and microbial (enzymatic) dechlorination of atrazine (Lin et al., 2008; Seffernick et al., 2007; Wackett et al., 2002).	98	198.1	156.4 (86.2, 69.2)
Desisopropylatrazine (DIA)	 CAS #1007-28-9	Toronto Research Chemicals	Cytochrome P450 dealkylation (liver microsome metabolism), microbial dehalogenation of atrazine (Graymore et al., 2001; Papadopoulos et al., 2012) and terbuthylazine dealkylation (Du Preez et al., 2005).	99	174.1	68.2 (79.2, 62.2)
Atrazine desisopropyl-2-hydroxyl (AD-2OH)	 CAS # 7313-54-4	Dr Ehrenstorfer	Microbial dechlorination and dealkylation of atrazine (Singh and Cameotra, 2014).	98	156.1	69.2 (114.3, 86.3)
Atrazine desethyl-desisopropyl (ADD) (Didealykylatrazine)	 CAS #3397-62-4	Dr Ehrenstorfer	Microbial dehalogenation of atrazine (Graymore et al., 2001; Singh and Cameotra, 2014) and terbuthylazine metabolism (Du Preez et al., 2005)	96	146	68.2 (104.2, 62.2)
Hydroxyterbuthylazine (HT)	 CAS # 66753-07-9	Dr Ehrenstorfer	Chemical and microbial dechlorination of terbuthylazine with concomitant hydroxylation (Palm and Zetzsch, 1996; Papadopoulos et al., 2012)	98	212.1	156.4 (69.2, 86.2)
Desethylterbuthylazine (DET)	 CAS # 30125-63-4	Dr Ehrenstorfer	Cytochrome P450 dealkylation of terbuthylazine (liver microsome metabolism) (Du Preez et al., 2005; Papadopoulos et al., 2012)	98	202.1	146.4 (68.2, 62.2)
Prometryn	 CAS # 7287-19-6	Dr Ehrenstorfer	Herbicide	99	241	68.2 (200.5, 74.2)
Propazine	 CAS # 139-40-2	Dr Ehrenstorfer	Herbicide	99	214	68.2 (79.2, 62.2)

Netherlands under frozen conditions for extraction and analysis. The SPE method was validated against precision and reproducibility using D7 deethylatrazine as an internal standard. For GC analysis, the cartridges were eluted with 6 × 500 μL DCM portions before evaporating under a gentle stream of nitrogen and reconstituting with 1 mL toluene (J. T. Baker, Deventer, The Netherlands). For LC-MS/MS analysis the cartridges were eluted with 3 × 500 μL DCM followed by 3 × 500 μL methanol portions. Extra sample cleanup was performed by dispersive SPE (dSPE) after concentrating the DCM/methanol extract to near dryness with a gentle stream of nitrogen and changing the solvent to acetonitrile (Sigma-Aldrich, Missouri, USA). The acetonitrile extracts were added to a 10 mL Supel™ QUE PSA/C18(EN) (Deisenhofen, Germany) cleanup tube before adding 6 mL acetonitrile and vortexing for 30 s at 35 Hz, centrifuging for 8 min at 3800*g* (4000 rpm) and transferring the acetonitrile extract to a new test tube. The extract was evaporated to near dryness under a gentle stream of nitrogen and reconstituted in 200 μL of 10% methanol (Milli-Q water: Methanol, 90:10 v/v).

2.3.1.2. Fish samples. Three 5 year old catfish (*Clarias gariepinus*) and three 5 year old carp (*Cyprinus carpio)* were caught on 14 August 2015 using mobile nets and transported to the laboratory where internal organs were removed and separated prior to frozen storage for 2 days at −20 °C before analysis. Gravimetric measurements of the fish are shown in SI Table S2. The freezing process increases membrane permeability and causes phase separation of membrane components (Wolkers et al., 2007). The age of the catfish was estimated using weight-length ratios and the age of the carp was estimated by using a combination of weight-length ratios and scale annuls, using scales taken from between the lateral line and pectoral fin. Five year old fish were selected as their long life could have potentially lead to significant

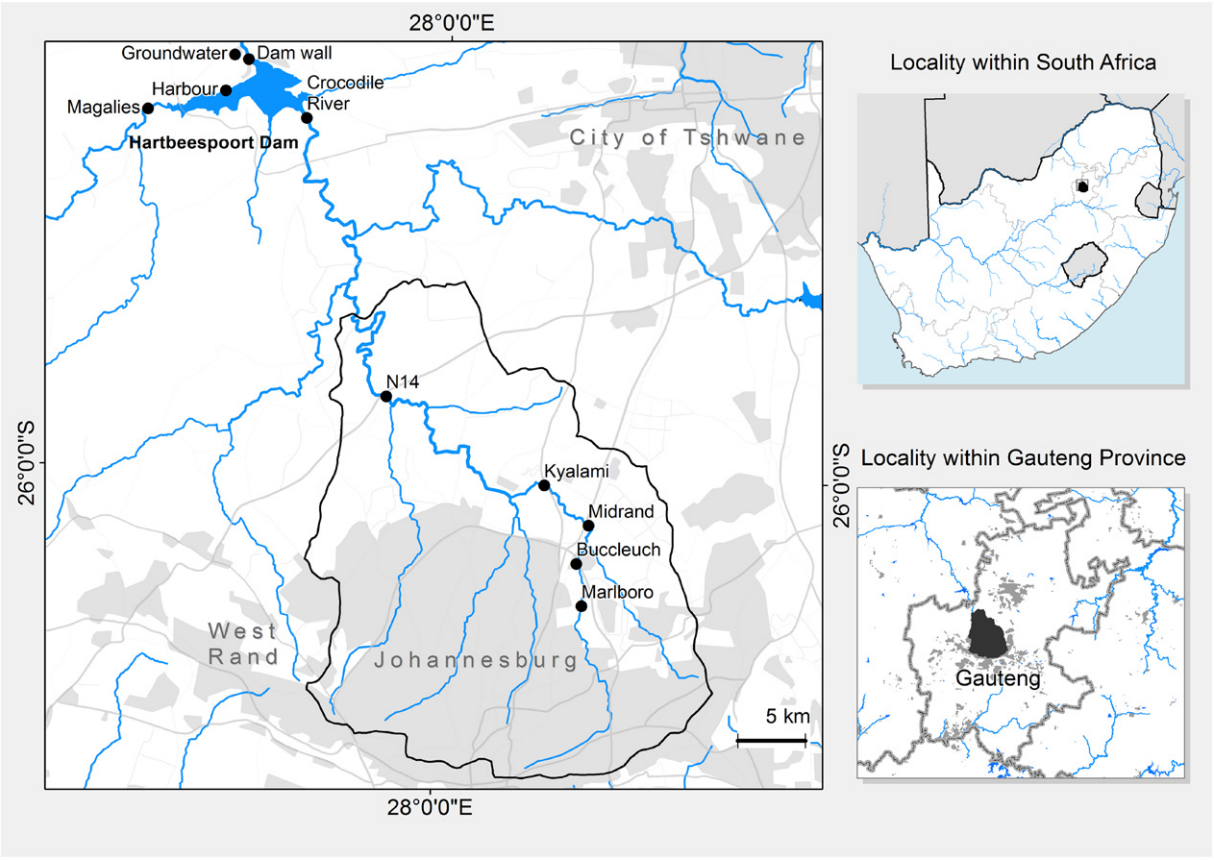


Fig. 1. Sampling area- Hartbeespoort Dam catchment The GPS coordinates of the sampling sites (as marked by the dots) can be found in the SI (Table S1). The Jukskei River catchment is shown in black border.

levels of exposure and bioaccumulation of pollutants. Fish muscle (15 g) from each of the 3 fish was pooled together and homogenized using a blender to create a representative sample. Fish muscle samples (28 g fresh weight [fw]) were weighed into two equal portions of 14 g into two 50 mL fluoroethylenepropylene centrifuge tubes. A 40 μL internal standard with a concentration of 1 mg L^{-1} D7 deethylatrazine was added to each centrifuge tube. Acetonitrile (8 mL) and 0.2 g NaCl (Merck, Darmstadt, Germany) were added before manually shaking vigorously for 15 s, vortexing for 15 s at 35 Hz and allowing the mixture to equilibrate for 12 h. The samples were centrifuged for 15 min at 3800*g* (4000 rpm) and the upper organic layer was removed before adding another 8 mL acetonitrile, vortexing for 15 s at 35 Hz and centrifuging for 15 min at 3800*g* (4000 rpm). The second organic layer was combined with the first organic layer in a clean centrifuge tube.

A modified Quick Easy Cheap Efficient Rugged Safe (QuEChERS) method was developed based on prior work conducted by Vudathala et al. (2010). A QuEChERS kit (Agela Technologies, Delaware, USA) containing 50 mg primary secondary amine (PSA), 50 mg C_{18}, 150 mg $MgSO_4$ was added to the fish extracts, together with an additional 200 mg $MgSO_4$ (Sigma-Aldrich, Missouri, USA), 200 mg PestiCarb (Agela Technologies, Delaware, USA), 100 mg diatomaceous earth (Sigma-Aldrich, Missouri, USA) and 250 mg basic alumina (Sigma-Aldrich, Missouri, USA) in a centrifuge tube. The sample extracts were vortexed for 15 s at 35 Hz before being left to settle for 5 min and centrifuged for 8 min at 3800 g (4000 rpm). The supernatant was transferred to a clean test tube, combining the two 14 g sample extracts and evaporated in a water bath at 38 °C to near dryness. Extracts for GC–MS analysis were reconstituted with 1 mL toluene and for LC-MS/MS analysis with 200 μL of 10% methanol.

Table 3
Sampling seasons and dates.

Calendar dates	Season	Sampling dates
1 September to 30 November	Spring	14 November 2014
1 December to 28/29 February	Summer	13 February 2015
1 March to 31 May	Autumn	15 May 2015
1 June to 31 August	Winter	14 August 2015

(South African Weather Service a).

2.3.2. Chemical analysis

Analysis of atrazine, simazine, terbuthylazine, ametryn, prometon and gesatamin was performed with gas chromatography coupled to a mass-spectrometer (GC–MS, GC 6890 N, MS 5975, Agilent Technologies, CA, USA) with oven temperature programming 70 °C (2 min), 25 °C min^{-1} to 150 °C, 3 °C min^{-1} to 200 °C, 8 °C min^{-1} to 280 °C (17 min) with a 1 μL splitless injection and an injector temperature of 280 °C. A Zebron ZB-Multiresidue 1 column (30 m × 0.25 mm × 0.25 μm; Phenomenex, USA) was used with constant pressure of 151 kPa.

Analysis of propazine, prometryn, deethylatrazine (DEA), hydroxyatrazine (HA), desisopropylatrazine (DIA), atrazine desisopropyl-2-hydroxyl (AD-2OH), atrazine desethyl-desisopropyl (ADD), hydroxyterbuthylazine (HT) and desethylterbuthylazine (DET) was performed with liquid chromatography coupled to a triple quadrupole mass-spectrometer (LC-MS/MS, 1200 series LC system, 6410 triple quadrupole MS; Agilent Technologies, Amstelveen, The Netherlands). After comparing different C_{18} columns with different ionization modes at different pH, buffer conditions and mobile phase ratios, the method was finally optimized on a Biphenyl 100 Å LC-column (2.6 μm, 100 × 2.1 cm) with gradient mobile phase comprising of 5 mM ammonium formate (pH 4, solvent A) and 1.5% formic acid in methanol (solvent B) with a 10 μL sample injection volume. A 0.3 mL min^{-1} flow rate was

used with a gradient of 0% B 0–2 min, 20% B 2 min; 95% B 10 min, 0% B 15.5–30 min. The LC-MS/MS was performed using an electrospray ionization (ESI) source in positive mode, source spray voltage 4 kv, transfer capillary temperature 350 °C, gas flow 9 mL min^{-1} and nebulizer pressure 40 psi. Transitions are listed in Table 2.

Stock solutions for GC–MS analysis were made up to 100 mg L^{-1} in toluene. Stock solutions for LC-MS/MS analysis were made up to 1 mg mL^{-1} in methanol, with the exception of HT, ADD and HA which have very low solubility in methanol hence were made in a lower concentrations of 100 mg L^{-1} in Milli-Q water: methanol (50:50 v/v). A 7 point calibration curve was used for GC–MS analysis, ranging from 0.0156 mg L^{-1}, 0.0313 mg L^{-1}, 0.0625 mg L^{-1}, 0.125 mg L^{-1}, 0.25 mg L^{-1}, 0.5 mg L^{-1} to 1 mg L^{-1}. A 10 point calibration curve was used for LC-MS/MS analysis, ranging from 0.2 ng L^{-1}, 0.4 ng L^{-1}, 0.8 ng L^{-1}, 2 ng L^{-1}, 4 ng L^{-1}, 10 ng L^{-1}, 20 ng L^{-1}, 40 ng L^{-1}, 80 ng L^{-1} to 200 ng L^{-1}. All calibration curves had a regression of 0.998 or better. A combination of standard addition and labeled internal standard calibration was used to calculate recoveries and compensate for matrix effects. Recoveries for atrazine herbicides ranged from 80 to 102% and atrazine degradation products ranged from 69 to 112% (SI Table S3). Limit of detection (LOD) and limit of quantification (LOQ) were calculated at 3× and 10× the signal to noise ratio respectively. The analytical method robustness was successfully tested against precision and repeatability using 11 samples. No triazine compounds were detected in the blank samples ($n = 6$) analysed.

3. Results and discussions

The ∑triazine herbicide abundance concentrations in the Hartbeespoort Dam were in the order atrazine > simazine > terbuthylazine > propazine > ametryne > prometryn (Table 4). The results show that ∑triazine concentrations in the Hartbeespoort Dam peak at summer and gradually decrease throughout successive seasons of autumn, winter and spring. This can be explained as the vast majority of the maize crops have emerged by mid-summer and herbicide application to kill weeds has been conducted as pre- or post-emergence application. In the Hartbeespoort Dam, atrazine was found in the highest concentrations in summer with average concentrations around 800 ng L^{-1} where sampling was conducted at the height of the seasonal annual rains. The average atrazine levels declined to around 600 ng L^{-1} in autumn, further declining to around 500 ng L^{-1} in winter and declining again to around 400 ng L^{-1} in spring. Flow data for the Crocodile River (Fig. 2) obtained from gauging station A2H012 and flow data for the Magalies River (Fig. 3) obtained from gauging station A2H013 (DWA Hydrology, 2017) shows that the summer season had the highest flows as it is in the peak of the rainy season. The summer season recorded average flows of 21 and 0.54 m^3 s^{-1} in the Crocodile and Magalies Rivers respectively.

The ∑Crocodile River triazine herbicide concentrations were similar to the ∑Dam wall triazine herbicide concentration at 30 m below the water surface (2 to 5 m above the bottom) in autumn (2185 and 2378 ng L^{-1}, respectively) with a relative standard deviation (RSD) of 6% and winter (1863 and 1903 ng L^{-1}, respectively) with a RSD of 1.5%. This shows that the in the cold season, the colder water from the Crocodile River (Fig. 4 passes along the bed of the dam to the outflow from autumn through to spring as described by Hely-Hutchinson and Schumann (1998). In the spring and summer seasons where the Crocodile River water was warmer than the Hartbeespoort Dam water (Fig. 4), the ∑Crocodile River triazine herbicide concentrations showed much higher differences with the ∑Dam wall 30 m triazine herbicide concentrations. The ∑Crocodile River triazine herbicide and ∑Dam wall 30 m triazine herbicide concentrations were 760 and 1653 ng L^{-1} respectively in spring (RSD of 52%), and 2287 and 5061 ng L^{-1} respectively in summer (RSD of 55%).

Table 4
Triazine herbicides in the water samples from Hartbeespoort Dam catchment (ng L^{-1}).

	Atrazine	Simazine	Terbuthylazine	Ametryn	Prometon	Gesatamin	Propazine	Prometryn	∑Triazine herbicide
LOD 3×	5	5	5	5	5	5	0.1	0.1	
LOQ 10×	17	17	17	15	17	16	0.3	0.2	
Summer									
Crocodile R.	940	580	N.D	340	N.D	N.D	419	7	2287
Dam wall 30 m	1570	610	1969	306	N.D	28	574	4	5061
Harbour	1237	654	684	112	N.D	N.D	877	6	3570
Magalies R.	830	630	19	260	N.D	20	486	5	2250
Groundwater	180	30	80	N.D	N.D	N.D	77	N.D	367
Autumn									
Crocodile R.	631	556	547	86	N.D	N.D	362	3	2185
Dam wall 30 m	699	598	576	88	N.D	N.D	415	3	2379
Harbour	760	636	602	95	N.D	N.D	790	5	2888
Magalies R.	644	570	504	87	N.D	N.D	359	3	2166
Groundwater	138	217	61	N.D	N.D	N.D	75	N.D	491
Winter									
Crocodile R.	523	471	425	75	N.D	N.D	366	4	1863
Dam wall 30 m	576	485	460	78	N.D	N.D	301	3	1903
Harbour	543	480	433	75	N.D	N.D	415	5	1950
Magalies R.	503	438	412	77	344	N.D	231	6	2010
Groundwater	152	221	66	N.D	N.D	N.D	88	N.D	527
Spring									
Crocodile R.	483	N.D	117	N.D	N.D	N.D	150	10	760
Dam Wall 30 m	503	503	378	71	N.D	N.D	198	N.D	1653
Harbour	453	453	378	68	N.D	N.D	348	3	1703
Magalies R.	234	99	53	N.D	N.D	N.D	48	1	299
Groundwater	100	154	64	N.D	N.D	N.D	76	N.D	394
Dam wall 5 m	450	395	360	67	N.D	N.D	167	2	1442
Jukskei river winter									
N14	923	480	134	N.D	N.D	N.D	66	<0.2	1602
Kyalami	210	<5	74	N.D	N.D	N.D	208	6	499
Midrand	<5	<5	148	N.D	N.D	N.D	65	1	214
Buccleuch	<5	<5	110	N.D	N.D	N.D	92	1	202
Marlboro	<5	658	130	N.D	N.D	N.D	80	1	869

N.D. = Not detected.
< = Below LOQ.

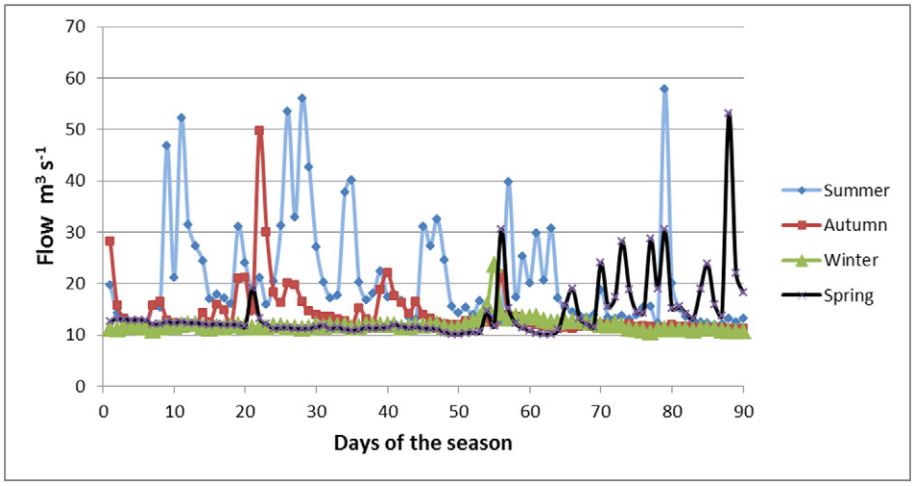


Fig. 2. Crocodile river flow.

With 8 days recording flow rates >40 $m^3 s^{-1}$ in the summer season, the Crocodile River has much higher flows that the Magalies River which recorded flows <3.5 $m^3 s^{-1}$ in all seasons (Figs. 2 and 3). The Magalies River showed similar ∑ triazine herbicide concentrations with the Crocodile River, however the much lower Magalies River flows into the Hartbeespoort Dam (Fig. 3) means it has a less significant effect on the Hartbeespoort Dam water chemistry compared to the Crocodile River (Fig. 2). The high triazine compound concentrations in summer (Tables 4 & 5), coupled with increased summer flow rates into the Hartbeespoort Dam shows that the summer season has the largest contribution of triazine herbicides into the Hartbeespoort Dam. The last month of spring experienced high flows into the Hartbeespoort Dam whilst the first month of autumn experienced high flows of water as these mark the beginning and end of the rainy season, respectively. The Crocodile River flow is on average 38 times higher than the Magalies River flow, year on year. This shows that the Crocodile River has a much higher influence on the water quality of the Hartbeespoort Dam. The hydraulic residence time of water in the Hartbeespoort Dam is one year, with water at the bottom having a lower residence time as the outflow port is 11 m above the bottom of the dam (Hely-Hutchinson and Schumann, 1998). The high summer ∑ triazine herbicide concentration (5061 ng L^{-1}) at the Dam wall at 30 m depth (Table 4) can be explained by the combined effect of thermal stratification with loading of the hypolimnion by triazines from the Crocodile and Magalies Rivers and resuspension of pollutants in the sediments by bottom feeding fish.

The lower Jukskei River catchment sampling sites of Midrand, Buccleuch and Marlboro which are located in highly industrialised areas recorded very low atrazine levels, below the LOD as there are no significant undeveloped land areas available for growing plants. The furthermost downstream point of Marlboro which is located downstream of two golf courses, recorded a significant simazine concentration of 658 ng L^{-1}. However the immediate successive three downstream points had no significant simazine concentrations as they were below the LOD. This may be attributed to the dilution effect as the Jukskei River flow gets significantly stronger downstream of the Marlboro point. The terbuthylazine concentrations through the Jukskei River transect was fairly consistent, approximately 100 ng L^{-1} and propazine concentrations were between 65 and up to 208 ng L^{-1} (Table 4).

In the Jukskei River, terbuthylazine and propazine were quantified at all the Jukskei River points. The N14 site recorded high atrazine, and simazine concentrations of 923, and 480 ng L^{-1} and the Marlboro point recorded the highest simazine concentration of 658 ng L^{-1}. The highest Jukskei River atrazine winter concentration of 923 μg L^{-1} was recorded in the N14 site, being higher than the downstream Crocodile River inlet point winter concentration of 523 ng L^{-1} as well as the Magalies River inlet point winter concentration of 503 ng L^{-1}. This indicates that the Jukskei River contributes significantly to Hartbeespoort Dam atrazine herbicides. A comparison of historical data shows a decline in atrazine concentrations over the past three decades as values recorded by Ansara-Ross et al. (2012) show surface water concentrations of 730 to 14,970 ng L^{-1} and groundwater concentrations of 490 to 3890 ng L^{-1}

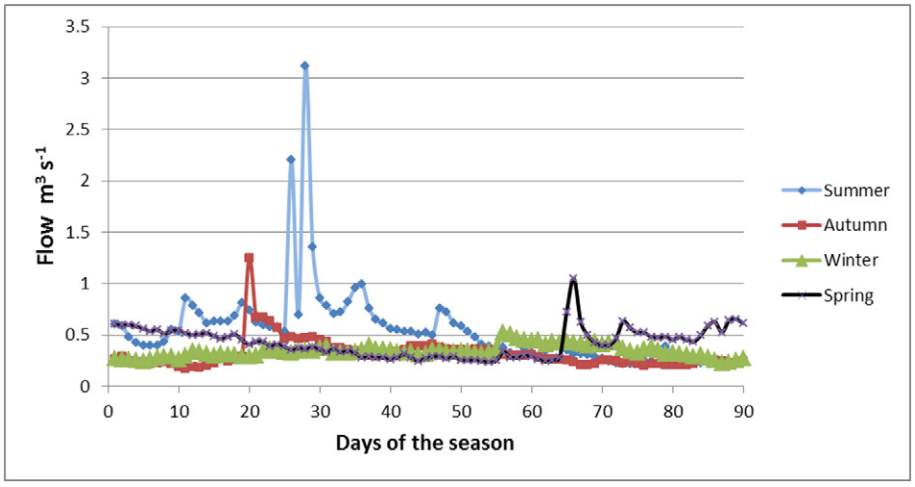


Fig. 3. Magalies river flow.

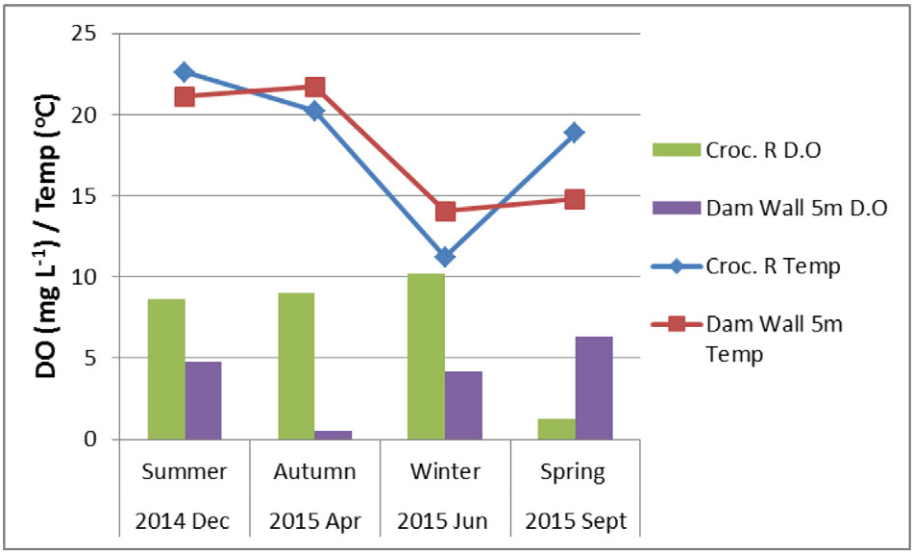


Fig. 4. Temperature and DO profiles of the Crocodile river and Dam wall epilimnion.

during the early 1990's maize growing areas in Gauteng Province. The major cause of the decrease is due to expanded urban development in the province where city developments have taken over previous farming areas especially along the Jukskei River.

Groundwater triazine concentrations were similar throughout the four seasons, recording averages of 156, 155 and 67 ng L^{-1} for atrazine, simazine and terbuthylazine respectively. Groundwater atrazine concentrations did not follow the surface water trends of a gradual decrease from summer season, as the highest concentration of 180 ng L^{-1} was recorded in summer followed by the winter season with a concentration of 152 ng L^{-1}. The average atrazine groundwater concentration for the four seasons of 156 ng L^{-1} was much lower than the atrazine concentrations detected in the Hartbeespoort Dam surface water but higher than the European maximum permissible atrazine level for drinking water of 100 ng L^{-1} and lower than the USA and Indian maximum permissible levels of 3000 ng L^{-1}. There are currently no maximum permissible levels for atrazine in drinking water prescribed in South Africa, though a target range of 0 to 2000 ng L^{-1} is stated in the South African Water Quality Guidelines for domestic water use. The highest atrazine concentration recorded was at the Dam wall site in summer which peaked at 1570 ng L^{-1} sampled at a depth of 30 m which is within the target range.

Table 5
Atrazine and terbuthylazine degradation products (DP) in water samples from the Hartbeespoort Dam catchment (ng L^{-1}).

	DIA	HA	DEA	HT	DET	ADD	AD-2OH	∑DP
LOD 3×	0.2	0.1	0.1	0.1	0.1	0.1	0.1	
LOQ10×	0.5	0.3	0.2	0.3	0.3	0.3	0.2	
Summer								
Crocodile R.	16	4	184	16	447	N.D	N.D	667
Dam wall 30 m	17	6	164	20	388	N.D	N.D	594
Harbour	18	4	127	17	402	N.D	N.D	569
Magalies R.	14	4	162	13	277	N.D	N.D	470
Groundwater	6	1	85	1	136	N.D	N.D	229
Autumn								
Crocodile R.	14	3	157	13	313	N.D	N.D	500
Dam wall 30 m	14	4	144	18	321	N.D	N.D	501
Harbour	15	3	171	12	377	N.D	N.D	578
Magalies R.	13	3	114	11	247	N.D	N.D	387
Groundwater	5	1	52	1	99	N.D	N.D	157
Winter								
Crocodile R.	22	4	166	13	303	N.D	N.D	509
Dam wall 30 m	18	4	147	13	239	N.D	N.D	421
Harbour	19	4	172	14	282	N.D	N.D	491
Magalies R.	10	2	129	7	225	N.D	N.D	373
Groundwater	2	1	33	1	122	N.D	N.D	159
Spring								
Crocodile R.	10	2	106	6	197	N.D	N.D	322
Dam Wall 30 m	9	2	105	6	174	N.D	N.D	297
Harbour	6	1	80	5	251	N.D	N.D	343
Magalies R.	2	1	19	2	30	N.D	N.D	53
Groundwater	6	1	68	1	129	N.D	N.D	204
Dam wall 5 m	6	2	91	8	232	167	2	507
Jukskei river winter								
N14	3	1	45	5	104	N.D	N.D	158
Midrand	8	3	82	11	187	N.D	N.D	291
Kyalami	3	1	46	4	120	N.D	N.D	174
Marlboro	15	5	92	9	149	N.D	N.D	271
Buccleuch	9	2	81	4	133	N.D	N.D	230

ND = Not detected.

Data on atrazine and terbuthylazine degradation products in South Africa is scanty and has never been studied in the Hartbeespoort Dam, though DIA, DEA and ADD have been assessed upstream of the Magalies River (Du Preez et al., 2005). DET is the degradation product found in the highest concentrations in the Hartbeespoort Dam sites with concentrations >300 ng L^{-1} for the Crocodile River site in summer, autumn and winter seasons (Table 5). Seasonal trends for triazine degradation products follow the triazine herbicide trends with the highest seasonal triazine ∑ degradation product concentrations of 2529 ng L^{-1} being recorded in summer and gradually decreasing in successive seasons through to spring which recorded the lowest ∑ degradation product concentration of 1220 ng L^{-1} (Table 5). Of the triazine degradation products tested in the Jukskei River, DET concentrations were highest, recording concentrations >100 ng L^{-1} at all sites. DEA had the second highest triazine ∑ degradation product concentrations in both the Hartbeespoort Dam and Jukskei River points. DET has been described as the main degradation product of terbuthylazine and is usually found in high concentrations in impoundments in close proximity to areas treated with terbuthylazine (Bottoni et al., 2013; Stara et al., 2016) The triazine ∑ degradation product concentration was in the order DET > DEA > DIA > HT > HA. ADD and AD-2OH could not be detected in any of the water samples tested. The data reveals a wide variation in ∑ degradation product concentration contribution from different sites with the Crocodile River showing the highest ∑ degradation product concentration in summer and winter of 667 and 509 ng L^{-1} respectively, whilst the Harbour site had the highest ∑ degradation product concentration in the autumn and summer seasons of 578 and 569 ng L^{-1} respectively.

3.1. Mixing and pollutant stratification

Density mixing in the Hartbeespoort Dam has been studied intricately in the past three decades and has been reported to occur as early as in April, and can repeat after further combinations of cold weather, wind and gravity (Hely-Hutchinson and Schumann, 1998; Robarts et al., 1982). This leads to cool surface water that is otherwise warm in other seasons, to move down to the bottom of the dam as it becomes denser, causing an overturn (Robarts et al., 1982). The dissolved oxygen (DO) and temperature data (Fig. 4) shows that the overturn occurred in autumn as characterized by sudden and concurrent drops in DO and epilimnion water temperature. Consistent Dam Wall point vertical depth temperatures recorded in the months April to June at 0 to 30 m below the water surface show that overturn had occurred in April and mixing continued from autumn to winter.

Fig. 4 shows DO and temperature measured using a calibrated YSI multiparameter reading instrument. There are no significant differences in the triazine concentrations between the 30 m and 5 m level below the surface in spring, suggesting that the water was well mixed in spring after the April mixing as described by Robarts et al. (1982). This shows that there is adequate mixing of the water between the epilimnion and hypolimnion. The temperatures recorded for the Crocodile River and Dam Wall 5 m below the surface (Fig. 4) correspond well with historical data. The Crocodile River water temperature was cooler than the Dam wall epilimnion in autumn and winter, but warmer in spring and in summer. The April overturn is characterized by the epilimnion becoming warmer than the Crocodile River water, as shown by the Dam wall 5 m depth which becomes warmer than the Crocodile River in winter (Fig. 4). This causes the cooler and denser Crocodile River water to sink down to the epilimnion as re-stratification commences after winter, creating optimum conditions for the Crocodile River water to flow through the dam fairly rapidly as it exits the dam out of the outlet 11 m above the surface of the bed of the dam. This creates a lower residence time for the Crocodile River water. To some extent, this may assist to offload large volumes pollutants from the Crocodile River out of the dam. The Dam wall DO epilimnion concentration in mid-April 2015 reached 0.5 mg L^{-1} which is consistent with historical measurements and maintained the mixing throughout the autumn-winter season before re-stratifying in spring.

3.2. Groundwater interaction

The presence of atrazine in groundwater may point to the presence of a surface water and groundwater hydraulic link. The contamination of groundwater is a major concern as many communities in South Africa rely on groundwater for drinking water purposes, as they have no access to treated water (Dabrowski, 2015). It is difficult to confidently formulate sound hypotheses on surface water-groundwater interactions unless stable isotopes with ^{18}O, ^{2}H, ^{3}H dyes or salts are used (Abiye et al., 2015). Surface water-groundwater interaction in the Hartbeespoort Dam precinct is poorly understood (DWA Groundwater, 2010). The Hartbeespoort Dam has been described by different authors as lying on quartzite rock consisting mainly of shale and homfels with 62% SiO_2, 19% Al_2O_3 and 8% Fe_2O_3 (Abiye et al., 2011; Coetzee, 1993). The Hartbeespoort Dam quartzite is located adjacent to an expanse of a dolomitic water bearing aquifer from the West Rand area up to further north in the City of Tshwane (Abiye et al., 2015; Abiye et al., 2011). There is evidence from isotope studies that some groundwater within the Hartbeespoort Dam catchment has been circulating underground for >50 years. Hence it is possible that groundwater contaminants may prevail for decades (Abiye et al., 2011).

The low permeability of the Hartbeespoort Dam quartzite rock without evidence of significant fractures and fissures in the Hartbeespoort Dam quartzite rock (Abiye et al., 2015) makes it difficult to confidently draw interactions between the aquifer water and the Hartbeespoort Dam surface water. The groundwater site is located on a slope with the borehole descending vertically, primarily between sheets of sloping quartzite and hybrid conglomerate granophyric rock, but appears to be sealed off from interaction with the Hartbeespoort Dam by impervious rock that can be accurately described as quartzite, locally embedded with hornfels and slate (Abiye, 2011; Scott et al., 1977; Schifano, 1971, Map). The low concentration of atrazine in groundwater compared to the Hartbeespoort Dam may indicate that seepage of irrigation water from top soil is the main source of the groundwater pollution and that there is little or no groundwater-Hartbeespoort Dam surface water interaction.

3.3. Triazines and degradation products in fish muscle

African sharptooth catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) were selected for triazine pollution monitoring as they are major fish species in the Hartbeespoort Dam by biomass. They are also the most consumed fish by humans as they are the major catch from the dam (Koekemoer and Steyn, 2005). Catfish and carp have a high ecological significance as they are edible piscovorous fish which are high up the food chain and have the potential to bioaccumulate organic pollutants to concentrations higher than those found in the rest of the aquatic environment (Squandrone et al., 2013a; Squandrone et al., 2013b). No triazine herbicides were detected in fish muscle by GC–MS or LC–MS/MS analysis (Table 6). This indicates that triazine herbicide bioaccumulation is negligible.

Bioturbation of the hypolimnion due to large quantities of bottom feeding carp and catfish fish in the Hartbeespoort Dam is known as the cause of resuspension of pollutants in the Hartbeespoort Dam (Hart and Harding, 2015). Only DET and DEA were detected in catfish muscle with concentrations of 0.3 and 0.2 ng g^{-1} respectively. As with the Hartbeespoort Dam water concentrations, DET was found in the highest concentration in catfish. Only DEA was detected in carp muscle with a concentration of 0.3 ng g^{-1} Table 6). Toxicity data on triazine herbicide metabolites is scanty but is thought to be equivalent to parent triazine herbicides (Ralston-Hooper et al., 2009).

3.4. Statistical analysis on factors affecting Hartbeespoort Dam triazine concentrations

The influence of season, sampling point and triazine pollution on the Hartbeespoort Dam triazine pollution was statistically tested using the software package Statistical Package for Statistical Sciences (SPSS) version 16 to determine the factors which have the largest influence on the triazine load in the Hartbeespoort Dam. A test for the homogeneity of variances between the season, sampling point and triazine produced a *p*-value of 0.961 which indicates that equal variances are assumed. A one way ANOVA (Table 7) was used to determine statistically significant differences between groups and to prove/disprove that the differences between the triazine concentrations is independent of the season, sampling site and triazine type.

A test for homogeneity of variances between the different seasons indicates that there are no statistically significant differences between the total triazine concentrations within the seasons as the *p*-value is 0.82 (Table 7). To test the assumption of homogeneity of variances between the five Hartbeespoort Dam sampling points, the *p*-value of 0.04 (Table 7) computed indicates that there are statistically significant

Table 6
Triazine degradation products in fish muscle (ng g^{-1} fw).

Fish sample	ADD	AD-2OH	DIA	HA	DEA	HT	DET
LOD 3× (ng g^{-1})	0.1	0.1	0.2	0.1	0.1	0.1	0.1
LOQ 10× (ng g^{-1})	0.3	0.2	0.5	0.3	0.2	0.3	0.3
Catfish	N.D	N.D	N.D	N.D	0.2	N.D	0.3
Carp	N.D	N.D	N.D	N.D	0.3	N.D	N.D

N.D. = not detected.

Table 7
One way ANOVA results.

		Sum of squares	df	Mean square	F	*p*-Value
Seasons	Between groups	250	204	1.22	0.667	0.82
	Within groups	11	6	1.83		
	Total	261	210			
Sampling points	Between groups	400	204	1.96	3.921	0.043
	Within groups	3	6	0.50		
	Total	403	210			
Triazine	Between groups	3323	204	16.29	5.43	0.019
	Within groups	18	6	3.00		

$p < 0.05$ is statistically significant.

differences between triazine concentrations at different sampling points. To test if there are statistically significant differences between the different triazine compounds detected and quantified throughout the seasons, across all Hartbeespoort Dam sampling points, a *p*-value of 0.02 (Table 7) was computed. This indicates that there are indeed statistically significant differences between different triazine compounds detected and quantified. The differences between the different triazine concentrations in the Hartbeespoort Dam are therefore statistically independent of the sampling season but dependant on the sampling point location as well as the specific triazine compound.

4. Conclusions

Terbuthylazine had the highest detection rate in the Jukskei River and its degradation product DET was the most abundant triazine degradation product in the Jukskei River catchment as well as the Hartbeespoort Dam surface water and groundwater. The N14 site downstream of the Jukskei River recorded high atrazine concentrations which were higher than concentrations in the majority of the sampling sites in the Hartbeespoort Dam. The significantly higher flow from the Crocodile River into the Hartbeespoort Dam indicates that the Jukskei River has the highest influence on the Hartbeespoort Dam triazine load. In fish muscle, no triazine herbicides were detected. In the Hartbeespoort Dam, the summer season recorded highest $\sum$ triazine herbicide and $\sum$ triazine degradation product concentrations in the water samples with a declining trend in the following successive seasons. Groundwater $\sum$ triazine and $\sum$ triazine degradation product concentrations were fairly consistent throughout the seasons. Groundwater atrazine concentrations recorded values greater than the European maximum permissible level for drinking water of 100 ng L^{-1} in all seasons except spring, which recorded a value of 100 ng L^{-1}. This indicates that there is a significant risk in consumption of the contaminated groundwater. The analytical and geohydrological data show no evidence of groundwater-Hartbeespoort Dam surface water interaction. Thus, the source of triazines to the groundwater needs further investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.09.119.

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