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Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa

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ABSTRACT

Concentrations of total aluminium (Al) and iron (Fe) were determined in Lake Loskop over a period of four months in 2009 in samples of phytobenthos, phytoplankton, macroinvertebrates, amphibians and fish. The highest concentrations of Al and Fe were measured in the filamentous algae *Spirogyra fluviatilis* (Hillse) and *Spirogyra adanata* (Kütz), (Al=18,997.5 mg kg^{-1} dry weight and Fe=22,054.2 mg kg^{-1} dry weight) in the riverine zone of the lake with a near-neutral water average pH of 7.3. However, a negative correlation exists between the Al and Fe concentrations measured in the filamentous algae in comparison with the corresponding concentrations of these elements in the water column of the riverine zone. The Al concentrations in the macroinvertebrate families collected ranged from 140.6 to 385.7 mg kg^{-1} dry weight, with the highest values measured for Al and Fe in the family Gomphidae (385.7 and 1710.0 mg kg^{-1} dry weight, respectively) in comparison to other macroinvertebrate families sampled. Al and Fe concentrations (2580 and 10,697 mg kg^{-1} dry weight) in the stomach contents of adult *Oreochromis mossambicus* fishes were much higher in comparison with adult *Micropterus salmoides* fishes (98.5 and 439.6 mg kg^{-1} dry weight), respectively. In all cases of dissected fish species either white or yellow body fat was observed, thus in none of the samples both type of body fats occurred simultaneously. The concentrations of total Al and Fe in the different organs of *O. mossambicus* were along a mean sequence of intestine > yellow body fat > brain > gills > liver > heart > white body fat, while the mean sequence of total Al and Fe in *M. salmoides* was: intestine > gills > liver > heart > brain > white body fat. From the levels of Al detected in the yellow body fat of the studied fish species *O. mossambicus*, we suggest that this phenomenon may be related to the feeding habits of this species. Furthermore, the intake of certain species of phytobenthos by *O. mossambicus* could have played a role in the bioaccumulation of Al in the food chain and the possible development of pansteatitis in predators at higher trophic levels.

1. Introduction

In the upper Olifant's River catchment, acid mine drainage, untreated sewage and industrial pollution and extensive agriculture are the main sources of anthropogenic stressors on the aquatic environment (Driescher, 2008). The situation is exacerbated by a historic lack of policy implementation to address acid mine drainage (AMD), especially at mine closure. As a consequence, AMD from closed ownerless coal mines in the catchment creates tremendous environmental liabilities for the South African government (Adler et al., 2007). The Department of Water Affairs and Forestry (DWAF) has spent more than US$20 million over the last decade to investigate and clean up the historic pollution caused by abandoned or liquidated mines (Schwab, 2002).

A number of flooded underground coal mines such as the Middelburg Colliery – located to the west and northwest of the city of Witbank – commenced decanting acidic water in the mid-1990s. These mines contribute large volumes of AMD to the upper reaches of the Olifant's River catchment upstream of Lake Loskop, which acts as a repository for pollutants from the upper catchment (Oberholster et al., 2010). To the authors' knowledge, only one earlier study has been carried out on the upper Olifant's River to determine metal bioaccumulation in fish, and which included aluminium (Al) as a toxic agent of AMD (Coetzee et al., 2002). The authors found in their study that Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe

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preferentially accumulated in the skin, muscle, liver and gill tissues of fish with the highest metal concentrations localised to the liver and gill tissues of *Clarias gariepinus* and *Labeo umbratus*. Aluminium chemistry depends strongly on pH and under normal environmental conditions (circum-neutral pH values) it is mostly insoluble. However, the solubility of Al increases significantly under both acidic ($pH < 6.0$) and alkaline ($pH > 8.0$) conditions (Scancar and Milacic, 2006). Several studies have addressed the issue of increasing Al concentrations in surface waters due to acidification, which results in Al toxicity to fish and other aquatic organisms, such as benthic macroinvertebrates when toxic thresholds were exceeded (Herrmann, 1990; Witters, 1998; Soucek, 2006). However, no previous studies have been conducted on the bioaccumulation or bio-magnification of Al and Fe in the food chain of river or lake water with a neutral pH using different trophic levels (phytoplankton, phytobenthos, macroinvertebrates, amphibian and fish) downstream of areas impacted by a mixture of mining and industrial activities, as well as untreated sewage inflow.

In a recent study conducted by Oberholster et al. (2010) elevated concentrations of Al and Fe were detected in the surface water of Lake Loskop during the late summer rainy season of 2008. High concentration of Al can have detrimental effects on the aquatic environment (Soucek et al., 2001), as well as on humans that may consume fish containing high levels of Al. At a circum-neutral pH as in the case of Lake Loskop, Al can accumulate in significant amounts in some organisms in particular filter-feeders and grazers (Elangovan et al., 1999). From an ecological perspective, the incidents of Nile crocodile (*Crocodylus niloticus*) and serrated hinged terrapin (*Pelusios sinuatus*) mortalities in Lake Loskop during the past five years (2003–2008) have resulted in a decline in the crocodile population from approximately 30 animals to a total of 6 in 2008, and coincided with an incident where fourteen tonnes of fish in Lake Loskop died during this time (Driescher, 2008; Paton, 2008). The crocodile and terrapin mortalities in Lake Loskop were ascribed to pansteatitis (hardening of body fat, changes in body fat colour from white to yellow or orange), causing stiffness in the crocodiles and resulting in their death which appears to be associated with the intake of rancid fish fat after a fish die-off (Oberholster et al., 2010). However, to date no unequivocal answer could be formulated on the precise cause(s) of pansteatitis or the die-off of crocodiles and terrapins in Lake Loskop (Lubick, 2009) (Fig. 1). In a previous study by Yoshino et al. (1999) the authors reported that aluminium mediated inhibition of the oxidation of ferrous ion causing oxidative damages to membrane lipids. This observation may provide a possible explanation for the link between fish sampled with only white or yellow body fat in the present study. During the same time of this study, the crocodile population in the Olifant's River Gorge downstream of Lake Loskop in the Kruger National Park, which is one of the largest conservation areas in Africa, declined from 1000 to 400 due to pansteatitis. No obvious fish die-off was, however, reported before the pansteatitis outbreak in the Kruger National Park indicating that this phenomenon may not be the only cause of the crocodile mortalities (Paton, 2008). The objectives of the study were (1) to compare the concentrations of Al and Fe in representative biota at different trophic levels to determine the extent to which these metals are bioaccumulated or bio-magnified in the food chain of Lake Loskop; (2) to report the spatial heterogeneity between different biota sampled in the area and (3) to determine the possible association between bioaccumulation of Al and Fe in the food chain and the occurrence of fish yellow body fat in fish, and the possible association with the occurrence of pansteatitis at higher levels in the food chain.

2. Materials and methods

2.1. Sampling sites

The dam wall that impounds Lake Loskop is situated at 25° 26′ 57.05″ S; 29° 19′ 44.36″ E in the Mpumalanga Province of South Africa and receives inflows from the Olifant's and Wilge rivers (Fig. 2). At full supply capacity, the impoundment has an area of 2427 ha and a volume of 374.3 Mm^3; the lake supplies water to large irrigation schemes located downstream of the dam wall and the lake is also used for recreational activities such as boating and angling. Lake Loskop forms part of the 25,000 ha Loskop Nature Reserve, most of which is situated in the upper Olifant's River catchment. The total area of the catchment which drains into Lake Loskop is 11,464 km^2 (Midgley et al., 1994). Lake Loskop is eutrophic to hypertrophic and has a pH that oscillates between 5.9 and 7.3 in the inflowing riverine zone, while pH values increase to between 8.9 and 10.1 in the main basin of the lake (Oberholster et al., 2010).

Four permanent sites were selected for sample collection from June to September 2009 when low river flows occurred. This is also the time period when previous crocodile mortalities were recorded downstream of Lake Loskop in the Kruger National Park. The first sampling site was located in the riverine zone of Lake Loskop; this site was characterised by reedbeds of *Phragmites mauritianus* while the substratum consisted predominantly of boulders and sand (for sampling of benthic filamentous algae, macroinvertebrates, amphibia and fish). The second site chosen was a small undisturbed natural stream known as the "Fountain-without-end" (reference site), which originated from the mountains near the gorge of Lake Loskop (sampling of phytobenthos, macroinvertebrates and amphibian) (Fig. 1). The substratum of this small stream was dominated by bedrock and embedded cobbles and no in-stream vascular aquatic plants were present. The substratum type and cover of both site 1 and the reference stream were determined visually following the methodology of Stevenson and Bahls (1999). Fish specimens were not sampled at this site, since the shallow water of this site only contain young fish specimens with an average body length of 3 cm. The third sampling site was located in the transitional zone of Lake Loskop (sampling of water column phytoplankton and fish), while the fourth sampling site was located in the main lake basin (sampling of water column phytoplankton and fish).

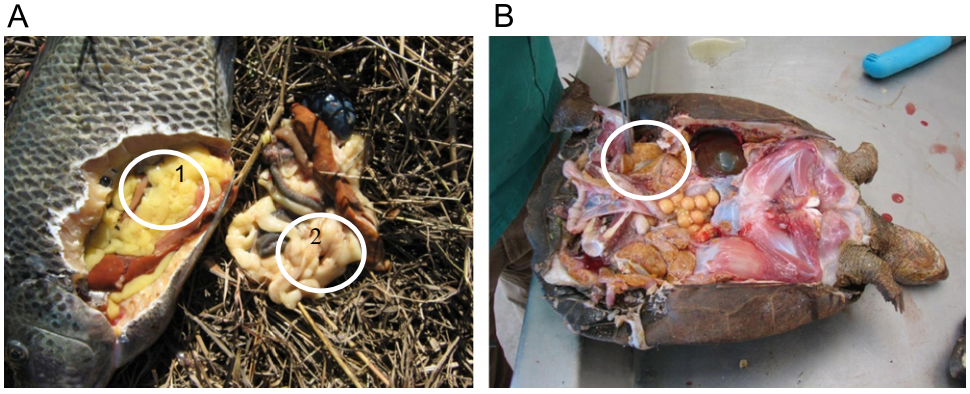

Fig. 1. (A) Yellow (1) and white body fat (2) of collected *Oreochromis mossambicus* specimens collected in Lake Loskop. (B) Fat of a serrated hinged terrapin (*Pelusios sinuatus*) that died of pansteatitis in 2007. (For interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)

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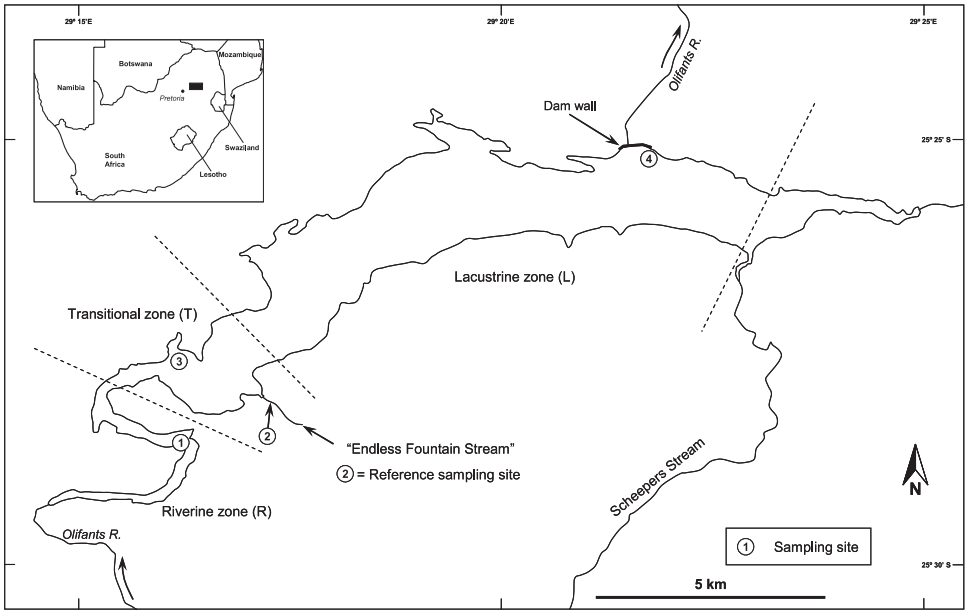

Fig. 2. Map of Lake Loskop showing the location of the four sampling sites, and the position of inflowing Olifants River. Inset shows the location of the map area in South Africa. The dashed lines indicate the approximate boundaries of the inflowing riverine zone (R), the transitional zone (T) and the lacustrine zone (L) of the lake.

Approval for sampling and analysis of fish and amphibian stomach content, tissue and organs, was obtained from the Animal Use and Care Committee (AUCC), a subcommittee of the Committee for Research Ethics and Integrity of the University of Pretoria. The authors affirm that all sampling and analysis was conducted in such a manner that it adheres to the standard operating procedures as prescribed by National Standards and according to Animals Protection Act (Act 71 of 1962) of South Africa.

2.2. Sampling of macroinvertebrates and amphibia

Macroinvertebrates were collected once a month over a period of four months (June to September 2009) at sampling sites 1 and 2. Samples were taken from macrophytes and boulder/sand substrates at each sampling site with a pond net (25 cm diameter and 500 μm mesh), by pushing the net through the loose upper part (1–2 cm) of the substrate over a distance of 1 m. A random sampling procedure was used to reduce hydrobiological variability between sites (Voelz and Ward, 1991). Samples were immediately preserved in 70% ethanol and later washed through a 75 μm mesh sieve to remove fine particles. The samples were then sorted and identified according to Merrit and Cummins (1996) to the lowest possible taxonomic category, using an Olympus dissection microscope. Sorting continued until the entire sample was sorted. Invertebrate diversity was calculated using the Shannon Diversity Index (Shannon and Weaver, 1949). The Berger–Parker dominance index (Berger and Parker, 1970) was used to estimate the dominance of organisms at each sampling site.

In addition, an alternate classification technique that involved the functional analysis of invertebrate feeding based on their morpho-behavioural mechanisms of food acquisition, was also used (Merrit and Cummins, 1996). The functional feeding groups consisted of scrapers, shredders, predators, collectors, collector–atherers and collector–filterers. For the analysis of Al and Fe concentrations within macroinvertebrate tissue, the five most abundant families present at sampling sites 1 and 2 during the four months sampling were collected, namely Atyidae, Coenagrionidae, Gomphidae, Belastomatidae and Potamonautidae.

Amphibia were sampled with a soft 1 mm-gauge mesh net (30 cm in diameter). Since we could not identify the different tadpole species, only adults were used in the study. All amphibian species collected were identified according to Carruthers (2001) and Channing (2001).

2.3. Sampling of periphyton and watercolumn phytoplankton

Five stones were collected from the submerged part (30–60 cm depth) of the river bank at sampling sites 1 and 2. The attached algae were removed by brushing an area of 5 cm^2 of the stone and the material was resuspended in 200 ml deionised water. Aliquots (100 ml) were used for metal and trace metal detection, while another aliquot (50 ml) was fixed with formaldehyde at a final concentration of 4% (v/v) for phytoplankton microscopic examination. All phytoplankton identifications were made by using a compound microscope at 1250x magnification (Van Vuuren et al., 2006). A total of 50 ml of each of the samples were sedimented in a chamber and were analysed using the strip-count method (American Public Health Association, 1992). Algal abundance in the epilithon samples was evaluated by counting the presence of each species (as cells in a filament or equal number of individual cells).

For Lake sampling (sites 3 and 4) integrated phytoplankton samples were taken from the top 2 m of the water column (epilimnion) using a 4 cm internal diameter weighted polyethylene hose. All samples were stored in acid washed polyethylene bottles and kept cool and in the dark during the 2 h period of transfer from the field to the laboratory. On return to the laboratory, all sampled biota for bioaccumulation analysis were rinsed three times with ultrapure (67%) 1 NHCl and deionised water to remove surface metals after which samples were dried to constant weight at 60 °C. Triplicate subsamples (50–100 mg dry weight) were digested in concentrated nitric acid (ultrapure 67%) to extract Al and Fe. Digest blanks were included as controls. Al and Fe concentrations were then determined by inductively coupled plasma atomic emission spectroscopy (ICP–AES). The instrument was calibrated using internal standards.

2.4. Fish sampling and analysis of stomach content, tissue and organs

A total of ten adult fishes of two different fish species (*Oreochromis mossambicus* and *Micropterus salmoides*) with different body lengths (300–480 mm) were caught by means of gill nets (120 mm stretched mesh size). These fishes were caught near the vicinity of each sampling site during our survey from June to September 2009 ($n=4$). Organ and tissue samples (liver, gills, heart, body fat, brain and intestine) were dissected from each fish specimen, and placed in 50 ml acid cleaned polyethylene bottles. The two fish species used in this study to determine bioaccumulation of total Al and Fe concentrations were identified according to Skelton (1993). The dissected organ and tissue samples were kept cool (4 °C) in a cooler box during the 2 h period of transfer from the field to the laboratory. On arrival to the laboratory the samples of liver, gills, hart, body fat, brain and intestine from the different fish species were dissolved separately in quartz beakers using nitric acid. The samples were finally diluted with MillQ water. The concentrations of total Al and Fe in the organs and tissue were analysed using ICPOES (Jobin–Yvon, JY24 Instrument SA, France). Sample-based standards were used as described by Jugdaohsingh et al. (1998). A subsample of the stomach contents of each fish was fixed with formaldehyde at a final concentration of 4% for microscopic examination of phytoplankton content and abundance, while the remainder of the sample was digested in concentrated nitric acid to extract Al and Fe for analysis by ICPOES.

2.5. Physical and chemical variables

Dissolved oxygen, water temperature, pH and electrical conductivity were measured *in situ* at each sampling site using a Hach sension^{TM} 156 portable multiparameter (Loveland, USA). Water samples for chemical measurements were collected in 1 L acid clean polyethylene bottles. Nutrient concentrations (NH_4^+–N, NO_3–N, NO_2–N and PO_4–P) were determined *in situ* using the colorimetric methods adapted from standard methods for the examination of water and wastewater (APHA, 1992). On return to the laboratory, water samples from the

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Table 1
Comparison of the average physical and chemical water characteristics recorded at four sampling sites in Lake Loskop from June to September 2009 ($n=4$).

Characteristic	Unit	Site 1	Site 2	Site 3	Site 4
Alkalinity	mg l^{-1} $CaCO_3$	44 ± 8	5.5 ± 3	59 ± 6	55 ± 4
Aluminium	mg l^{-1} Al	0.09 ± 0.03	0.07 ± 0.02	0.08 ± 0.01	0.06 ± 0.03
Ammonia nitrogen	mg l^{-1} N	0.4 ± 0.05	0.1 ± 0.02	1.3 ± 0.06	0.1 ± 0.08
Cadmium	mg l^{-1} Cd	0.03 ± 0.04	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Calcium	mg l^{-1} Ca	38 ± 4	0.41 ± 0.08	27 ± 5	26 ± 3
Chloride	mg l^{-1} Cl	18 ± 3	5 ± 2	12 ± 3	11 ± 2
Fluoride	mg l^{-1}l F	1.6 ± 0.8	0.2 ± 0.07	1.1 ± 0.6	0.9 ± 0.4
Iron	mg l^{-1} Fe	0.26 ± 0.4	0.12 ± 0.3	0.11 ± 0.6	0.18 ± 0.2
Magnesium	mg l^{-1} Mg	22 ± 5	0.5 ± 0.3	17 ± 4	12 ± 3
Manganese	mg l^{-1} Mn	0.09 ± 0.02	0.03 ± 0.01	0.07 ± 0.03	0.1 ± 0.07
Total nitrogen	μg l^{-1} N	6200 ± 387	1100 ± 115	3600 ± 294	3780 ± 189
Total phosphate	μg l^{-1} P	221 ± 41	6.9 ± 0.7	135 ± 21	167 ± 28
Potassium	mg l^{-1} K	6 ± 2	0.5 ± 0.2	4 ± 2	2 ± 0.09
Silica	mg l^{-1} Si	0.4 ± 0.06	4.4 ± 2	4.3 ± 2	3.7 ± 1
Sodium	mg l^{-1} Na	23 ± 5	1 ± 0.09	18 ± 4	17 ± 6
Sulphate	mg l^{-1} SO_4	172 ± 41	85 ± 2	116 ± 14	108 ± 19
Zinc	mg l^{-1} Zn	0.09 ± 0.03	0.03 ± 0.02	0.05 ± 0.01	0.06 ± 0.01
Electrical conductivity (μS cm^{-1} at 25 °C)		389 ± 31	18 ± 7	311 ± 23	286 ± 17
pH (negative log [H^+] at 25 °C)		7.3 ± 4	8.4 ± 3	8.6 ± 2	8.9 ± 3

different sampling sites were filtered through 1 μm Gelman glassfibre filters and preserved in nitric acid, after which total Al and Fe were determined by ICPOES.

2.6. Statistical analysis

Regression analysis was used to examine the relationship (proportionality) between environmental parameters and concentrations of Al and Fe in different tissues of the collected specimens. A significant level of at least $p<0.05$ was used in all analyses. The Pearson's correlation coefficient was used between mean pH, conductivity and the Al, Fe, TP and Si concentrations in the inflowing river, stream and lake water. Concentration factors for accumulated Al and Fe were expressed as the ratio of the mean concentration in the tissue (mg kg^{-1} dry weight) of the different collected biota (Table 1) to the mean concentrations in the water column (mg l^{-1}).

3. Results

3.1. Water chemistry

A distinct difference was observed between the four water quality variables measured *in situ* at the 4 sampling sites (Table 1). The pH values at sampling site 1 ranged between 7.1 and 7.6 indicating that the water at this site was almost neutral, while the pH values at sites 2, 3 and 4 ranged between 8.7 and 9.2. Electrical conductivity values ranged between 306 and 411 μ Scm^{-1} at sites 1, 3 and 4 (Table 1). The electrical conductivity values at site 2 (selected as the unimpacted reference site for this study) varied between 14.3 and 24.1 μ Scm^{-1} during the study period. The total Fe and Al concentrations in the water column at all 4 sampling sites ranged between 0.06 and 0.18 mg l^{-1} for Fe, and 0.07–0.09 mg l^{-1} for Al. The Fe and Al concentrations in the water column correlated strongly ($p<0.05$) with each other and with the near-neutral average pH of 7.3 at sampling site 1. Total fluoride concentrations in the water column of Lake Loskop range between 0.28 and 2.1 mg l^{-1} at the 4 sampling sites. The maximum concentrations of total Al detected at all sites were higher than the values allowed by the South African Water Quality Guidelines for Al (≤ 0.005 μg l^{-1}) for ecosystem health (DWAF, 1996). Total phosphate (TP) and nitrogen concentrations at sampling sites 1, 3 and 4 ranged from 221 to 6200 μg l^{-1} and 135 to 3600 μg l^{-1}, respectively, during the four-month study period (Table 1). During the study period there exist a strong correlation ($p<0.05$) between the Al concentrations at sampling sites1; 3 and 4 and TP concentrations sampled at these lake sites.

3.2. Macroinvertebrates

The macroinvertebrate communities at sites 1 and 2 varied widely in terms of species composition and abundance. The highest values for macroinvertebrate diversity were recorded at site 1 ($H=2.17$), while the numbers ($n=98/m^2$) of shredders (crustacea and plecoptera) were reduced at site 2 in comparison with site 1, where more abundant leaf litter was visually observed during the study. A higher abundance ($n=127/m^2$) of grazers were also recorded at site 1 with a much higher filamentous algal growth (biovolume 11 mm^3 l^{-1}) than at site 2. A total of 18 macroinvertebrate families were recorded from the 2 sampling sites during the four months. The macroinvertebrate populations at site 2 were characterised by a lower family abundance ($n=12$) than observed at site 1 ($n=18$). However, we did sample specimens of the more pollution sensitive macroinvertebrate family Barbarochthondae at site 2. Large numbers of dragonfly larva (Gomphidae) were present at sites 1 and 2, and this was the dominant family throughout the study (Berger and Parker Index, 0.480 and 0.441, respectively).

3.3. Amphibia

Two species of amphibian *Afrana angolensis* (9 individuals) and *Strongylopus fasciatus* (5 individuals) were sampled during our 4-month sampling survey, however, the amphibian *A. angolensis* was the only species that occurred at both sampling sites (1 and 2), and therefore, we only used *A. angolensis* to compare total Al and Fe bioaccumulation between these two sites.

3.4. Phytoplankton

The relationship between the abundance of filamentous algae and number of periphyton species at site 1 did reflect the association between a decrease in number of species and an increase in filamentous algae abundance such that a few, very abundant species was present at this sampling site. The dominant (Berger and Parker Index, 0.421 and 0.403) phytobenthos species at site 1 was the filamentous green alga *Spirogyra fluviatilis* (Hillse), and *Spirogyra adanata* (Kütz) with average biovolumes of 11–8.2 mm^3 l^{-1}, respectively, while the cyanobacterium *Oscillatorial tenuis* (*M. Gomont*) was present at a much lower biovolume (4 mm^3 l^{-1}). The dominant phytobenthos (Berger and Parker Index, 0.211 and 0.194) at site 2 was *Ulothrix punctata* (lagerh)

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and *Plectonema dangeardii* (Fremy), while the green alga *S. fluviatilis* (Hillse), occurred at a much lower biovolume (2 $mm^3 l^{-1}$). The cyanobacterium *Oscillatorial* species which occurred at sampling site 1 was absent at site 2, and was replaced by the cyanobacteria *Nostoc paludosum* which correlated significantly ($p < 0.05$) with the lower total nitrogen concentrations (0.339 $mg l^{-1}$) at this site. The diatoms *Gomphonema affine* (Kütz) and *Craticula cuspidata* (Kütz) also occurred at low biovolumes in the phytobenthos at site 1, while *Tabellaria flocculosa* (Kütz) and *Eunotia formica* (Ehrenberg) were the dominant diatom (Berger and Parker Index, 0.102 and 0.132) species at site 2. Both these diatom species are indicators of oligotrophic waters with low electrolyte content (Taylor et al., 2007). The dominant phytoplankton species in the transitional and main basin zone of Lake Loskop from July to September 2009 was the larger and slower growing species of *Ceratium hirundinella* (Müller) with an average biovolume of 13 $mm^3 l^{-1}$, which correlated positively ($p < 0.05$) with the higher average concentrations (3.0 and 3.7 $mg l^{-1}$) of Si in the water column of sites 3 and 4 in comparison with lower concentrations of 0.4 $mg l^{-1}$ at site 1 in the riverine zone of Lake Loskop. Other species that occurred in much lower biovolumes at sampling sites 3 and 4 were *Peridium bipes* (Stein) (1 $mm^3 l^{-1}$), and the diatoms *Asterionella formosa* (Hassal) and *Fragilaria crotonensis* (Kitton) as the dominant diatom species (biovolumes 1.8 and 2.1 $mm^3 l^{-1}$). The lower average biovolume (1.6 $mm^3 l^{-1}$) of the different species of diatoms observed at all sites during the winter and spring sampling period correlated negatively ($r = -0.8587$, $p \leq 0.04$) with the average silica concentration (3 $mg l^{-1}$) measured during the four-month study period (Table 1).

3.5. Bioaccumulation of Fe and Al in different biota

The highest concentrations of Al and Fe were measured in the filamentous algae (Al = 18,997.5 $mg kg^{-1}$ dry weight and Fe = 22,054.2 $mg kg^{-1}$ dry weight.) collected at site 1, with a near-neutral average pH of 7.3. However, there were no relationship between the Al and Fe concentrations measured in filamentous algae compared to the corresponding concentrations of these elements in the water column at site 1 (Tables 1 and 4). The Al concentrations in macroinvertebrates collected at site 1 ranged from 140.6 to 385.7 $mg kg^{-1}$ dry weight, with the highest values of Al and Fe measured in the family Gomphidae (385.7 and 1710.0 $mg kg^{-1}$ dry weight, respectively) in comparison to other macroinvertebrate families collected at this site.

The highest Al and Fe concentrations measured in the filamentous algae from site 2 were 4782.2 and 9947.1 $mg kg^{-1}$ dry weight, respectively, while the Al concentration in the macroinvertebrate family Coenagrionidae was the highest (31.7 $mg kg^{-1}$ dry weight) in comparison to the other families at this site. The average concentrations of Al measured in macroinvertebrates were not related to the average concentrations measured in stream and river water (0.08 $mg l^{-1}$) at site 2. A significant correlation ($r = 0.8735$; $p \leq 0.005$) was observed between the bioaccumulation of Al (4.6 $mg kg^{-1}$ dry weight) in the family Hydraenidae (minute moss beetles) and the low concentrations of Al (0.8 $mg l^{-1}$) sampled in the water column of sites 1 and 2. From our observations it seems that the Hydraenidae family had the lowest body burden of this metal.

Bioaccumulation of total Al and Fe concentrations in the amphibian *A. angolensis* was much higher (5399 and 4245 $mg kg^{-1}$ dry weight) at site 1 in comparison with site 2 (1957 and 3471 $mg kg^{-1}$ dry weight) but because of the small sample size ($n = 9$) this observation may not be significant. *C. hirundinella* (Müller) the dominant water column phytoplankton species throughout the study period at sites 3 and 4 contained average total Al concentrations of 1579 and 1262 $mg kg^{-1}$ dry weight, respectively (Table 4).

Analyses of the stomach content of *O. mossambicus* caught at sampling sites 1 and 3 revealed a mixture of large amounts of the filamentous algae *S. fluviatilis* and *S. adanata* (average biovolume of 9.1 and 4.4 $mm^3 l^{-1}$) with a much lower average biovolume of 2.3 $mm^3 l^{-1}$ of the algal *C. hirundinella*. In contrast, the stomach contents of *M. salmoides* specimens caught at sampling sites 1, 3 and 4 contained unidentified pieces of fish, macroinvertebrates and *Daphnia sp*. specimens, as well as a low average biovolume of 1.0 $mm^3 l^{-1}$ of *C. hirundinella* cells. Al and Fe concentrations (2580 and 10,697 $mg kg^{-1}$ dry weight, respectively) in the stomach contents of adult *O. mossambicus* fishes sampled at sites 1 and 3 were much higher in comparison with adult *M. salmoides* fishes (98.5 and 439.6 $mg kg^{-1}$ dry weight, respectively). In all cases of dissected fish species either white or yellow body fat was observed, thus in none of the samples both types of body fat occurred simultaneously. The concentrations of Al and Fe measured in the different organs of *O. mossambicus* at sampling sites 1 and 3 was along a mean sequence of intestine > yellow body fat > brain > gills > liver > heart > white body fat, while the mean sequence of total Al and Fe in *M. salmoides* sampled at sites 1, 3 and 4 was: intestine > gills > liver > heart > brain > white body fat. No yellow body fat was detected in any of the sampled specimens of *M. salmoides*, while all *O. mossambicus* specimens caught at sampling sites 1 (riverine zone) and 3 (transitional zone) contained yellow body fat. The stomach content of the *O. mossambicus* specimens collected at sites 1 and 3 contained predominantly filamentous algae, while specimens of *O. mossambicus* caught at sampling site 4 did not contain any yellow body fat. Furthermore, the stomach contents of these fish specimens only revealed high concentrations of *C. hirundinella* cells, as well as the cladoceran *Daphnia* sp. (Tables 2 and 3).

4. Discussion

Although the pH values were near-neutral in the riverine zone of Lake Loskop during our study, acid mine drainage is entering the system from a number of mines, principally via the waters of the Blesbok stream which is a tributary of the upper Olifants River. As a result, the water in the Blesbok stream upstream of Lake Loskop has a low average pH of 2.1 as well as high concentrations of total

Table 2
Statistics of Al and Fe concentrations ($mg kg^{-1}$ dry weight) in the different organs and tissue of *Oreochromis mossambicus*.

Organs and tissue	Aluminium							Iron						
	Heart	Brain	Intestine	Liver	Gills	Yellow body fat	White body fat	Heart	Brain	Intestine	Liver	Gills	Yellow body fat	White body fat
N	10	5	8	10	10	6	4	10	5	8	10	10	6	4
Range	11–24	58–78	1977–2760	16–32	19–33	97–141	9–23	184 – 241	395 – 481	9611–10,970	228 – 322	267 – 312	1901–2240	49–76
Median	17	69	2580	23	28	128	13	223	465	10,697	256	288	2128	60
SD	5.32	8.19	342.19	6.56	5.83	18.70	1.91	24.05	38.41	602.41	39.91	18.39	142.32	11.12

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Table 3
Statistics of Al and Fe concentrations (mg kg^{-1} dry weight) in the different organs and tissue of *Micropterus salmoides*.

Organs and tissue	Aluminium						Iron					
	Heart	Brain	Intestine	Liver	Gills	White body fat	Heart	Brain	Intestine	Liver	Gills	White body fat
N	8	6	8	8	8	8	8	6	8	8	8	8
Range	9–21	6–12	81–105	13–22	19–29	3–10	163–191	89–123	398–456	210–251	291–361	46–61
Median	14	9	98.5	17	23	6	177	112	439.6	237	334	54
SD	4.93	2.45	10.30	3.69	4.12	2.87	11.43	14.31	24.77	17.15	28.95	6.13

dissolved solids (Bell et al., 2002). The dominance of the phytoplankton species *C. hirundinella* in the transitional zone and main basin of Lake Loskop could be associated with the average higher Si concentrations at these two sampling sites in comparison with the other sampling sites, since this element is a major constituent of *Ceratium* cell walls (Sigee et al., 1999). The higher concentrations of Si measured in the water column at these sites may also have played a role in the Al uptake by *C. hirundinella*., due to the fact that metals generally enter plant tissue in ionic form and accumulate in cell walls (Crowder, 1991). The lower concentrations of Si observed in the riverine and transitional zone of Lake Loskop during this study may have led to a reduction in the abundance of benthic diatom species. However, Willen (1991) reported that Si concentrations as low as 200 μg l^{-1} – much lower than the concentrations (average 400 μg l^{-1}) measured in the water column of the riverine zone in our study – should be sufficient for diatom reproduction in eutrophic systems. Therefore, it seems unlikely that low silica concentrations could be the reason for the low number of benthic diatoms observed in the riverine zone of this study. However, numerous experimental studies have demonstrated that the toxicity of Al to aquatic organisms decreases when Si is added to culture medium (Campbell et al., 2000; Camilleri et al., 2003). The latter, may possible play a role in lower diatom counts at sites 1 and 2 with lower Si concentrations. The strong correlation between increasing Al concentrations and the reduction of TP at sampling sites 3 and 4 has also been observed in artificial algal growth media tested by Gensemer (1989). This phenomenon in part is due to the ability of Al to strongly bind phosphorus, thereby reducing the availability of this nutrient to algae (Vrba et al., 2006).

Douterelo et al. (2004) observed in their study that species in the epilithic cyanobacterial order Oscillatoriales were always associated with highly eutrophic water as observed in our study, while Fernandez-Pinas et al. (1991) found several species of the cyanobacterial genera *Calothrix, Scytonema* and *Nostoc* to be positively associated with low levels of nutrients. Our data show similar results with the cyanobacterium *Oscillatoria* occurring only at site 1, with much higher concentrations of water column nutrients in comparison with the reference site, where only heterocystous cyanobacterial (i.e., nitrogen-fixing) species occurred. The spatial heterogeneity of *Nostoc* and *Oscillatoria* between sites 1 and 2 were possibly due to the greater competitive ability of *Oscillatoria* compared to heterocystous species such as *Nostoc* in the nitrogen-enriched water of site 1 (Douterelo et al., 2004). The occurrences of *Oscillatoria* at site 1 may also have contributed to the higher levels of Al and Fe bioaccumulation in the algal mats dominated by filamentous green alga *S. fluviatilis* at this site, since Sheron and Bhandari (2005) reported that in a bench scale experiment with microbial mats predominated by *Oscillatoria*, metals and sulphides were removed from the water column over a very short period of time. From the observations in our study, it was evident that the bioaccumulation of Al and Fe in the mixture of different species of algae occurring at different sampling sites may have played a major role in the bioaccumulation of these metals in the food chain of resident biota at these sites.

Table 4
A comparison of different ranges of concentrations of Al and Fe (mg kg^{-1} dry weight) measured in dominant resident biota (phytoplankton, macroinvertebrates and amphibia) collected over a period of four months at four different sites.

Genus species or family name	Sampling site	Al mg kg^{-1} dry weight	Fe mg kg^{-1} dry weight
Phytobenthos			
Spirogyra fluviatilis	1	15,346–18,997	17,782–22,054
Ulothrix punctata	2	3111–4782	7632–9947
Phytoplankton			
Ceratium hirundinella	3	923–1579	794–1536
Ceratium hirundinella	4	121.4–262.0	624.3–831.0
Macroinvertebrate			
Gomphidae	1	280.1–385.7	912.4–1,710.0
Coenagrionidae	2	24.3–31.7	87.2–118.1
Amphibia			
Afrana angolensis	1	3642–5399	4102–4245
Afrana angolensis	2	1289–1957	2984–3471

Several studies have shown that algae in streams can accumulate Al and Fe (e.g. Bailey and Stokes, 1985; Sheoran and Bhandari, 2005). However, Novis and Harding (2007) and Das et al. (2009) reported that the absorption of metals by algae is highly variable, depending on the metal, the algal taxon, the age of the material and other environmental conditions which is in relation to data generated in our study. The concentrations of Fe that was measured in samples of the filamentous alga *S. fluviatilis* at site 1 was within the range of 0.01–27.0 mg g^{-1} dry weight recorded by Winterbourn et al. (2000) in the taxa *Microspora*, *Tribonema* and *Ulothrix* in New Zealand streams.

Elangovan et al. (1999) reported that the rate of accumulation of Al following initial exposure was higher in the crustacean *Asellus aquaticus* exposed to lower concentrations of Al in the water. They suggested that it may be due to the characteristic aqueous chemistry of Al polymerisation at a neutral pH. A study conducted by Balance et al. (1999) indicated that the rate of precipitation of Al is slower at lower added concentrations of 100 μg l^{-1} compared to higher concentrations. The number and taxonomic diversity of macroinvertebrates at site 2, which was lower in comparison with site 1, was possibly due to the fact that the predominantly bedrock substrate and the absence of aquatic macrophytes reduced habitat diversity and caused a reduction in the numbers of benthic invertebrate species present at site 2. However, the low numbers of macroinvertebrate families throughout the study in comparison with other South African rivers and streams impacted by acid drainage from coal field mines (Harrison, 1958; Kemp, 1967) could possibly be related to adverse effects of metal bioaccumulation in biota. An earlier study by Roux et al. (1996) indicated that inorganic Al concentrations between 0.1 and 0.3 mg l^{-1}, much lower than the concentrations detected in this study, were toxic to different benthic macroinvertebrate species. Furthermore, Kadar et al. (2002) reported that the filtration action in freshwater mussels was severely

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impaired by short-term (1 h) as well as long-term (15 days) exposure to Al at a neutral pH. A study by Csoti et al. (2001) demonstrated that Al altered voltage-activated sodium currents in the pond snail *Lymnaea stagnalis*, suggesting the interference with electrophysiology of ion channels in the plasma membrane.

Although there are many reports in literature (e.g. Vuorinen et al., 1990; Eeckhaoudt et al., 1996) on the effects of high concentrations of Al in acidic waters on fish, most of these studies have focussed on Al accumulation in the gills of the studied fish. However, little is known on the responses and bioaccumulation of this metal in fish in waters with near-neutral pH values (Soucek et al., 2001). The toxicity of Al is linked to its biological availability, but the processes by which Al is adsorbed into, excreted from or deposited and retained in the body of fish are still poorly understood (Takatsu et al., 2000). The bioaccumulation of Al and Fe in the two fish species investigated in this study may have been related to their feeding habits. For example, differences in acidity in the gut and in the quantity and composition of mucus secreted by the gut could affect the uptake of Al from their food source and from any water directly ingested (Elangovan et al., 1999). According to Deacon (1988) adult *O. mossambicus* feeds primarily on plant material such as filamentous green algae, which is comparable to our findings after analysis of the stomach contents of *O. mossambicus* caught at sampling sites 1 and 3. However, this particular fish species can also ingest aquatic insects, crustaceans, small fish and bottom sludge and naturally occupies shallow waters where benthic filamentous algae occurs (Kalff, 2002). In contrast, *M. salmoides* is an omnivorous scavenger and predator of other fish. It is a general fact that metals such as Al become more bioavailable and have increased toxicity with decreasing pH (Rengel, 2004). Dietary acids can extend the solubility of Al^{3+} and thus increase Al bioavailability during longer sections of the gastrointestinal tract, which also helps Al^{3+} diffusion across the intestinal epithelium through the formation of neutral complex species (Dayde et al., 2003). Deacon (1988) reported that the pH in the stomach of *O. mossambicus* decreased from 6 to as low as 2.9 after feeding commenced. Therefore, the intake by *O. mossambicus* of filamentous algae such as *S. fluviatilis,* with an average Al concentration of 18,997.5 mg kg^{-1} dry weight under low pH conditions in the intestine of this fish species, can increase the bioavailability of this trace metal. However, the direct ingestion of water containing different concentrations of fluoride from the water column of Lake Loskop by this fish species may also have played a role in Al absorption from filamentous algae by their gastrointestinal tract. In a previous study conducted on rats and mice by Allain et al. (1996), they showed that the addition of fluoride can cause higher Al absorption from the gastrointestinal tract.

From previous reports in the literature, it is evident that Al interferes with basic cellular functions such as the phosphoinositide and intracellular Ca^{2+} signalling pathways, which are involved in a myriad of cellular metabolic functions (Rengel, 2004). Furthermore, Al decreases the accumulation of inositol phosphates especially inositol-1.4.5-triphosphate (IP_3) causing changes in the membrane phospholipid composition (Shi et al., 1993). In addition, Al can potentiate Fe-induced oxidative stress through increased production of reactive oxygen species, resulting in lipid peroxidation and cytotoxicity of free-oxygen radicals (Strong et al., 1996; Crichton et al., 2002). Therefore, we suggest that the high levels of Al measured in yellow fat of the sampled *O. mossambicus* in the riverine and transitional zone of Lake Loskop may be one of the main contributing factors that resulted in the changes of body fat composition and colour, and may contribute to the development of pansteatitis in predators higher up in the food chain e.g. in crocodiles and terrapins. However, a shortfall of this study was that no crocodile and terrapin mortalities were reported in the area of Lake Loskop during the 4-month sampling period, which prevented the authors from determining the Al and Fe levels within the body fat of these animals. Nevertheless, tracking down carcasses of animals such as crocodiles and terrapins is a major problem when it comes to diagnosis of mortalities in wildlife, as there is almost no prior warning of a toxic pollution event. These carcasses are usually found decomposed or partially consumed by scavengers, many days after death has occurred (Oberholster et al., 2009).

Moreover, data generated from our study indicate that the water quality parameters of metals measured in Lake Loskop do not correlate with concentrations of metals measured in the different organisms. However, an earlier study conducted by Kar (1991) indicated that water quality parameters often do not indicate all human effects on streams, whereas the resident biota is thought to respond to an integration of all human impacts. For example, in Ohio, USA, assessments using the stream biota correctly identified the presence of human influence 49.8% of the time when it is not identified by water quality variables (Yoder, 1991).

5. Conclusion

The study demonstrated that the bioaccumulation of Al and Fe concentrations in biota are more sensitive than the dissolved concentration in the water column when used as indicators of contamination in hydrologic systems. Furthermore, the results from this study also indicated that no bio-magnification of Al and Fe concentrations in the food chain of Lake Loskop water with a neutral pH, using different trophic levels of the food chain downstream of areas impacted by mining, industry and sewage inflow, did occurred. The detection of higher concentrations of Al and Fe in the yellow body fat of *O. mossambicus* could be related to the intake of different phytoplankton species associated with higher levels of bioaccumulation of Al and Fe. From the data generated in this study we suggest that the feeding habits of *O. mossambicus* may have played a role in the bioaccumulation of Al in the food chain of Lake Loskop.

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References

Adler, R., Claassen, M., Godfrey, L., Turton, A.R., 2007. Water, mining and waste: A historical and economic perspective on conflict management in South Africa. Econ. Peace Secur. J. 2, 32–41.

Allain, P., Gauchard, F., Krari, N., 1996. Enhancement of aluminium digestive absorption by fluoride in rats. Res. Commun. Mol. Pathol. Pharmacol. 91, 225–231.

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF), 1992. Standard Methods for the Examination of Water and Wastewater19th edition APHA, AWWA, and WPCF, Washington, DC, USA.

Bailey, R.D., Stokes, P.M., 1985. Evaluation of filamentous algae as biomonitors of metal accumulation in softwater lakes: a multivariate approach. Aquatic Toxicology and Environmental Fate. ASTM Special Technical Publication 854, American Society for testing and materials, Philadelphia, PA, pp. 55–126.

Balance, S., White, K.N., McCrohan, C.R., Powell, J.J., 1999. Influence of extracellular polysaccharides on the behaviour and bioavailability of aluminium at neutral freshwaters. In: Lovejoy, D.A. (ed.), Heavy Metals in the Environment: An Integrated Approach. Proceedings of the First International Conference on

metals in the Environment, Vilnius, October 1997. Institute of Geology, Vilnius, pp. 261–265.
Bell, F.G., Hälbich, T.F.J., Bullock, S.E.T., 2002. The effects of acid mine drainage from an old mine in the Witbank Coalfield, South Africa. Q. J. Eng. Geol. Hydrogeol 35, 265–278.
Berger, W.H., Parker, F.L., 1970. Diversity of planktonic *Foraminifera* in deep sea sediments. Science 168, 1345–1347.
Camilleri, C., Markich, J.S., Noller, B.N., Turley, C.J., Parker, G., Dam, R.A., 2003. Silica reduce the toxicity of aluminium to tropical freshwater fish (*Mogurnda mogurnda*). Chemosphere 50, 355–364.
Campbell, M.M., White, K.N., Jugdaohsingh, R., Powell, J., McCrohan, C.R., 2000. Effect of aluminium and silicic acid on the behaviour of the freshwater snail *Lymnaea stagnalis*. Can. J. Fish. Aquat. Sci. 57, 1151–1159.
Carruthers, V., 2001. First Field Guide to Frogs of Southern Africa. Struiker Publishers, Cape Town, South Africa p.44.
Channing, A., 2001. Amphibians of Central and Southern Africa. Cornell University Press, Ithaca, NY pp.1–470.
Coetzee, L., Du Preez, H.H., Van Vuren, J.H.J., 2002. Metal concentrations in *Clarias gariepinus* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: Zinc, Copper, Manganese, Lead, Chromium, Nickel, Aluminium and Iron. Water SA 28, 433.
Crichton, R.R., Wilmet, S., Legssyer, R., Ward, R.J., 2002. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J. Inorg. Chem. 91, 9–18.
Crowder, A., 1991. Acidification, metals and macrophytes. Environ. Pollut. 71, 171–203.
Csoti, T., Gyori, J., Salanki, J., Erdelyi, L., 2001. pH-dependent actions of aluminium on voltage-activated sodium currents in snail neurons. Neurotoxicology 22, 109–116.
Das, B.K., Roy, A., Koschorreck, M., Mandal, S.M., Wendt-Potthoff, K., Bhattacharya, J., 2009. Occurrence and role of algae and fungi in acid mine drainage environment with special reference to metals and sulphate immobilization. Water Res. 43, 883–894.
Dayde, S., Brumas, V., Champmartin, D., Rubini, P., Berthon, G., 2003. Aluminium speciation studies in biological fluids. Part 9. A quantitative investigation of aluminium (III)-glutamate complex equilibria and their potential implications for aluminium metabolism and toxicity. J. Inorg. Chem. 97, 104–117.
Deacon, A.R., 1988. Die voedingsekologie en voedingsfisiologie van die Kurper, *Tilapia rendalii* (Bloulenger) en *Oreochromis mossambicus* (Peters) in 'n warm, rioolverrykte habitat. PhD thesis, RAU, Johannesburg, South Africa, pp. 1–193.
Douterelo, I., Perona, E., Mateo, P., 2004. Use of cyanobacteria to assess water quality in running waters. Environ. Pollut. 127, 377–384.
Driescher, A.C., 2008. A water quality study of Loskop Dam and the upper catchment of the Olifants River. Unpublished MSc thesis. 150 pages. University of the Free State, Bloemfontein, South Africa, pp. 1–150.
Department of Water Affairs and Forestry (DWAF), 1996. second editionSouth African Water Quality Guidelines, Volume 1. Domestic Use. Department of Water Affairs and Forestry, Pretoria, South Africa.
Eeckhaoudt, S., Van Grieken, R.E., Cholewa, M., Legg, G.J.F., 1996. Study of aluminium-exposed fish by scanning proton microprobe analysis. Mikrochim Acta 122, 17–25.
Elangovan, R., Balance, S., White, K.N., McCronhan, C.R., Powell, J.J., 1999. Accumulation of aluminium by the freshwater crustacean *Asellus aquaticus* in neutral water. Environ. Pollut. 106, 257–263.
Fernandez-Pinas, F., Leganes, F., Mateo, P., Bonille, I., 1991. Blue-Green Algae (cyanobacteria) as Indicators of Water Quality in Two Spanish Rivers. In: Whitton, B.A., Rott, E., Friedrich, G. (Eds.), Use of algae for monitoring riversInstitut für Botanik, Universität Innsbruck, Innsbruck, Austria, pp. 129–138.
Gensemer, R.W., 1989. Influence of aluminium and pH on cell size and silica content of silica-limited populations of Asterionella ralfi var. Americana. PhD dissertation. Ann Arbor, MI, University of Michigan.
Harrison, A.D., 1958. The effect of sulfuric acid pollution on the biology of streams in the Transvaal, South Africa. Verh. Int. Ver Limnol. 13, 603–610.
Herrmann, J., 1990. Physiological, Food Chain and Ecological Effects Among Benthic Invertebrates Exposed to Low pH and Associated Metal Concentrations. In: Mason, B.J. (Ed.), The surface water acidification programmeCambridge University Press, Cambridge, pp. 383–396.
Jugdaohsingh, R., Campbell, M.M., Thompson, R.P.H., McCrohan, C.R., White, K.N., Powell, J.J., 1998. Mucus secretion by the freshwater snail *Lymnaea stagnalis* limits aluminium concentration of the aqueous environment. Environ. Sci. Technol. 32, 2591–2595.
Kadar, E., Salanki, J., Powell, J., White, K.N., McCrohan, C.R., 2002. Effect of sublethal concentrations of aluminium on the filtration activity of the freshwater mussel *Anodonta cygnea* L. at neutral pH. Acta Bot. Hung. 53, 485–493.
Kalff, J., 2002. Limnology: Inland Water Ecosystems. Prentice-Hall Inc., Upper Saddle River, New Jersey, USA pp.1–592.
Karr, J.R., 1991. Biological integrity: A long-neglected aspect of water resource management. Ecol. Appl. 1, 66–84.
Kemp, P.H., 1967. Hydrobiological studies on the Tugela River System Part VI—Acid drainage from mines in the Natal coal fields. Hydrobiologia 29, 393–425.
Lubick, N., 2009. Hunting a croc killer: Mass deaths of South African's Nile crocodiles puzzle biologists. Sci. Am., 12–13.
Merrit, R.W., Cummins, K.W., 1996. An Introduction to the Aquatic Insects of North America, 3rd ed. Kendall/Hunt, Dubuque, Iowa, pp. 1–722.
Midgley, D.C., Pitman, W.V., Middleton, B.J., (1994.) Surface Water Resources of South Africa 1990. Appendices. First edition. Report 298/4.1/94. Water Research Commission. Pretoria.
Novis, P.M., Harding, J.S., 2007. Extreme Acidophiles: Freshwater Algae Associated with Acid Mine Drainage. In: Seckbach, J. (Ed.), Algae and cyanobacteria in extreme environment. Springer, The Netherlands, pp. 443–463.
Oberholster, P.J., Myburgh, G.J., Govender, D., Bengis, R., Botha, A.-M., 2009. Identification of toxigenic *Microcystis* strains after incidents of wild animal mortalities in the Kruger National Park, South Africa. Ecotoxicol. Environ. Saf. 72, 1177–1182.
Oberholster, P.J., Myburgh, J.G., Ashton, P.J., Botha, A.-M., 2010. Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicol. Environ. Saf. 73, 326–335.
Paton, C., 2008. Dam Dirty. F.M. BDFM Publishers (Pty) Ltd., Cape Town, South Africa, pp 32–39.
Rengel, Z., 2004. Aluminium cycling in the soil-plant-animal-human continuum. BioMetals 17, 669–689.
Roux, D.J., Jooste, S.H.J., Mackay, H.M., 1996. Substance-specific water quality criteria for the protection of South African freshwater ecosystems: Methods for derivation and initial results for some inorganic toxic substances. S. Afr. J. Sci. 92, 198–206.
Scancar, J., Milacic, R., 2006. Aluminium speciation in environmental samples: A review. Anal. Bioanal. Chem. 386, 999–1012.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communications, University of Illinois Press, Urbana, Illinois, USA.
Skelton, P., 1993. Freshwater Fishes of South Africa. Southern Boekuitgewers (Edms) Bpk, Halfweghuis, Cape Town, South Africa, pp 89–380.
Sheoran, A.S., Bhandari, S., 2005. Treatment of mine water by microbial mat: Bench scale experiments. Mine Water Environ. 24, 38–42.
Shi, B., Chou, K., Haug, A., 1993. Aluminium impacts elements of the phosphoinositide signalling pathway in neuroblstoma cells. Mol. Cell. Biochem. 121, 109–118.
Sigee, D.C., Levado, E., Dodwell, A.J., 1999. Elemental composition of depth samples of *Ceratium hirundinella* (Pyrrophyta) within a stratified lake: An X-ray microanalytical study. Aquat. Microb. Ecol. 19, 177–187.
Soucek, D.J., 2006. Effects of freshly neutralized aluminium on oxygen consumption by freshwater invertebrates. Arch. Environ. Contam. Toxicol. 50, 353–360.
Soucek, D.J., Cherry, D.S., Zipper, C.E., 2001. Aluminium dominated acute toxicity in neutral waters downstream of an acid mine drainage discharge. Can. J. Fish Aquat. Sci. 58, 2396–2404.
Stevenson, R.J., Bahls, L.L., 1999. Periphyton protocols. In: Barbour, M.T., Gerritren, J., Snyder, B.D., Stribling, J.B. (Eds.), Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic macroinvertebrates and fishSecond Ed EPA 841-B-99-002. U.S. Environmental Protection Agency, Washington, DC, pp. 6-1–6-22.
Strong, M.J., Garruto, R.M., Joshi, J.G., Mundy, W.R., Shafer, T.J., 1996. Can the mechanisms of aluminium neurotoxicity be integrated into a unified scheme? J. Toxicol. Environ. Health 48, 599–613.
Schwab, R. 2002. Government experiences with mine closure: meeting objectives rather than actions. WISA Mine Water Division. Mine Closure Conference. Randfontein, South Africa. 23–24 October 2002.
Takatsu, A., Ezoe, Y., Eyama, S., Uchiumi, A., Tsunoda, K., Satake, K., 2000. Aluminium in lake water and organs of a fish *Tribolodon hakonensis* in strongly acidic lakes with a high aluminium concentration. Limnology 1, 185–189.
Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2007. An illustrated guide to some common diatom species from South Africa. WRC Report, TT, 282/07. Pretoria: Water Research Commission, plates 1–178.
Van Vuuren, S., Taylor, J.C., Gerber, A., Van Ginkel, C., 2006. Easy Identification of the Most Common Freshwater Algae. North-West University and Department of Water Affairs and Forestry, Pretoria, South Africa, pp. 1–200.
Voelz, N.J., Ward, J.V., 1991. Biotic responses along the recovery gradient of a regulated stream. Can. J. Fisher. Aquat. Sci. 48, 2477–2490.
Vrba, J., Kopacek, J., Bittl, T., Nedoma, J., Strojsova, A., Nedbalova, L., Kohout, L., Fott, J., 2006. A key role of aluminium in phosphorus availability, food web structure and plankton dynamics in strongly acidified lakes. Biologia 61, 441–451.
Vuorinen, P.J., Vourinen, M., Peuranen, S., 1990. Long-term exposure of adult whitefish (*Coregonus wartmanni*) to low pH/aluminium: Effects on reproduction, growth, blood composition and gills. In: Kauppi, P., Anttila, P., Kenttämies, K. (Eds.), Acidification in Finland. Springer, Berlin, pp. 941–961.
Winterbourn, M.J., McDiffett, W.F., Eppley, S.J., 2000. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage effects of trophic level. Sci. Total Environ 254, 45–54.
Witters, H.E., 1998. Chemical speciation dynamics and toxicity assessment in aquatic systems. Ecotoxicol. Environ. Saf. 41, 90–95.
Willen, E., 1991. Planktonic diatoms–an ecological review. Algol. Stud. 62, 69–106.
Yoder, C.O., 1991. Answering some concerns about biological criteria based on experiences in Ohio. In: Water quality standards for the twenty-first century. US. Environmental Protection Agency, Washington, DC, USA, pp. 95–104.
Yoshino, M., Ito, M., Haneda, M., Tsubouchi, R., Murakami, K., 1999. Prooxidant action of aluminium ion-stimulation of iron-mediated lipid peroxidation by aluminium. BioMetals 12, 237–240.

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