Fate, Transport and Effects of Pollutants Originating from Acid Mine Drainage in the Olifants River, South Africa

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Abstract

Concentrations of pollutants were measured in water, sediment and algal samples collected along a longitudinal gradient from a stretch of the Olifants River, South Africa, that receives AMD from the Klipspruit River. The effects of AMD were determined through macroinvertebrate biotic indices (SASS5) and multivariate analysis of macroinvertebrate communities. The acidic Klipspruit River caused increased concentrations of total Al, Fe and Mn in the Olifants River. Upon mixing of the Klipspruit with that from the alkaline Olifants River, Al and Fe precipitate rapidly, leading to lower concentrations in the dissolved phase and higher concentrations in the suspended phase and in sediment at sites in close proximity to the confluence. Similarly filamentous algae accumulated high concentrations of Al, Fe and Zn immediately after the confluence. Mn remains in the dissolved phase and sediment and algal concentrations increase with increasing distance downstream. Metal speciation analysis indicate that Al is rapidly converted from more toxic forms (e.g., Al^{3+} and $Al(OH)^{2+}$) to less toxic forms (e.g., $Al(OH)_3$(aq) and $Al(OH)^{4-}$). In contrast, Mn remains in the soluble Mn^{2+} form. Macroinvertebrate metrics and community structure showed clear signs of deterioration in water quality in the Olifants River downstream of the point of AMD input. While total TDS concentrations at all sites fall within ranges likely to affect macroinvertebrates, the relative composition of major ions changes as a result of AMD input, which may also account for the observed changes in macroinvertebrate communities. Further downstream, the Wilge River discharges into the Olifants River, and significantly improves water quality downstream of the confluence. Future mining and development activities in the Wilge catchment should be carefully managed and monitored so as to ensure sufficient flows of acceptable quality to prevent further deterioration of water quality in the Olifants River and downstream reservoirs.

Keywords: acid mine drainage, metals, salinity, macroinvertebrates, fate, transport.

Introduction

The upper Olifants River catchment is the most important source of coal in South Africa, and acid mine drainage (AMD) originating primarily from old, abandoned mines has been identified as one of the major long-term water quality impacts in the catchment (Hobbs *et al.*, 2008). The Klipspruit River in particular is heavily impacted due to the extensive network of shallow, abandoned underground coal workings in the catchment and water quality is characterised by low pH and high concentrations of dissolved metals (aluminium, manganese, iron and zinc) and salts (Bell *et al.*, 2001; Dabrowski and De Klerk, 2013). The Klipspruit River discharges into the Olifants River approximately 13 km upstream of the confluence with the Wilge River and 45 km upstream of the inflow to Loskop Reservoir. Loskop Reservoir in particular has been heavily impacted by pollution originating from the upper Olifants catchment, with fish kills having been regularly reported in the mid-2000s and populations of Nile Crocodile (*Crocodylus niloticus*) declining sharply since 2009 (Botha *et al.*, 2012). Water quality in the reservoir has steadily declined (Dabrowski *et al.*, 2013) and recent studies have indicated bioaccumulation of aluminium and iron in the fat of *Oreochromis mossambicus* (Oberholster *et al.*, 2012).

The significance of elevated metal concentrations on aquatic ecosystem health is complicated to determine, because, while a comparison of total dissolved metal concentrations to water quality guideline values can give an indication of potential effects, the speciation of metals plays a significant role in their toxicity towards aquatic organisms (Driscoll, 1985). Speciation of metals, particularly aluminium, is complex and is related to a number of factors, including pH, the availability of complexing ligands (*e.g.*, F^- and SO_4^{2-}) and water temperature (Gensemer and Playle, 2010; Tipping and Carter, 2011). Furthermore, dissolved metals in acidic waters rapidly precipitate out of solution upon mixing with more neutral or alkaline rivers (Olías *et al.*, 2004). Therefore the influence of water originating from AMD-affected rivers on receiving rivers or reservoirs may be mitigated to lesser or larger degrees depending on the relative physico-chemical and hydrological characteristics of the mixing water bodies and the attenuation of metal concentrations along a longitudinal gradient (Kimball *et al.*, 2002). Field assessments of ecosystem health are therefore important with respect to interpreting the likely effects of observed water quality on the environment. Assessments of macroinvertebrates and their community structure are commonly used as indicators of water quality related to AMD, because of their varying tolerance towards pollutants (Winterbourn *et al.*, 2000; Solà *et al.*, 2004; Gray and Delaney, 2008; Van Damme et al., 2008). The absence or presence of sensitive species provides additional insight as to whether or not pollutants are affecting a system. This is the philosophy upon which the South African Scoring System, Version 5 (SASS5) biomonitoring approach is based (Dickens and Graham, 2002).

While the water chemistry of the Klipspruit River itself is relatively well understood, no studies have investigated the fate and transport of metals once introduced into the Olifants River and how they and other contaminants introduced by AMD (e.g. increased salinity) affect the downstream environment into Loskop Reservoir. The potential mediating influence of the Wilge River has also not been studied in detail. Given the water quality and ecological problems observed in the reservoir, this study aimed to fill these knowledge gaps in support of providing management guidelines aimed at preventing further deterioration in water quality in the Olifants River and Loskop Reservoir.

Methods

Site Selection

Sampling sites were selected so as to determine the longitudinal effects of water quality originating from the Klipspruit and Wilge rivers entering the Olifants River. A total of eight sites were chosen, six along the Olifants River and one each in the Klipspruit and Wilge rivers (Fig. 1). Site OL1 was the most upstream site in the Olifants River, located approximately 1 km upstream of the confluence with the Klipspruit River. It drains an area of approximately 6600 km^2, with approximately 8 % of the land covered by mining activities with relatively little AMD impact. Site KL2 was located in the Klipspruit River and drains a catchment area of approximately 240 km^2, of which 8.4 % is under mining. A large proportion of this area consists of abandoned mines that decant high volumes of AMD into the Brugspruit and Blesbokspruit rivers which are tributaries of the Klipspruit River. Sites OL3 to OL6 were located in the Olifants River downstream of the Klipspruit River and upstream of the confluence with the Wilge River, and were selected to determine the transport and fate of metals introduced by the Klipspruit River along a longitudinal gradient. Site WG7 was located in the Wilge River (catchment size of 4000 km^2, with less than 2 % under mining activity) and site OL8 was located downstream of the confluence between the Wilge and Olifants rivers. All sites were located within the Eastern Bankenveld ecoregion (Kleynhans et al. 2005) and aquatic macroinvertebrate communities are unlikely to be affected by geomorphological, geographical or longitudinal variation amongst sites (Dallas, 2007). Sampling was conducted during July 2012, a period of relatively low flow.

Water Chemistry Sampling and Analysis

Water temperature, dissolved oxygen, pH and electrical conductivity values were measured *in situ* at each site using a Thermo 5 star pH/RDO/Conductivity meter set. At each site, two 1 L water samples were collected for water chemistry assessments; one to assess total metal concentrations and one to assess the water dissolved fraction of metals. All water samples were collected in pre-rinsed, polyethylene bottles and placed on ice in the dark, without the addition of any preservatives. For each site one sample was filtered through 0.45 µm pore size Whatman filters prior to being analysed for dissolved nutrients, metals and major ions using standard methods (APHA, AWWA and WPCF, 1992). Increased concentrations of aluminium (Al), iron (Fe), manganese (Mn), and zinc (Zn) are commonly associated with AMD in this area (Bell et al., 2002; Dabrowski and De Klerk, 2103) and were determined using inductively coupled plasma mass spectrometry (ICP-MS). Detection limits were 1 µg L^{-1}. All major ions, except chloride, were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES). Chloride and all dissolved nitrogen and phosphorous forms were measured using a flow injection analyser (FIA). The second sample was not filtered prior to analysis and represented the combination of suspended and water dissolved metal concentrations in the water. For interpretive purposes, dissolved metal concentrations were compared to South African Water Quality Guidelines for the aquatic ecosystem (DWAF, 1996). The guidelines specify Acute

(AEV) and Chronic Effect Value (CEV) guidelines for each constituent, which provide an indication of the concentration at which there is expected to be a significant probability of measurable acute or chronic effects to 5 % of the aquatic community. The water quality data and in-stream field data were used as input into the VMinTeq software programme, version 3.0 (Gustafsson, 2011), which was used to estimate the theoretical distribution of species of Al, Fe, Mn and Zn.

Sediment and Algal Sampling and Analysis

Composite sediment samples were obtained from each site by combining five discrete samples of the upper 4-5 cm of river sediment in 60 ml polycarbonate containers. These were stored on ice in the field, and then frozen at -4ºC prior to analysis. Sediments were freeze-dried and then ball milled. Metals in the sediment were extracted by microwave digestion using nitric acid (HNO_3), perchloric acid ($HClO_3$) and hydrogen peroxide (H_2O_2) (Ip, 2007). Concentrations of metals were determined using methods described above.

In addition, five discrete epilithic filamentous macroalgae samples (Cladophora glomerata) were collected from cobbles and boulders at each site and combined in a composite sample. Algal samples were stored in river water in polycarbonate containers and stored on ice and later refrigerated at 4 ºC prior to analysis. Algal samples were rinsed three times with deionised water to remove any trapped suspended sediment and debris after which samples were dried at 60 ºC. The samples were ball milled and digested in HNO3 and H2O2 to extract metals and concentrations were determined using methods described above.

Macroinvertebrate Sampling and Data Analysis.

Stones in current (*i.e.*, riffle sections) and marginal vegetation were sampled at each site using a standard sweep-net (Dickens and Graham, 2002). Stones in current and sediment habitats (gravel, sand and mud) were sampled for a total of two minutes per biotope, placing the net downstream of the stones, and agitating the stones vigorously to dislodge macroinvertebrates into the net. Vegetation was sampled for two minutes, sweeping the net along vegetation hanging into the water, and just under it, for a total river length of about two metres. The collected macroinvertebrates were then placed in an open tray of water, where they were identified to family level and enumerated using a grid marked on the tray. No macroinvertebrate samples were collected at OL3 due to inaccessibility and lack of suitable sampling habitat.

Version 5 of the South African Scoring System (SASS5) rapid bioassessment method for rivers was used to evaluate ecosystem health based on the absence or presence of sensitive and tolerant species. In summary, each family is rated between 1 to 15, based on their sensitivity to pollutants (1 indicating lowest sensitivity or highest tolerance to pollutants and 15 indicating highest sensitivity or lowest tolerance to pollutants) (Dickens and Graham, 2002). The sum of all the ratings per family for a particular sample is the SASS5 score. The number of different taxa, (different families), is also taken into account as another measure of river condition. The Average Score per Taxon (ASPT) is calculated by dividing the SASS5 score by the number of different taxa identified, and is often the most meaningful metric.

In addition, a Canonical Correspondance Analysis (CCA) was used to explore the relationship between water chemistry variables and macroinvertebrate community structure. All water quality parameters measured in water and sediment were included in the analysis. Dissolved as opposed to total metal concentrations were used in the analysis as these are regarded as more available and biologically relevant. To remove the effect of measurement units, all water quality parameters were normalised into a 0-1 rank by dividing each parameter by the maximum value. Macroinvertebrate data was log transformed ($\log(X+1)$) prior to analysis. Analysis was performed using the Canoco for Windows package, version 4.5 (Ter Braak and Smilauer, 2002).

Results

Water Quality

Dissolved oxygen concentrations were relatively high across all sites (Table 1). For most sites in the Olifants River, pH values were also relatively high (> 8). The pH in the Klipspruit River was acidic (5.28) bringing the pH at OL3 down to neutral in comparison to a relatively alkaline pH (8.34) at site OL1. The pH increased again over a relatively short distance and at site OL6 was again comparable to site OL1. The Wilge River had a comparably lower pH (7.31) in comparison to the Olifants River but had no major influence on pH at site OL8. TDS was highest in the Klipspruit River resulting in an increase in the Olifants River downstream of site OL1. TDS was lowest in the Wilge River and the dilution effect reduced electrical conductivity at site OL8 to levels similar to that measured at site OL1. Sulphate was the dominant ion measured at all sites and, together with Na^+, increased markedly at OL3 and further downstream due to relatively high contributions from the Klipspruit River. Carbonate concentrations are highest at site OL1 and decrease further downstream. The dilution effect of the Wilge River can again be seen at site OL8 with concentrations of all parameters decreasing in comparison to OL6. Concentrations of ammonium and ortho-phosphate were generally below detection limits (< 0.1 mg L^{-1}) at all sites. Nitrate concentrations in the Olifants River were comparably higher with concentrations ranging from 4.6 mg L^{-1} (at site OL1) to 3.4 mg L^{-1} (at site OL6). The Wilge River had significantly lower concentrations (0.8 mg L^{-1}) resulting in a reduced concentration at site OL8.

Metal Concentrations in water, sediment and algae

Concentrations of metals in water at site OL1 were generally very low compared to other sites (Fig. 2) with Al and Mn being below detection limits (< 5 µg L^{-1}). Concentrations of Fe and Zn were also relatively low, with only a small proportion being in the dissolved phase. The Klipspruit River had the highest dissolved metal concentrations for all sites included in the analysis. The dissolved concentrations of Al and Mn in particular were very high in comparison to other sites (and other metals), and resulted in increased concentrations at sites OL3 to OL6. KL2 also resulted in elevated total concentrations of Fe from OL3 to OL6, although dissolved concentrations were mostly below detection limits at all these sites. Dissolved Zn concentrations were elevated at KL2 and OL3, but were below detection limits at all other sites. With the exception of Mn, dissolved concentrations of metals accounted for a small proportion of total metal concentrations at sites OL3 to OL6. In contrast to site KL2, metal concentrations in the Wilge River were significantly lower. Total and dissolved concentrations of all metals decreased at site OL8, downstream of the confluence with the Wilge and Olifants rivers.

The pattern in sediment contamination complemented that observed in the water samples (Fig. 3a). Concentrations of Fe and Al in particular increased markedly at OL3, and then decreased over distance further downstream and were comparable to site OL1 from site OL5 downwards. Mn appeared to increase in sediment at sites located further downstream, with the highest concentrations being observed at OL4 and OL6. Mn concentrations in sediment at OL3 were considerably lower than Al and Fe. Concentrations of Zn were relatively low at all sites. With the exception of Zn, metal concentrations in algae showed similar patterns to those observed in the sediment. In comparison to site OL1, concentrations of Al, Fe and Zn increased significantly in algae collected from site OL3 (Fig. 3b). At sites located further downstream these metals were detected at only slightly higher concentrations in comparison to site OL1. The concentrations of Mn in algae increased with increasing distance further downstream, with the highest concentrations being detected in samples collected from site OL6. In general, Mn concentrations were markedly higher than other metals measured in algae.

Metal Speciation

Metal speciation modelling suggests that while total dissolved aluminium exceeded the CEV guideline at sites upstream and downstream of the confluence of the Klipspruit and Olifants rivers, the majority of Al was in the non-toxic $Al(OH)_4^-$ form, accounting for almost 100 % of the species composition (Table 2). The exception was site OL3, which, while still being dominated by $Al(OH)_4^-$, had low concentrations of $Al(OH)_2^+$ and $Al(OH)_3$ (aq) and alumina-fluoride, -sulphate and -phosphate complexes. Approximately 1 % of Al was in the Al^{3+} form and was below guideline levels. The composition of Al species at site KL2 differed markedly from the other sites. The majority of Al was estimated to be in alumino-fluoride and –sulphate complexes, with no presence of $Al(OH)_3$ or $Al(OH)_4^-$. Although total dissolved Al exceeded the AEV at KL2, the toxic species (Al^{3+} and $AlOH^{+2}$) only exceeded the CEV for Al. At all sites, the majority of Mn was estimated to be predominantly in the Mn^{2+} form and exceeded the CEV at KL2 and OL3. Similarly, while dissolved concentrations of Zn and Fe were only detected at a few sites, these metals were predominantly in the Zn^{2+} and Fe^{2+} form, with Zn^{2+} exceeding the CEV at KL2 and OL3.

Macroinvertebrate Sampling

The number of macroinvertebrate taxa clearly declined at site OL4 in comparison to site OL1 after the input of the Klipspruit River (Table 3). The number of taxa at site KL2 was the lowest of all sites sampled in the study area. The decreased number of taxa continued from sites OL4 to OL6. The Wilge River (WG7) had the largest number of taxa in comparison to other sites. The number of taxa increased from site OL6 to OL8 (downstream of the confluence with the Wilge River).The number of taxa at sites WG7 and OL8 was higher than at site OL1. The lower number of taxa at sites KL2 and OL4 to OL6 resulted in decreased SASS5 scores in comparison to site OL1. SASS5 scores at sites WG7 and OL8 were the highest recorded amongst sites - even higher than those measured at site OL1. The decreased SASS5 scores were mirrored by slightly decreased ASPT scores at sites OL1 to OL6 (including site KL2). The ASPT score at site WG7 was markedly higher than at other sites in the study area.

Results from the CCA support the SASS5 indices (Fig. 4). The first and second axes explain 29.8 % and 26.3 % of the variation, respectively. Concentrations of TDS, Al, Mn and Zn had the highest positive loading (correlation) on the 1st axis and pH and alkalinity had the highest negative loadings (Table 4). Loadings for dissolved metals were higher than for sediment associated metals indicating that dissolved metals play a more important role in explaining the variation in macroinvertebrate community structure. Most taxa plotted towards the left side of the graph, indicating that these taxa were negatively affected by dissolved metals and low pH. There were fewer taxa plotted on the right hand side of the graph. These taxa were generally associated with sites KL2 and OL4 to OL6. KL2 was characterised by high abundances of Leptoceridae in particular. Oligochaeta and the sensitive ephemeropteran families Leptophlebidae and Heptagenidae, were only present at OL1, WG7 and OL8. Potamonautidae were associated with OL1 and OL8 and were absent from other sites. OL1, OL8 and WG7 all recorded a number of unique taxa that did not occur at any other sites (these taxa fall directly on a line drawn from the origin of the plot through the site in question). For example the sensitive Perlidae (a plecopteran family), Chlorocyphidae and Psephenidae were all only collected at WG7.

Discussion

Our results clearly indicate that site KL2 is heavily affected by AMD, with low pH values and high concentrations of mining-related water quality indicators, most notably sulphate (Table 1) and dissolved metals (Fig.2). The acidic conditions result in the majority of metals being available in the dissolved fraction which are more available and toxic. More importantly, the acidic conditions result in elevated concentrations of toxic species, most notably Al^{3+} and $Al(OH)_2^{+}$ (Table 2), which are known to be particularly toxic to fish and aquatic macroinvertebrates (McCahon *et al.*, 1987; Dangles and Guérold, 2000). Additionally, dissolved concentrations of Mn and Zn were primarily predicted to be in the bioavailable Mn^{2+} and Zn^{2+} forms. The effects of these high metal concentrations were reflected in the macroinvertebrate sampling which showed the lowest number of taxa and SASS5 scores amongst all sites sampled (Table 4).

Poor water quality originating from the Klipspruit River clearly influences water quality in the Olifants River, with concentrations of dissolved metals and TDS (especially sulphate) increasing markedly downstream of the confluence. Dissolved Al, Fe and Zn in acidic waters are known to precipitate rapidly out of solution upon mixing with more neutral to alkaline water bodies (Kimball *et al.*, 2002; Olías *et al.*, 2004; Balistrieri *et al.*, 2007). While total concentrations of Al, Fe and Zn increased in comparison to OL1, the proportion in the dissolved concentrations were lower than at KL2 (Fe and Zn were mostly below detection limits) indicating that these metals rapidly precipitate out of solution into the suspended or colloidal phase. Additionally metal concentrations increase markedly in sediment at OL3 and gradually decline with increasing distance downstream, indicating that the majority of metals precipitate out of solution almost immediately after mixing of the acidic Klipspruit with the alkaline Olifants River. The spike in total Fe and Al concentrations at site OL4 could be as a result of turbulence or localised sediment disturbance resulting in suspension of colloidal or sediment bound metals (Fig. 2). In contrast, Mn appeared to increase in the sediment further downstream. This is most likely due to the fact that in contrast to Al and Fe, while Mn concentrations showed a decreasing trend from site OL3 downstream to site OL8, the majority of this metal remained in the dissolved form, indicating that Mn takes longer to precipitate out of solution. This observation is supported by the fact that the oxidation and precipitation of Mn^{2+} in oxic environments is known to be slow, taking a number a days in natural waters (Lasier *et al.*, 2000).

This trend was also confirmed by metal concentrations in filamentous algae. The results indicate that benthic filamentous algae accumulate high concentrations of all metals. Similar to sediment, concentrations of Al, Fe and Zn in algae were highest immediately after the confluence (at site OL3) and decreased further downstream. In contrast, Mn concentrations in algae increased along a longitudinal gradient with highest concentrations measured in samples collected from site OL6. Lawrence *et al.* (1998) through use of a scanning electron microscope, showed that filamentous algae reduced metal concentrations originating from AMD due to the formation of mineral precipitates around individual filaments and suggested that factors such as the pH proximal to the algae may play an important role in mineral production. However, studies have also documented the ability of filamentous algae to accumulate high concentrations of metals (Mehta and Gaur, 2005).

The macroinvertebrate assessment indicates that the Klipspruit River negatively affects ecosystem health, leading to a decrease in taxa and total SASS5 scores further downstream of its confluence with the Olifants River. Speciation results indicate that toxic species of Al associated with water from the Klipspruit River are rapidly transformed into less toxic species, with ($Al(OH)_3(aq)$ and $Al(OH)_4^-$) becoming more prevalent already at site OL3 and further down to site OL8 (Table 2). The deterioration in macroinvertebrate assemblage assessments (as reflected in decreased SASS5 scores) suggests that water quality originating from the Klipspruit River may result in chronic toxicity. While speciation analysis indicates that Al is primarily in a non-toxic form at these sites, Mn was predominantly in the dissolved phase in forms that are primarily responsible for aquatic toxicity, although below the CEV guideline (Mn^{2+}; Table 4) (Lasier *et al.*, 2000). Additionally dissolved Zn concentrations were comparatively high at site OL3. In addition to the inherent toxicity of the metal species themselves, which are known to affect macroinvertebrate communities (Schmidt *et al.*, 2002), the physical process of precipitation that occurs after mixing of acidic and neutral water could also play an important role in decreased macroinvertebrate diversity. Continuous precipitation of metalliferous compounds could result in unsuitable macroinvertebrate habitat as well as physical clogging of gills of sensitive species (*e.g.*, Ephemeroptera) (Gower *et al.*, 1994; Schmidt *et al.*, 2002; MacCausland and McTammany, 2007). Furthermore studies indicate residual toxicity of AMD contaminated sediment and precipitates to macroinvertebrates (Dsa *et al.*, 2008).

The results of the CCA support the SASS results, showing higher numbers of taxa and diversity associated with sites less affected by AMD (OL1, WG7 and OL8). AMD effects on macroinvertebrate density and diversity have been documented in numerous other studies (Gerhard *et al.*, 2004; Van Damme *et al.*, 2008). Site KL2 was characterised by high numbers of Leptoceridae and Chironomidae in particular. Leptoceridae are known to be relatively tolerant of acidic conditions (Jooste and Thirion, 1999) while Chironomidae are commonly regarded as being relatively tolerant to metal pollution (Cranfield *et al.*, 1994; Dickman and Rygiel, 1996). Sites OL1, WG7 and OL8 were all characterised by relatively good water quality with high pH and alkalinity, low metal concentrations, and a higher number of macroinvertebrate taxa, although each with quite distinct macroinvertebrate communities. In general, these sites had higher numbers of sensitive families that are regarded as being sensitive to poor water quality. These include the Heptagaenidae and

Leptophlebidae (Order Ephemeroptera) and Perlidae (Order Plecoptera) families, commonly regarded as sensitive families in local and international biotic indices (Dickens and Graham, 2002 Maret *et al.*, 2003; Winterbourne *et al.*, 2000) as well as other sensitive families such as Elmidae and Psephenidae. These families were absent in between the confluences of the Olifants and Klipspruit and Olifants and Wilge rivers. The fact that the dissolved metals had a relatively high influence on the CCA loadings in comparison to sediment bound metals indicates that that these variables have a stronger influence on the distribution of macroinvertebrates. However it is interesting to note that Oligochaeta which inhabit the sediment were also only found upstream of the Klipspruit confluence and downstream of the Wilge confluence, indicating that precipitated metals may influence sediment-dwelling macroinvertebrate communities. The decline in macroinvertebrate at sites KL2 and OL3 to OL6 could also possibly be related to increased salinity introduced by AMD. The TDS levels measured at all sites included in the study fall within guideline levels (300 to 800 $mg.L^{-1}$) that are expected to result in measurable chronic toxicity to aquatic macroinvertebrates (Scherman *et al.*, 2003). It is therefore impossible to account for changes in macroinvertebrate community structure amongst sites based on TDS concentrations. The relative composition of major ions and cations comprising salinity are however important to consider with respect to potential toxicity (Mount *et al.*, 1997). Salts commonly found in freshwater systems, in decreasing order of toxicity, are magnesium sulphate ($MgSO_4$), sodium sulphate (Na_2SO_4), calcium chloride ($CaCl_2$), and sodium chloride (NaCl) (Palmer *et al.*, 2004). This means that water bodies with similar TDS but different ionic composition could pose different levels of threat to aquatic ecosystems (Palmer *et al.*, 2004). Assuming the toxic effect of salts can be deduced from the relative composition and concentration of their respective dissolved anions and cations (Mount *et al.*, 1997), the deterioration in macroinvertebrate assemblages at sites OL3 to OL6 is unlikely to be related to increased $MgSO_4$ toxicity, as, while SO_4^{2-} increased, Mg^{2+} did not show any increase downstream from OL1 (Table 1). Na^+ did however increase markedly from OL3 to OL6 and, together with increased concentrations of SO_4^{2-} could potentially explain the deterioration in macroinvertebrate assemblages, especially considering the fact that concentrations of toxic metal ions rapidly decreased to those below relevant guideline values (Table 2). Previous studies conducted in the Blesbokspruit (a small tributary that flows into the Klipspruit further upstream from KL2) indicated that Na^+ is the dominant cation in AMD originating from abandoned mines in the area (Bell *et al.*, 2001).

The importance of tributaries, both as refugia for aquatic biota and for dilution of poor water quality is seldom taken into account in either IWRM or conservation planning, yet is regarded as an essential regulating service provided by aquatic ecosystems (Millenium Ecosystem Assessment, 2005). In this respect, the Wilge River clearly provides these essential services through its ability to improve aquatic ecosystem health in the Olifants River. This river clearly has relatively good water quality, which is reflected by the highest macroinvertebrate index scores (high number of taxa, SASS5 and ASPT) of all sites included in the study. Furthermore, the river delivers comparatively low concentrations of dissolved metals and TDS to the Olifants River and improves both water quality and macroinvertebrate indices measured at site OL8, which had the highest recorded number of taxa and showed higher values for SASS5 metrics in comparison to other sites in the Olifants River, downstream of the confluence with the Klipspruit River.

Conclusions

While the impact of AMD on aquatic biota is due to a number of different factors namely acidity, metal toxicity, metal precipitation and salinization (Gray, 1997), aquatic ecosystem effects associated with AMD are often interpreted using only total dissolved metal concentrations. This study emphasises the speciation of metals and the relative composition of major anions and cations that make up salinity as being additional factors to consider when assessing the effect of AMD on aquatic biota. The relative importance of these factors varies according to the nature of the AMD, its dilution, the nature of the receiving water, especially its buffering and assimilative capacities, species tolerance to pollutants, and other ecological and environmental factors (Gray and Delaney, 2008).

While concentrations of dissolved Al exceeded relevant guidelines, speciation analysis indicates that these are likely to be in a non-toxic form. Fe and Zn rapidly precipitated resulting in very low dissolved concentrations (below guideline levels). Mn remains in the dissolved, toxicologically relevant form over a long distance from the point of input. The concentrations, although comparatively higher than other metals, are significantly lower than those expected to result in toxic effects. It is therefore possible that the relative change in composition of major anions and cations due to AMD input, leading to increased concentrations of more toxic salts (e.g. Na_2SO_4), may also play a significant role in driving the observed macroinvertebrate response. This has implications for treatment of AMD, as the reduced metal toxicity associated with neutralisation may be compromised by the addition of neutralizing agents that result in salinity with a more toxic composition of ions.

It is however possible that the observed decline in macroinvertebrate community metrics may be indicative of historical events when more toxic species of dissolved metals may have been present. Annual variations in hydrology could result in acidic water from the Klipspruit River exerting a stronger influence on the Olifants River downstream of the confluence (MacCausland and McTammany, 2007). The Olifants, and Wilge rivers are heavily utilised (*e.g.*, for irrigation and industrial activities) and have large reservoirs in their upper catchments. In contrast, the Klipspruit River has no major impoundment along its length and is not used for irrigation because of its poor quality. Therefore, particularly during the dry season or periods of drought, highly regulated flows in the Olifants and Wilge rivers may not be sufficient to dilute or buffer AMD introduced by the Klipspruit River (DWA, 2014). Acidic conditions (pH 5.9 – 6.8) were reported at the inflow of Loskop Reservoir from January to June 2008 (Oberholster *et al.*, 2010).

The Wilge River is crucial in terms of improving and restoring water quality and ecological health downstream of its confluence with the Olifants River up to Loskop Reservoir. This is an important finding within the context of future development plans in the Wilge catchment. The upper Olifants River catchment hosts the majority of South Africa's coal-fired power plants and the Wilge River catchment has been ear-marked for significant coal mining activities to support the 4800 MW coal-fired Kusile power station currently under construction. Considering the current water quality of the

Olifants River and the Klipspruit River, it is therefore vital that mining operations are planned and operated in such a manner that they have minimum effects on water quality and environmental flows in the Wilge catchment in the future. In spite of the mediating effect of the Wilge River on water quality it is likely that Loskop Reservoir acts as a sink for metal enriched sediments washed down the Olifants River during high flow periods and further research is required to determine the fate of these metals in the reservoir. Acidic conditions can also mobilise metals from the sediments (Calmano *et al.*, 1993). Furthermore, the reservoir experiences regular algal blooms and studies indicate that under anoxic conditions, exacerbated by eutrophication, Mn and Fe in particular can be released from the sediments into the hypolimnion (Balistrieri *et al.,* 1992).

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References

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 Wastewater. (19th edition). APHA, AWWA, and WPCF, Washington, D.C., USA.

Balistrieri LS, Murray JW, Paul B. 1992. The cycling of iron and manganese in the water column of Lake Sammamish, Washington. *Limnology and Oceanography* **37**(3): 510-528.

Balistrieri L., Seal II RR, Piatak NM, Paul B. 2007. Assessing the concentration, speciation, and toxicity of dissolved metals during mixing of acid-mine drainage and ambient river water downstream of the Elizabeth Copper Mine, Vermont, USA. *Applied Geochemistry* **22**: 930-952.

Bell FG, Bullock SET, Hälbich TFJ, Lindsay P. 2001. Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. *International Journal of Coal Geology* **45**(2-3): 195-216.

Calmano W, Hong J, Forstner U. 1993. Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. *Water Science and Technology* **28**(8-9): 223-235.

Cranfield TJ, Kemble NE, Brumbaugh WG, Dwyer FJ, Ingersoll CG, Fairchild JF. 1994. Use of benthic community structure and the sediment quality triad to evaluate metal-contaminated sediment in the upper Clark Fork River, Montana. *Environmental Toxicology and Chemistry* **13**: 1999-2012.

Dabrowski J, Oberholster PJ, Dabrowski JM, Le Brasseur J, Gieskes J. 2013. Chemical characteristics and limnology of Loskop Dam on the Olifants River, in light of recent fish and crocodile mortalities. *Water SA*, in press.

Dabrowski JM, De Klerk LP. 2013. An assessment of the impact of different land use activities on water quality in the upper Olifants River catchment. *Water SA* **39**: 231-244.

Dallas HF. 2007. River Health Programme: South African Scoring System (SASS) Data Interpretation Guidelines. Report produced for the Department of Water Affairs and Forestry (Resource Quality Services) and the Institute of Natural Resources. Available (online) at: [http://www.dwa.gov.za/iwqs/rhp/methods/SASS%20Interpretation%20Guidelines.pdf]

Dangles O, Guérold FO. 2000. Structural and functional responses of benthic macroinvertebrates to acid precipitation in two forested headwater streams (Vosges Mountains, northeastern France). *Hydrobiologia* **418**(1): 25-31.

Dickens CW, Graham PM 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. *African Journal of Aquatic Science* **27**(1): 1-10.

Dickman M., Rygiel G. 1996. Chironomid larval deformity frequencies, mortality, and diversity in heavy-metal contaminated sediments of a Canadian riverine wetland. *Environment International* **22**: 693-703.

Driscoll CT. 1985. Aluminium in acidic surface water: chemistry, transport and effects. *Environmental Health Perspectives* **63**: 93-104.

Dsa JV, Johnson KS, Lopez D, Kanuckel C, Tumlinson J. 2008. Residual toxicity of acid mine drainage-contaminated sediment to stream macroinvertebrates: relative contribution of acidity vs. metals. *Water Air and Soil Pollution* **194**: 185-197.

DWA (Department of Water Affairs). 2014. Hydrological Services – Surface Water (Data, Dams, Floods and Flows). Available (online) at: http://www.dwaf.gov.za/hydrology/

DWAF (Department of Water Affairs and Forestry). 1996. South African Water Quality Guidelines: Volume 7 – Aquatic Ecosystems. Department of Water Affairs and Forestry, Pretoria, South Africa.

Gensemer RW, Playle RC. 2010. The bioavailability and toxicity of aluminium in aquatic environments. *Environmental Science and Technology* **29**(4): 315-450.

Gray NF, Delaney E. 2008. Comparison of benthic macroinvertebrate indices for the assessment of the impact of acid mine drainage on an Irish river below an abandoned Cu-S mine. *Environmental Pollution* **155**: 31-40.

Gower AM, Myers G, Kent M, Foulkes ME 1994. Relationships between macroinvertebrate communities and environmental variables in metal-contaminated streams in south-west England. *Freshwater Biology* **32**(1): 199-221.

Gustafsson JP. 2011. Visual Minteq 3.0 User Guide. KTH, Department of Land and Water Resources Engineering, Stockholm, Sweden. Available (online) at: [http://www2.lwr.kth.se/English/OurSoftware/vminteq/download.html]

Hobbs P. Oelofse SHH, Rascher J. 2008. Management of environmental impacts from coal mining in the upper Olifants River catchment as a function of age and scale. *Water Resources Development* **24**(3): 417-431.

Ip CCM, Li X-D, Zhang G, Wai OWH, Li Y-S. 2007. Trace metal distribution in sediments of the Pearl River Estuary and the surrounding coastal area, South China. *Environmental Pollution* **147**: 311–323.

Jooste S., Thirion C. 1999. An ecological risk assessment for a South African acid mine drainage. *Water Science and Technology* **39**: 297-303.

Kimball BA, Runkel RL, Walton-Day K, Bencala KE 2002. Assessment of metal loads in watersheds affected by acid mine drainage by using tracer injection and synoptic sampling: Cement Creek, Colorado, USA. *Applied Geochemistry* **17**(9):1183-1207.

Kleynhans CJ, Thirion C, Moolman J. 2005. A Level I River Ecoregion classification System for South Africa, Lesotho and Swaziland. Report No. N/0000/00/REQ0104. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa.

Lasier PJ, Winger PV, Bogenrieder KJ. 2000. Toxicity of manganese to Ceriodaphnia dubia and Hyalella azteca. *Archives of Environmental Contamination and Toxicology* **38**: 298-304.

Lawrence JR, Swerhone GDW, Kwong YTJ. 1998. Natural attenuation of aqueous metal contamination by an algal mat. *Canadian Journal of Microbiology* **44**: 825-832.

MacCausland A, McTammany ME 2007. The impact of episodic coal mine drainage pollution on benthic macroinvertebrates in streams in the Anthracite region of Pennsylvania. *Environmental Pollution* **149**(2): 216-226.

Maret TR, Cain DJ, McCoy DE, Short TM. 2003. Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society* **22**: 598-620.

McCahon CP, Pascoe D, Mc Kavanagh C. 1987. Histochemical observations on the salmonids *Salmo salar* L. and *Salmo trutta* L. and the ephemeropterans *Baetis rhodani* (Pict.) and *Ecdyonurus venosus* (Fabr.) following a simulated episode of acidity in an upland stream. *Hydrobiologia,* **153**(1): 3-12.

Mehta SK, Gaur JP. 2005. Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Critical Reviews of Biotechnology* **25**(3): 113–152

Millennium Ecosystem Assessment 2005. Ecosystems and Human Well-Being: General Synthesis. Island Press, Washington, DC.

Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). *Environmental Toxicology and Chemistry*. **16**(10): 2009-2019

Oberholster PJ, Myburgh JG, Ashton PJ, 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. *Ecotoxicology and Environmental Safety* **73**: 326-335.

Oberholster PJ, Myburgh JG, Asthon PJ, Coetzee JJ, Botha A-M. 2012. Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. *Ecotoxicology and Environmental Safety* **75**: 134-141.

Olías M, Nieto JM, Sarmiento AM, Cerón JC, Cánovas CR. 2004. Seasonal water quality variations in a river affected by acid mine drainage: the Odiel River (South West Spain). *Science of the Total Environment* **333**(1-3): 267-281.

Palmer CG, Muller WJ, Gordon AK, Scherman P-A, Davies-Coleman HD, Pakhomova L, De Kock E. 2004. The development of a toxicity database using freshwater macroinvertebrates, and its application to the protection of South African water resources. *South African Journal of Science* **100**(11-12): 643-650

Scherman P-A, Muller WJ, Palmer CG. 2003. Links between ecotoxicology, biomonitoring and water chemistry in the integration of water quality into environmental flow assessments. *River Research and Applications* **19**(5-6): 483-493

Schmidt TS, Soucek DJ, Cherry DS. 2002. Integrative assessment of benthic macroinvertebrate community impairment from metal-contaminated waters in tributaries of the upper Powell River, Virginia, USA. *Environmental Toxicology and Chemistry* **21**(10): 2233-2241.

Solà C, Burgos M, Plazuelo Á, Toja J, Plans M, Prat N. 2004. Heavy metal bioaccumulation and macroinvertebrate community changes in a Mediterranean stream affected by acid mine drainage and an accidental spill (Guadiamar River, SW Spain). *Science of the Total Environment* **333**(1-3): 109-126.

Ter Braak CJF, Smilauer P. 2002. CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, New York. 500pp.

Tipping E, Carter HT. 2011. Al speciation in streams and lakes of the UK Acid Waters Monitoring Network, modelled with WHAM. *Science of the Total Environment* **409**(8): 1550-1558.

Van Damme PA, Hamil C, Ayala A, Bervoets L. 2008. Macroinvertebrate response to acid mine drainage in rivers of the High Andes (Bolivia). *Environmental Pollution* **156**: 1061-1068.

Winterbourn MJ, McDiffett WF, Eppley SJ. 2005. Aluminium and iron burdens of aquatic biota in New Zealand streams contaminated by acid mine drainage: effects of trophic level. *Science of the Total Environment* **254**(1): 45-54.

Table 1. In-stream field measurements recorded during July 2012 at eight sites in the Olifants (OL), Klipspruit (KL) and Wilge (WG) rivers (units are in mg L^{-1} unless otherwise noted).

Field Measurement	Site							
	OL1	KL2	OL3	OL4	OL5	OL6	WG7	OL8
Ca^{2+}	55	51	50	50	53	53	68	55
K^{+}	9	12	10	11	11	11	5	9
Mg^{2+}	38	26	33	32	34	33	23	29
Na^{+}	42	99	68	65	68	65	24	53
Cl^{-}	31	44	37	34	37	33	12	28
SO_4^{2-}	235	372	301	296	303	301	247	283
$CaCO^{3}$	77	2	44	42	46	44	35	35
DO	11.73	10	9.39	12.17	10.09	10.72	10.16	11.53
DO (% saturation)	123.1	100.1	93.9	128.1	104.7	116.4	112	128.4
TDS	475	645	555	539	545	533	388	487
pH	8.34	5.28	7.03	8.12	8.01	8.4	7.31	8.34

Table 2. Theoretical concentrations (µg L^{-1}) of dissolved aluminium, manganese, zinc and iron species measured at eight sites in the Olifants (OL), Klipspruit (KL) and Wilge (WG) rivers (modelled using VMinTeq software). Concentrations exceeding guideline values are highlighted in bold.

Species	OL1	KL2	OL3	OL4	OL5	OL6	WG7	OL8
Aluminium[1]								
$Al(OH)_2^+$	0.001	-	5.43	0.07	0.06	0.03	0.07	0.01
$Al(OH)_3$ (aq)	0.02	-	7.01	1.28	0.77	0.97	0.22	0.3
$Al(OH)_4^-$	2.48	-	**35.86**	**81.65**	**38.17**	**120**	2.18	**32.69**
$AlOH^{2+}$	-	**17.47**	0.63	-	-	-	0.003	-
Al^{3+}	-	**39.88**	0.03	-	-	-	-	-
Al DOM1	-	**33.79**	0.03	-	-	-	-	-
AlF^{2+}	-	**240.24**	1.84	-	-	-	0.004	-
AlF_2^+	-	**73.90**	6.62	-	-	-	0.02	-
AlF_3 (aq)	-	0.84	0.89	-	-	-	-	-
$AlSO_4^+$	-	**119.66**	0.08	-	-	-	-	-
$Al(SO_4)_2^-$	-	6.11	-	-	-	-	-	-
$AlHPO_4^+$	-	2.38	0.05	-	-	-	-	-
$Al_2(OH)_2CO_3^{2+}$	-	5.31	7.53	-	-	-	-	-
Manganese[2]								
Mn^{2+}	0.82	**491.75**	228.6	181.33	101.95	36.55	68.94	37.50
$MnCO_3$ (aq)	0.26	-	2.09	19.62	9.04	7.82	1.08	5.88
$MnCl^+$	-	0.28	0.13	0.10	0.07	0.02	0.02	0.02
$MnSO_4$ (aq)	0.11	64.66	29.77	23.98	16.79	6.17	10.33	6.22
$MnHCO_3^+$	0.01	0.26	2.29	1.71	1.03	0.35	0.58	0.30
Zinc[3]								
Zn^{2+}	nd	**11.61**	3.22	nd	nd	nd	nd	nd
Zn DOM1		0.35	0.14					
$ZnCl^+$		0.02	-					
ZnSO4 (aq)		1.97	-					
Iron[4]								
Fe^{2+}	nd	31.23	nd	nd	nd	3.93	7.40	nd
$FeSO_4$ (aq)		5.75				0.93	1.55	

[1]AEV=100 µg.L^{-1}; CEV = 10 µg.L^{-1}

[2]AEV = 1300 µg.L^{-1}; CEV = 370 µg.L^{-1}

[3]AEV = 3.6 µg.L^{-1}; CEV = 36 µg.L^{-1}

[4]No guideline values available for Fe

Table 3: Results of SASS5 analysis at eight sites in the Olifants (OL), Klipspruit (KL) and Wilge (WG) rivers.

	OL1	KL2	OL3	OL4	OL5	OL6	WG7	OL8
No. Taxa	17	7	-	10	12	10	20	22
SASS Score	96	38	-	53	57	54	139	125
ASPT	5.6	5.4	-	5.3	4.8	5.4	7	5.7

Table 4: Water quality variable loadings on the 1st and 2nd axis of the Canonical Correspondence Analysis.

Parameter	1st Axis	2nd Axis
COD	0.26	0.75
DOC	0.19	0.32
pH	-0.74	0.27
TDS	0.96	-0.01
Al	0.83	-0.34
Fe	0.66	-0.44
Mn	0.87	-0.16
Zn	0.75	-0.31
Nitrate	0.41	0.24
SS	0.05	0.57
Alkalinity	-0.67	0.24
Al-S	0.38	0.66
Fe-S	0.49	0.28
Mn-S	0.20	0.23
Zn-S	-0.17	-0.50

Figure Captions:

Fig. 1. Location of eight sampling sites in the Klipspruit, Olifants and Wilge rivers.

Fig. 2. Metal concentrations (µg L^{-1}) measured in the Olifants Klipspruit and Wilge Rivers. Grey bars represent the dissolved proportion of the total metal concentrations.

Fig.3: Metal concentrations measured in sediments (A) and benthic algae (B) collected from the Olifants, Klipspruit and Wilge rivers.

Fig. 4. A Canonical Correspondance Analysis biplot showing the influence of metals in water and sediment (indicated by "-s" after element symbol) and other selected water quality parameters (COD

– Chemical Oxygen Demand; DOC – Dissolved Organic Carbon) on macroinvertebrate family composition at sites in the Olifants (OL), Klipspruit (KL) and Wilge (WG) Rivers.

Fig. 1

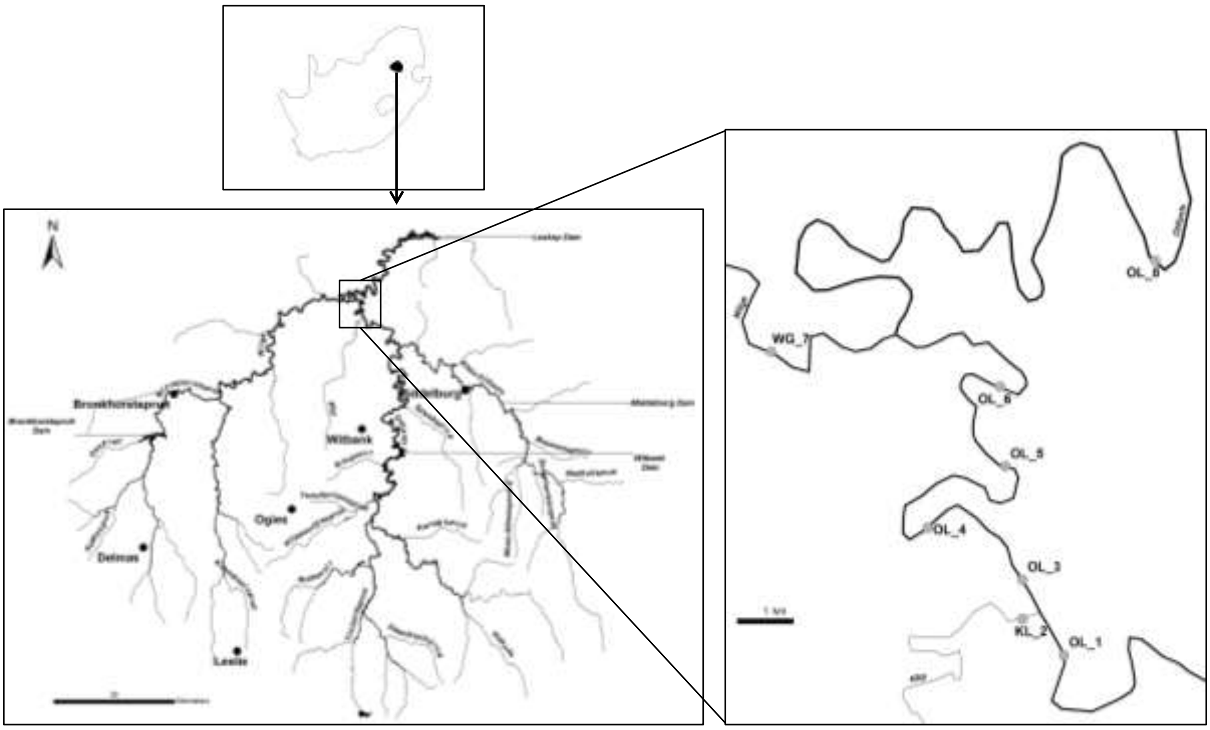

N
Bronkhorstspruit
Witbank
Ogies
Delmas
Leslie
WG_7
OL_8
OL_6
OL_5
OL_4
OL_3
KL_2
OL_1

Fig. 2

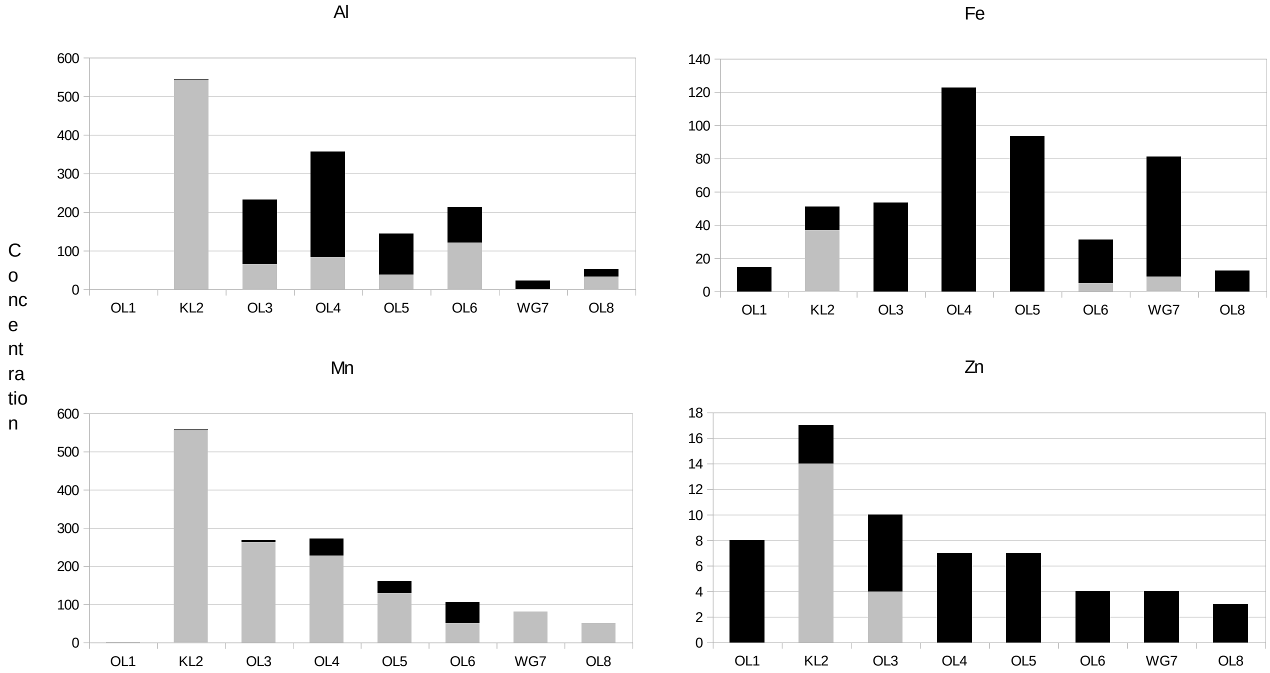

Al
Fe
Mn
Zn
Concentration
0
100
200
300
400
500
600
0
20
40
60
80
100
120
140
0
2
4
6
8
10
12
14
16
18
OL1
KL2
OL3
OL4
OL5
OL6
WG7
OL8

Fig. 3

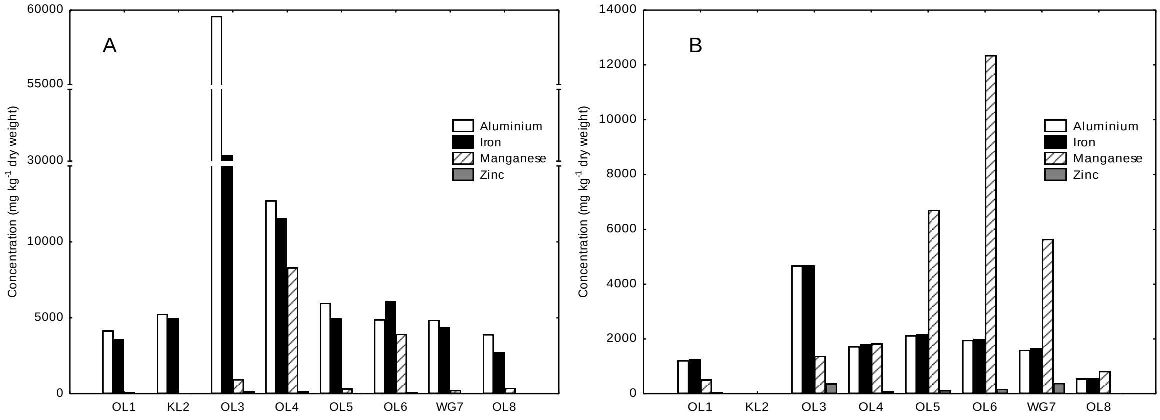
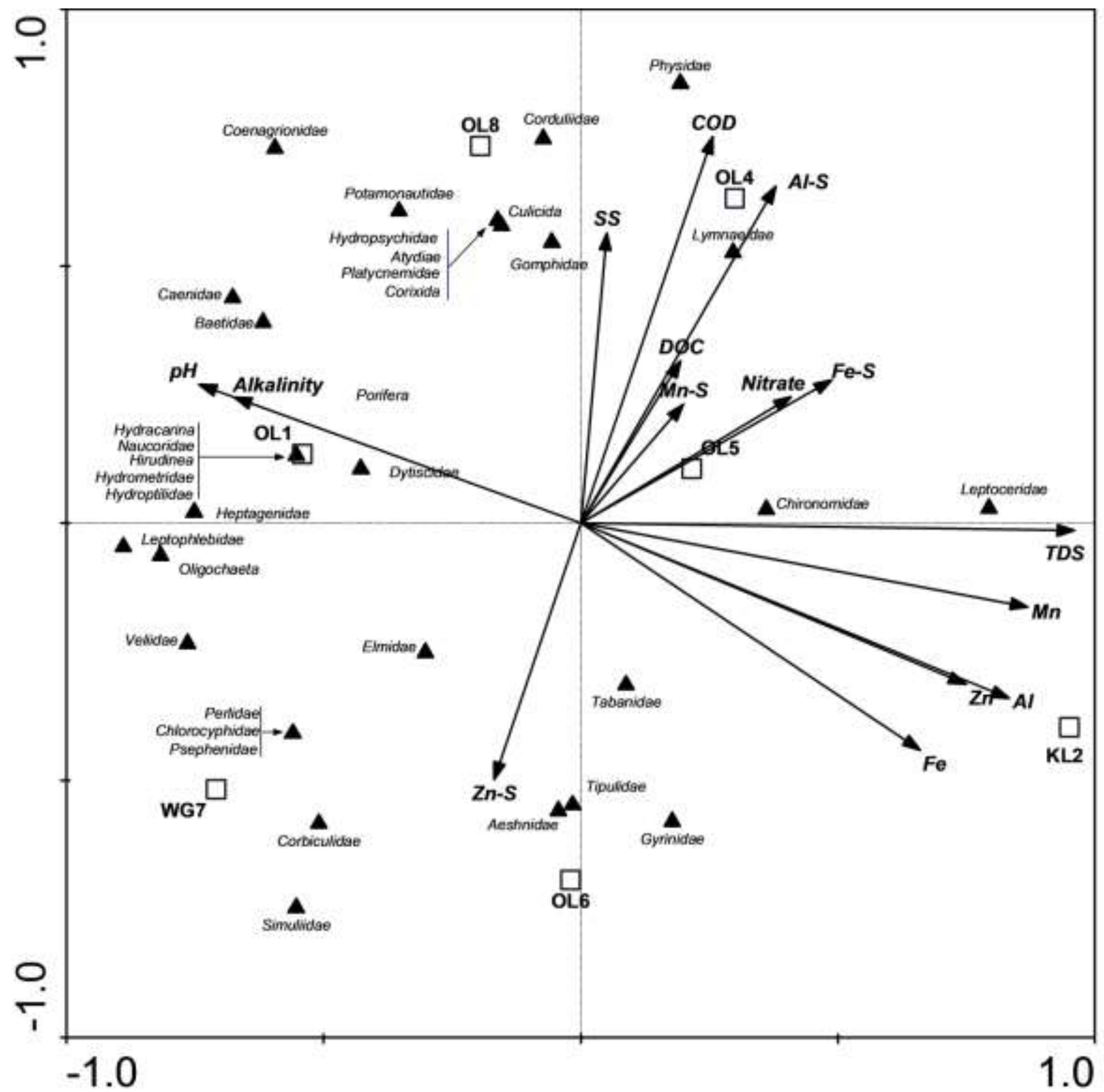

A
B
Concentration (mg kg-1 dry weight)
Aluminium
Iron
Manganese
Zinc
OL1
KL2
OL3
OL4
OL5
OL6
WG7
OL8
0
5000
10000
30000
55000
60000
2000
4000
6000
8000
10000
12000
14000

Fig. 4


1.0
-1.0
-1.0
1.0
Physidae
COD
Cordulliidae
OL8
Coenagrionidae
OL4
Al-S
Potamonautidae
Culicida
SS
Hydropsychidae
Atydiae
Platycnemidae
Corixida
Lymnaeidae
Gomphidae
Caenidae
Baetidae
DOC
pH
Alkalinity
Porifera
Nitrate
Fe-S
Mn-S
Hydracarina
Naucoridae
Hirudinea
Hydrometridae
Hydroptilidae
OL1
OL5
Dytiscidae
Heptagenidae
Chironomidae
Leptoceridae
Leptophlebidae
Oligochaeta
TDS
Mn
Velidae
Elmidae
Tabanidae
Zn
Al
Perlidae
Chlorocyphidae
Psephenidae
KL2
Fe
WG7
Zn-S
Tipulidae
Aeshnidae
Gyrinidae
Corbiculidae
OL6
Simuliidae