River catchment responses to anthropogenic acidification in relationship with sewage effluent: an ecotoxicology screening application

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Abstract

Rising environmental pressures on water resources and resource quality associated with urbanisation, industrialisation, mining and agriculture are a concern globally. In the current study the upper Olifants River catchment as case study was used, to show that acid mine drainage (AMD) and acid precipitation were the two most important drivers of possible acidification during a four-year study period. Over the study period 59% of the precipitation sampled was classified as acidic with a pH value below 5.6. Traces of acidification in the river system using aquatic organisms at different trophic levels were only evident in areas of AMD point sources. Data gathered from the ecotoxicology screening tools, revealed that discharge of untreated and partially treated domestic sewage from municipal sewage treatment works and informal housing partially mitigate any traces of acidification by AMD and acid precipitation in the main stem of the upper Olifants River. The outcome of the study using phytoplankton and macroinvertebrates as indicator organisms revealed that the high loads of sewage effluent may -have played a major role in the neutralization of acidic surface water conditions caused by AMD and acid precipitation. Although previous multi–stage and microcosm studies confirmed the decrease in acidity and metals concentrations by municipal wastewater, the current study is the first to provide supportive evidence of this co-attenuation on catchment scale. These findings are important for integrated water resource management on catchment level, especially in river systems with a complex mixture of pollutants.

Keywords: AMD, acid precipitation, sewage effluent, neutralization capacity

Introduction

The eutrophication and acidification of surface waters has become an endemic global problem. Nutrient loads from sewage treatment plants, agriculture activities and acidity from acid mine drainage and acid precipitation is major drivers, but it remain unclear how they interact on catchment level (López-Doval et al., 2012; Withers et al., 2014). The Olifants River, South Africa as case study has a number of strategically important water use activities that take place (Oberholster et al., 2013). These activities rely heavily on a variety of goods and services derived from the aquatic ecosystems in the catchment to sustain their processes. At the same time pollutants from point and non-point sources cause a great deal of pressure on the water resources in this catchment. The Olifants River system has been described as one of the most polluted rivers in southern Africa due to the intensity and variety of land use activities that are present, particularly in the upper catchment, and the changes to water quality that have resulted from these activities (Grobler et al., 1994).

South Africa possesses approximately 5% of the global recoverable coal reserves and is the world's sixth largest coal producer (220 Mt coal per year) (DME, 2004). The Witbank (eMalahleni) coal fields, situated in the Upper Olifants River catchment, represents the largest conterminous area of active coal mining in South Africa, producing coal for power generation (Tshwete et al., 2006). Many mines in the area are abandoned with underground workings that are flooded, while others are burning as a result of spontaneous combustion (McCarthy et al., 2011). Most of these mines are decanting large volumes of 50 ML/d of acid mine drainage (AMD) that are mostly left untreated and end up in groundwater and surface water resources. In addition, noxious pollutants for intance nitrogen oxides (NO_x)and sulfur dioxide (SO_x) related to burning fossil fuels from the coal mining industry are emitted into the atmosphere, which have direct consequences for water resources in the catchment (Bell et al., 2001).

Low pH and metals from AMD adversely affect the structure and functioning of aquatic systems via toxicity or –metals accumulate in aquatic organisms posing a health risk to aquatic organisms and people consuming these organisms (Cherry et al., 2001; Yim et al., 2006). Impacts on aquatic ecosystems may vary at different localities and among aquatic species inhabiting these ecosystems, affecting productivity, abundance in biomass or may result in complete elimination (Økland and Økland, 1986; Winterbourne et al., 2000).

Elevated concentrations of metals including Al and Fe are characteristic in many AMD contaminated streams (Winterbourne et al., 2000). Al in particular is a highly toxic metal at low pH ranges. The latter is commonly released via processes such as acid mine drainage and acid precipitation (Dise et al., 2001, Oberholster et al., 2013).

Usually, atmospheric precipitation provides a dilution effect on contaminants within a river system (Martins et al., 2014). However, acid precipitation is caused by emissions of SO_2, primarily from fossil-fuel power stations, metal smelters while NO_x is caused from industrial sources and power plants forming sulphuric and nitric acid in precipitation. Typical pH values of acid precipitation caused by anthropogenic emissions may be in the range of 3.5-5.0. In addition to wind direction and distance from the source, wet deposition depends on a number of factors, including the amount of precipitation and rate of SO_2 oxidation. (Menz and Seip, 2004, Huston et al., 2009).. Therefore, it is important to understand what the impact and consequences of these and other pollutants are in order to protect and manage aquatic ecosystems and goods and services that they provide.

According to Karr and Chu (1999), aquatic ecosystem studies that focus solely on water column chemistry cannot be relied on to provide an understanding of the health of an aquatic ecosystem. Water column chemistry alone may only give a snapshot view of stream conditions and do not provide an integrative measure of the overall health of aquatic ecosystems (Barbour et al., 1999). To overcome this shortfall, bioindicators from different trophic levels in the aquatic foodweb are often utilised as ecotoxicology screening tools to determine impacts such as acidification of aquatic systems. Aquatic organisms are adapted to live within certain environmental conditions and changes within their environment may adversely affect the composition and abundance characteristics of a specific biological community (DWAF, 1998). For this reason, biological measures (*in situ* and *ex situ*) provide an integrated and comprehensive assessment of the health of a water body over time (Karr, 1999). Diatoms in particular are useful ecological indicators because they are found in abundance in most lotic ecosystems (Rimet 2012). The great numbers of diatom species provide multiple, sensitive indicators of environmental change and the specific conditions of their habitat (Bray et al., 2008; Oberholster, 2011). Furthermore, benthic macroinvertebrates as indicators are widely distributed in lotic and lenthic systems and are the most popular and commonly used group of freshwater organisms in assessing water quality (Dabrowski et al., (2014). Macroinvertebrates represent an extremely diverse group of aquatic organisms, and the large number of species possesses a wide range of responses to stressors such as organic pollutants, acidification, sediments, and toxicants (Oberholster et al., 2005, 2008).

A key advantage of *ex situ* toxicity testing is that it detects toxic compounds based on their biological activity without any prior knowledge required of the toxicant to identify its presence (Leusch and Chapman, 2011). One of the preferred, and widely used, invertebrate species recommended for acute or chronic toxicity testing is the cladoceran *Daphnia magna* (Parlak et al., 2010). Algae on the other hand are selected as test organisms because they are the dominant primary producers in the aquatic food chain (Finlay et al., 2002). Disruptions to this production base would likely cause effects at higher trophic levels. Although there are numerous studies on the impact of atmospheric acid precipitation and AMD on water resources in the literature, very little is known about the impacts of a combination of these stressors in tandem with domestic sewage and agriculture activities on the aquatic environment. Thus, the objectives of the current study were to used the upper Olifants River catchment as case study to: (a) establish the current state of the aquatic ecosystems affected by acidification by using different aquatic indicator organisms as screening tool, and (b) to determine the response of the system to a mixture of AMD and acid precipitation in tandem with domestic sewage effluent and agriculture activities at a large scale.

2. Materials and methods

2.1. *Study area*

The Olifants River catchment is under severe water stress with the demand for water exceeds the available amount during a certain periods due to multiple land use activities in its catchment (Oberholster et al., 2010). The total upper Olifants River catchment area is 11,464 km^2, with a mean annual precipitation of 683 mm and a mean annual runoff of 10,780 Mm^3 (Midgley et al., 1994). In the upper catchment, coal has been mined for more than a century, and is being exported and used locally in coal-fired power plants. As a result large numbers of AMD decanting abandoned mines occured in the upper catchment as well as coal related industries which include steel and Vanadium manufaturers as well as coal washing plants. The upper Olifants River catchment is furthermore the most urbanised region of the whole catchment with the majority of the urban population located in the industrial city of Witbank (eMalahleni) and the town of Middelburg. In the city of Witbank, industrial activities are totally based upon coal mining, coal combustion power plants, smelters and several industries using coal as energy. The twelve power stations in the region provides 56 % of the electricity for South Africa, while Ferrometals near the city of Witbank is one of the largest individual ferrochrome plants in the world and produces chrome for the steel markets. A previous study by Jossipovic et al. (2011) showed that large portions of the industrial infrastructure is concentrated on the Highveld plateau which include the upper Olifants River catchment and accounts for approximately 90% of South Africa's scheduled emissions of industrial dust, sulphur dioxide and nitrogen oxides.

Other land use activities in the upper catchment that have major impacts on water resources are effluent from wastewater treatment plants (WWTPs). Thirty two of WWTPs are not operated optimally according to the National Green Drop (GD) Report of 2010 (DWA, 2010;

Oberholster et al., 2013). It was evident from this report that most of the WWTP's in the catchment under study had an overall score of less than 30%, showing that many of these plants are in a critical, dysfunctional condition. Previous assessments of individual river basins around the world have found that WWTP inputs can contribute 50% to 90% of annual nutrient inputs in river systems (Marti et al., 2004).

2.2. Selection of sampling sites for ecological status of the aquatic systems and atmospheric precipitation.

The ecological status of the upper Olifants River catchment was assessed at 14 sampling sites distributed between the main stem and its tributaries. Sampling of these sites was conducted over a period of four years on 3-monthly bases during high and low flow regimes **(**Table 1). These sites included a range of stream orders including lower orders ($\leq$3) that function as the source of surface water for downstream reaches, and higher orders ($\geq$ 4) that transport water along the main stem with no net gain or loss of materials (Oberholster et al., 2013a).

Atmospheric precipitation (wet deposits) was collected from a total of 11 sites using permanent standard V-cone plastic rain gauges graduated to 100 mm. Samples were collected by civilian volunteers during raining spells and kept at 4°C till collection (Figure 1). Atmospheric precipitation from the different rain gauges were collected in 1 litre acid clean polyethylene bottles.. Multiple sample bottles collected during a calendar month for a specific sampling site were pooled in a collective sample for chemical analysis, after which physical measurements e.g. pH, salinity were made. Sites were selected in the vicinity of coal-fired power plants, smelters agriculture land use, urban, industrial and rural areas taking account of dominant wind direction provided by the South African Weather Bureau and using 1: 50 000 land use maps to aid the selection process.

2.3. Physico-chemical Parameters of river and atmospheric precipitation

Surface river or stream water samples werecollected quarterly over a period of four years at each of the 14 selected sampling sites. At each site the pH and electrical conductivity (EC) were measured *in situ* using a Hach sension^{TM} 156 portable multiparameter (Loveland, USA) while turbidity of the different river sampling sites were measured *in situ* using a Hach 2100P Turbidimeter (Loveland, USA). Surface water samples for chemical analyses were collected in 1 litre acid clean polyethylene bottles by employing a grab bottle sampler. The Standard Methods for the Examination of Water and Wastewater (APHA, 1992) were used to determine the physico-chemical characteristics of the collected river or stream water and atmospheric precipitation.The entire sample collection were adjusted for temperature (specific conductance) to compare site by site.. All samples were kept on ice and in the dark during transportation to the laboratory. Total nitrogen (TN) and total phosphorus (TP) concentrations were determined with the persulphate digestion technique. Nitrate concentrations (NO_3 as N) were determined on an autoanalyzer with the cadmium reduction method, while soluble reactive phosphorus concentrations were determined by the ascorbic acid method. Sulphate concentrations were analyzed turbidimetrically, and alkalinity by titrimetry (USEPA, 1983). Total metal concentrations within the rain and river water samples were determined using an inductively coupled plasma spectometer (ICP-AES Optima 2000 DV spectrometer, Perki Elmer with an AS 93 autosampler) according to Chochorek et al. (2010).

2.4 Ecological status of the aquatic systems

2.4.1 Macroinvertebrate sampling

Macroinvertebrate integrity and the extent to which the habitat quality affected the occurrence of benthic macroinvertebrate taxa at each site were assessed following the methods described by the South African Scoring System version 5) (SASS5) (Dickens and Graham, 2002) and the Invertebrate Habitat Assessment System (IHAS) developed by McMillan (1998). SASS5 is a qualitative, multi-habitat, rapid, field-based method that requires identification of macroinvertebrates mostly to family level. Standard sensitivity weightings were used to calculate the biotic index.. Macroinvertebrate sampling was conducted quarterly over a period of four years.

2.4.2 *Periphytic algae sampling*

Periphytic algae were sampled over a period of four years during high and low flows according to Hauer and Lamberti, (2006). Five samples were taken along a river transect at each sampling site and pooled together to form a well-mixed composite sample for each sampling site and sampling event. The composite sample was divided into 2 unpreserved subsamples for benthic chlorophyll analysis and the culturing of doubtful filamentous algae in the laboratory for identification, respectively, and 2 preserved subsamples for microscope epilithic soft algae identification; and (d) preserved for microscope diatom identification (Oberholster et al., 2013b).

Diatom subsamples were preserved with ethanol to a final concentration of 20% for microscopic analyses. The samples were cleared of organic matter by heating in a potassium dichromate and sulphuric acid solution and the cleared material was rinsed, diluted and mounted in Pleurax medium for microscopic examination. All algae species (Table 3) were identified using a compound microscope at 1250 times magnification according to Truter, (1987); Komarek and Anagnostidis, (1999, 2005); Van Vuuren et al.(2006); Taylor et al.

(2007) and Wehr and Sheath, (2003). The samples were sedimented in an algae chamber and analysed using the strip-count method (APHA, AWWA and WPCF, 1992). The Berger-Parker dominance index (Berger and Parker, 1970) was used to measure the evenness or dominance of each algal species at each sampling site. In order to evaluate the benthic algae biomass of the selected sampling sites, benthic chl-*a* mg m^2 was used as proposed by Steinman and Lamberti (1996) and Biggs (1996) which includes the following categories: oligotrophic -(chl-*a* mg m^2 0-1.7); mesothropic- (chl-*a* mg m^2 1.7-21); eutrophic **(**chl-*a* mg m^2 21-84) and hypertrophic **(**chl-*a* mg m^2 > 84). For the determination of benthic chl-*a* (mg m-2) the protocol of Porra et al. (1989) was followed.

2.5 Toxicity tests

Toxicity tests, using a daphnid (*D. magna*) and an algal species (syn. *Raphidocelis subcapitata*), was conducted on water samples collected at the various sites within less than 72 hours after collection. The acute, 48-hour *Daphnia magna* screening assay was employed under static conditions to establish the short-term toxicity potential of the water samples from selected sites. *D. magna* toxicity test was performed in accordance with the U.S. Environmental Protection Agency's Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (USEPA, 2002a). A 96-hour growth inhibition test using the unicellular green alga *Selenastrum capricornutum* (syn. *Raphidocelis subcapitata*) was performed according to the USEPA (2002b) method. *Selenastrum capricornutum* (strain ATCC 22662, Canada) was used as the test organism and exposed to test waters collected from the different sampling sites. Cell growth inhibition or stimulation as endpoints was quantified (as cells ml^{-1}) after the 96-hour exposure period using a spectrophotometer.

3. Data interpretation

3.1Benthic periphyton assemblage

The data of the total number of phytoplankton taxa associated with mining pollutants, and their frequency of occurrence at each sampling site were categorized as followed: 1 = ≤ 50 (rare), 2 = 51- 250 (scarce), 3 = 251-1000 (common), 4 = 1001-5000 (abundant), and 5 = 5001-25 000 (predominant) cells 5 cm^2). The algal abundance in the samples was evaluated by counting the presence of each species (as cells in a filament or equal number of individual cells) according to Oberholster (2011). For the classification of the benthic periphyton assemblage, only species indicative of mining activities or acidification were considered. A class was assigned, based on the abundance of species present associated with mining pollutants. The higher the species abundance, the more impacted the site is due to mining pollutants. Therefore sites with an abundance of ≤ 50 cells 5 cm^2 were least impacted (Class A), while sites with an abundance of 5 001 – 25 000 cells 5 cm^2 were most impacted (Class E/F) (Figure 4).

3.2Toxicity testing

A risk/hazard class was determined per alga and daphnid test by applying the hazard classification system for natural waters (Persoone et al., 2003). This hazard class equates to the level of acute or chronic risk posed by the water sample tested. A percentage effect (PE) is determined for each toxicity test by measuring immobility / mortality or inhibition / stimulation, depending on the type of test. The sample result was then ranked into one of five classes, based on either screening or definitive testing protocols. Percentage effect: 10% effect = slight toxicity for *Daphnia*; 20% effect = slight toxicity for algae; 50% and > effect = toxicity for both test organisms. The hazard classification was originally developed to assess a battery of tests. For the purpose of this study a hazard class was separately assigned to *S.capricornutum* and *D. magna* (Figure 4).

3.3Macroinvertebrate assessment

Results were categorised based on ecoregions (Kleynhans, 1999), SASS5 total score, and Average score per taxon (ASPT), as developed by Dallas (2007). This system takes the SASS5 and ASPT scores into account per level 1 ecoregion, and rates them accordingly, from an un-modified water body to a highly impacted water body. The categories are summarised in Figure 4.

3.4. *Statistical analysis*

Statistical differences between dominant periphyton species indicating nutrient enrichment and catchment acidification as well as physical and chemical variables of the surface water (water column pH and phosphate) were analysed by computing the Pearson correlation and a *t*-test using Sigma-Plot (Jandel Scientific) program. A *p*-value of less than 0.05 was considered significant. A correlation coefficient of r near zero was regarded as unrelated.

Barker and Barker (1984)

4.Results

4.1Ecological status of the aquatic systems

The results of the ecological status of the upper Olifants River catchment are summarized in Figure 3. It was evident from the data collected that the river was seriously modified. The highest values of EC in comparison to other sampling sites were measured in the Brugspruit (FAN 6) and Blesbokspruit streams (B 2), both tributaries of the Klipspruit River. Lowest average pH values (< 3.5) were measured at the sampling site in the Klipspruit River (FAN 2) and Grootspruit stream (G1) indicating acidic conditions cause by AMD. These sites contained some of the highest concentrations of metals in the study area, including Al, Fe, Mn, V and Zn (Table 2). Concentrations of metals at both sites well exceed aquatic ecosystem guideline values set out by the Department of Water Affairs (DWA, 1996) and are likely to be toxic to aquatic life. However, we can not exclude the fact that precipitation of metal hydroxides (e.g. ferric hydroxides ($FeOH_3$) commonly known as yellow boy) could have smother intolerant biota in down stream AMD impacted areas with an increase in pH (Jarvis and Young, 2000). Aluminium was of particular concern because it is known to be toxic to aquatic organisms at relatively low concentrations and low pH values (Roux et al. (1996). The high concentrations of metals at these sites, in combination with low pH and intensive mining in the surrounding catchment suggest that AMD is the most likely cause of elevated Al concentrations. A high benthic biomass of periphyton species associated with mining impacts were identified at both sites (Class E/F); macroinvertebrates were severely modified while site FAN 2 furthermore poses an acute toxicity hazard to both daphnids and algae as shown in Figure 3. The dominant macroinvertebrate family at site FAN 2 was Leptoceridae (Cased Caddis flies), which have been observed to be prevalent in acidic waters, while the family Chironomidae dominated sampling site FAN 6. The low water turbidity (NTU) measured at site FAN 2 and G1 was in agreement with observations by Hogsden and Harding (2012). The authors reported in their study that streams downstream from abandoned mines appear clear since metals remain in solution under highly acidic conditions. The NTU correlated positively ($r = 0.7128$, $p \leq 0.04$) with the low pH values at these sites. The contamination of the main stem of the Olifants River (sites, FAN 3, FAN 5, and FAN 8) by metals was relatively low in comparison to the tributaries impacted by AMD that enter the main stem of the Olifants River (Table 2). This was also reflected by the benthic periphyton species present (Class B, class B and class A). Little AMD related impacts were detected at these sites that had average pH values of >7. The toxicity hazard posed to daphnids and unicellular green algae were also low according to the toxicity tests (Figure 2). The macroinvertebrates classes for these sites varied between largely to moderatedly modified (Figure 3). Both, the Wilge River (FAN 1) and the Koffiespruit stream (FAN 12) which are tributaries of the upper Olifants River, was moderately impacted with average pH value > 7. The dominant land use activity at the latter sites was agriculture and no acidophilic organisms as indicators of acidic precipitation were observed during the study period. The sensitive macroinvertebrate family Heptageniidae was observed during all sampling occasions at FAN 1 and FAN 12, but the most dominant family detected was Baetidae. Both the latter families were may flies (Ephemeroptera).

4.2 Benthic periphyton autecology as indicators of system acidification

Most of the algae samples were mainly dominated by species belonging to three taxonomic groups namely Chlorophyceae, Bacillariophyceae and Zygnemataceae. From the microscopic analysis a total of 156 species were identified in the upper Olifants River catchment of which 15 species were indicators of acidification. The occurrence of these species across the rivers in the different sampling locations is summerized in Table 3. The Bacillariophyceae species *Nitzschia littorea* was the most widely distributed and known as an indicator of mine effluent (Table 3). The biomass of the benthic communities was strongly dominated by filamentous green algae in AMD impacted sites. A maximum benthic filamentous algae biomass of 98 mg m^{-2} chl-*a* and 92 mg m^{-2} chl-*a* were measured at sampling sites FAN 2 and GI, respectively during the dry season month of June (mid winter) which correlated positively ($r = 0.8587$, $p \leq 0.05$) with low pH values of < 3.5. The filamentous algae *Ulothrix punctate* and *Klebsormidium acidophilum* dominated (Berger and Parker index, 3.84; 4.76) these sites. Site FAN 6, impacted by both AMD and sewage effluent, was dominated (Berger and Parker index, 1.96) by the filamentous algal *Stigeoclonium tenue*. The green filamentous algal *Oedegonium* spp. dominated (Berger and Parker Index 2.23) sampling sites B2; B1S02 and VZ2 with an average benthic algae biomass of 39.1 mg.m^{-2} chl-*a*, 45.3 mg m^{-2} chl-*a* and 65.3 mg m^{-2} chl-*a* respectively, during the dry season. These sites was impacted by AMD, sewage effluent and agriculture activities. Although the diatom *Nitzschia littorea* occurred at most of the sampling sites, it was detected in very low numbers at sites in the main stem of the upper Olifants River. Overall, the surface water of the main stem of the Olifants River were dominated (Berger and Parker Index 2.13; 1.87; 2.21) by planktonic green algae species *Euglena sociabilis*, *Phacus pleuronectes* and *Scenedesmus quadricauda* indicating nutrient enrichment, especially during the summer season. The green filamentous algae that occurred in abundance on the submerged pebbles, boulders and bedrock of the main stem of the upper Olifants River were the generas such as *Spirogyra*, *Stigeoclonium*, *Oscillatoria* and *Cladophora*. All the latter species are known indicator species of nutrient enriched conditions (Oberholster, 2010; Oberholster et al., 2013b). The filamentous algae *Stigeoclonium tenue* and *Cladophora glomorus* that dominated (Berger and Parker Index 3.53; 4.13) the periphyton throughout the study at sampling sites downstream of WWTPs and informal housing (FAN 6 and 8) correlated positively ($r = 0.9143$, $p \leq 0.04$; $r = 0.8941$, $p \leq 0.05$) with phosphate concentrations. *Melosira variance,* an indicator of eutrophic conditions (Taylor et al 2007), was the dominant diatom (Berger and Parker Index 1.93) in the main stems of the upper Olifants and Wilger rivers and Koffie stream.

4.3 Atmospheric precipitation

A total of 66 samples were analysed for physical attributes during the course of the rainy season. The EC values were low throughout the collection period. Two thirds (59%) of the samples tested could be classified as being acid rain, with the worst site, site 5 and the least effected, site 10. Unfortunately, precipitation values were not always available from all selected sites due to rainfall variation. However, estimates based on the analyses volumes confirmed that the most rainfall took place during the summer months (November, December and January). The atmospheric precipitation measured during the current study was the second highest since 1989/1990 at sampling site 5 located in the industrial city of Witbank. The 1,290 mm recorded at site 5 was just 30 mm from the recorded maximum of 1,320 mm, measured for the 1995/1996 seasons. Ammonium-nitrogen concentrations were present in 67% of the samples analysed, and were higher than the recommended aquatic ecosystem guidelines of the Department of Water Affairs (1996). V, Mn, Zn, Al, Cd, Fe and F were observed in atmospheric precipitation of some of the sites analysed, with Al, F, Mn and Zn often exceeded the recommended guidelines of DWA (1996) (Al$\leq$ 5 $\mu g l^{-1}$; F $\leq$ 750 $\mu g\ l^{-1}$; Mn $\leq$ 180 $\mu g l^{-1}$ and Zn $\leq$ 2 $\mu g\ l^{-1}$) for aquatic ecosystems (Table 4).

4.4 Buffer capacity of the upper Olifants River system

Previous studies in the Highveld region by Josipovic et al. (2011) which include the upper Olifants River catchment, showed an exceedance of critical loads of acidity (meq/m^2 per year) for soils and consequently possible impacts to terrestrial and aquatic ecosystems. According to Bowman (1991) surface waters with alkalinity values of less than 10 mg l^{-1} as $CaCO_3$ are regarded as being acid sensitive. However, the only acid-sensitive streams with a poor buffer capacity that were sampled in the current study was sites FAN 2, 6, 7 and G1 which were characterized by a low alkalinity ($\leq$ 10 mg l^{-1} as $CaCO_3$). All these sampling sites were directly impacted by point source acid mine drainage. Site FAN 3, 4 and 5 that were impacted by a mixture of AMD and sewage effluent comprise of a much higher alkalinity ($\leq$ 56 mg l^{-1} as $CaCO_3$).

5. Discussion

In the upper Olifants River catchment, land use impacts of post coal mining activities, sewage effluent, agriculture activities and acid precipitation were the main sources of pollutants identified. However, a previous study by Dabrowski (2014) applying the SWAT (soil and water assessment tool) to predict ortho-phosphate loads in the upper Olifants catchment showed that poorly operating WWTWs were the major contributors to catchment nutrient enrichment. Understanding the interplay between the combination of these pollutants and the biotic and abiotic factors in this river system provided the basis for determining the possible acidification of the system. However, the ecological processes affected by anthropogenic activities showed a general acidification of certain river stretches and tributries of the upper Olifants River system and the possible point source inputs or mobilisation of metal ions and other contaminants mainly via AMD related to the coal industry. The ecological effects of acidification of these river stretches and tributries may act directly through toxicity to biota resulting in increased mortality and/or physiological stress or changes to more tolerant species. It was evident from the current study that the macroinvertebrate assemblage gave a good indication of habitat degradation, while the *ex situ* toxicity tests indicated general toxicity. The periphyton algal assemblage on the other handgave a good indication of pH and EC values of the system. The outcome of the macroinvertebrate data of the current study was in relationship with a previous study by Soucek (2001). Soucek (2001) reported a significant reduction in the richness and abundance of Ephemeroptera-Plecoptera-Trichoptera (EPT) families, as well as a decrease in total taxon at sites receiving AMD water. Soucek (2001) further observed high water and sediment toxicity at sites impacted by mining due to high dissolved metal concentration and high iron (III) hydroxide precipitate deposition, respectively.

It was apparent from the water quality and the phytoplankton autecology data within the main stem of the upper Olifants and Wilge rivers, as well as the Koffiespruit stream, that very little or no acidophilic indicator species were observed that can be related to AMD and acid precipitation during high or low flow regimes. This phenomenon can possibly be related to two factors. The first being the self-purifying capacity of the Olifants River which enables it to increase pH values and reduce metal concentration within a relative short distance from post mining impacts through dilusion or through alluvial deposits and sand bottom substrate that can act as sink for metals. However the Olifants River catchment is under severe water stress making this factor unlikely especially during winter months with low flow regimes (Srinivasan et al., 2012). Furthermore, the upper river catchment is comprised mostly of bedrock as bottom substrate (red-bed sandstones, mudrock and conglomerates) lacking alluvial deposits that can act as a zink for pollutants in comparison to the middle catchment (WRC, 2001; Oberholster et al. 2013). Secondly, high loads of sewage effluent from unfunctional WWTPs and unformal housing possibly neutralized the acidity from AMD and acidic precipitation. From the data gathered in the current study the second senario seems to be the most plausible explanation, since the treatment of AMD by municipal wastewater is well documented (Roetman, 1932; McCullough et al., 2008).

In 2013, Oberholster et al. (2013) reported elevated TP, TN and chloride concentrations from the point source discharge of the non-functional Riverview WWTP in the upper Olifants River catchment which persisted up to a distance of 40 km downstream. In this study, the authors also compared their chemical data to the nutrient ranges of conventional activated sludge and different advanced treatment processes (Carey and Migliaccio, 2009). The total nitrogen measured at Riverview WWTP (80 mg l^{-1}) exceeded the total nitrogen of untreated water (70 mg l^{-1}) and was more than double the amount released from conventional activated sludge treatment plants (15-35 mg l^{-1}). It was also 10 times higher then what is released from activated sludge with BNR processes (Carey and Migliaccio, 2009). Although previous multi–stage and microcosm studies of co-treatment of synthetic AMD or acidic pit lake water and municipal wastewater conducted by McCullough et al. (2008) and Strosnider et al. (2011a and b) confirmed the decrease in acidity and metal concentrations, the current study is the first to provide evidence for co-attenuation on sub catchment scale using physical, chemical and biological indicator data. Although the study showed that sewage from WWTPs neutralise acification from AMD and acid presipitation, it must not been seen as a treatment obsion on catchment scale, since sewage can cause spread of disease, high oxygen demand, adverse effects from EDC's and pharmaceutical and personal care products.

Conclusion

The outcome of the study using indicator organisms showed that the high loads of sewage effluent might have played a major role in neutralizing acidic conditions caused by AMD and acid precipitation in the upper Olifants River catchment. Although neither AMD and acid precipitation nor untreated sewage is beneficial to an ecosystem, the combination of the two adverse effects appears to have an advantage. However, this should not be seen as an accepted manner for in situ AMD treated. The current study is the first to provide supportive evidence for co-treatment of AMD and municipal wastewater on sub catchment scale using chemical and aquatic biota data. These findings are important for integrated water resource management on sub catchment level especially in river systems that receive a complex mixture of pollutants.

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