Title: An ecotoxicological screening tool to prioritise acid mine drainage impacted streams for future restoration

Corresponding author: Prof A-M Botha

Department of Genetic, University of Stellenbosch, Private Bag X1,

Stellenbosch 7600, South Africa

(+27)-21-808-5832,

ambo@sun.co.za

Tables
5 Figures

An ecotoxicological screening tool to prioritise acid mine drainage impacted streams for future restoration

P.J. Oberholster[a.b.c], B. Genthe[a], P. Hobbs[c], P.H. Cheng[a.d], A.R. de Klerk[c], A-M Botha[d*]

[a]CSIR Natural Resources and the Environment, P.O. Box 320, Stellenbosch 7599, South Africa;

[b]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa;

[c]CSIR Natural Resources and the Environment, P.O. Box 395, Pretoria 0001, South Africa

[d]Department of Genetic, University of Stellenbosch, Private Bag 2600, Stellenbosch 7600, South Africa

*Corresponding author: A-M Botha (+27)-21-808-5832, ambo@sun.co.za

Department of Genetic, University of Stellenbosch, Private Bag X1, Stellenbosch 7600, South Africa

Abstract

Streams impacted by acid mine drainage (AMD) typically present water exhibiting low pH and high metal concentrations. These factors result in the environmental degradation of watercourses. The objective of this study was to develop and evaluate an ecotoxicological screening tool (EST) to prioritise future remediation of streams impacted by AMD. The Bloubank stream drainage system in South Africa, served as study area for this purpose. In the initial EST development phase physicochemical variables were assessed while in the second phase, epilithic filamentous green algae biomass (chl-*a* mg m^{-2}), diatoms and filamentous green algae community structures were employed as bioindicators as well as *Daphnia magna* toxicity assays.. Using a weight of evidence approach, , the first three sites receiving AMD

were critically and seriously modified, followed by site 4 that was modified.. Sites 1 to 3 with EST scores ≤70 % were assessed as priority candidates for future restoration.

Keywords: AMD, epilithic filamentous algae, diatom diversity, precipitate deposition

Capsule: An ecotoxicological screening tool combining physical chemical variables and bioindicators was developed and employed to prioritise future remediation of streams impacted by AMD.

Introduction

South Africa accounted for 12 % of global gold production in 2005, while 50 % of the world's gold reserves are found in South Africa (USGS, 2005). The Witwatersrand Basin presents the most immediate and urgent risks associated with acid mine drainage (AMD) in South Africa. Substantial thought and resources have been directed at understanding the AMD threat in this basin, and particularly the Western Basin where decant of AMD has been occurring since August 2002 (DWAF, 2010). AMD is formed when sulphide minerals are exposed to atmospheric, hydrological and biological elements (oxygen, water and chemoautotrophic bacteria), the resulting oxidation generating sulphuric acid that imparts a low pH and net acidity to water containing elevated sulphate and, dissolved metal concentrations, low alkalinity and high conductivity (Hogsden and Harding, 2012). Dilution may reduce metal concentrations while not markedly influencing pH. At a higher pH ($\geq$ 4.0) precipitation of metal hydroxides (e.g. ferric hydroxides ($FeOH_3$) commonly known as yellow boy) can smother biota, whereas at lower pH the dissolved metal ions can penetrate biota membranes and cause toxicity (Jarvis and Young, 2000; DeNicola and Stapleton, 2002, Van Ho et al., 2002). The AMD effect on aquatic ecosystems is threefold, namely (a) impacted communities experience lethal levels of pH and metals, which lead to a decrease in biota richness and diversity, (b) communities are restricted to tolerant organisms which are able to survive in these conditions, and (c) alteration in nutrient cycles and abiotic changes may occur. Another adverse effect of AMD is that the acidity of the water destroys the bicarbonate buffering capacity of an aquatic system (Gray, 1997). Many investigations of AMD impacted streams and rivers have made use of one or more biological indices (Jarvis and Young, 2000). The index most often applied in the United Kingdom to assess the impact of mine water pollution in recent years is the Biological Monitoring Working Party (BMWP) score. This index, like many other biological indices, makes use of benthic macroinvertebrates as bioindicators to determine

AMD impacted stream sites. Currently in South Africa, no specific AMD screening tool is favoured and none exists which employs both physicochemical and bioindicator parameters conjunctively.

In the proposed ecotoxicological screening tool (EST), use is made of epilithic filamentous green algae biomass (chl-*a* mg m^{-2}) and diatoms in conjunction with physicochemical parameters and visual interpretation based on Gray (1999). Although a variety of organisms are employed as biological indicators of AMD impacted aquatic systems, algae in particular are rapidly being implemented in assessments of stream ecosystems (Stevenson and Pan, 1999). Epilithic filamentous green algae and diatoms were selected as biological indicators for the EST on the basis that it is stationary, and therefore directly indicative of the physicochemical conditions of their immediate habitat (Stevenson and White, 1995).In previous studies Hill et al. (2000) and Verb and Vis (2005) have used algae as indicators to determine the adverse effects of AMD, these indices concentrate on algal community composition and species diversity. Furthermore, in recent studies by Archibald and Taylor (2007) and Zajack et al. (2010) diatom indices were used as a biomonitoring tool to determine biotic integrity of acid mine drainage impacted streams and the assessment of diffuse pollution from AMD. In contrast, macroinvertebrate drift or fish movement may render these organisms less suited for AMD biomonitoring. Fauna may also be generally less tolerant to AMD in areas where stresses are severe (Harding and Boothroyed, 2004). Previous studies indicated that algae as bioindicator may be more suitable than macroinvertebrates for water chemistry and land use impacts, while macroinvertebrates are better indicators of stream hydrology and oxygen depletion (Johnson et al., 2006; Hering et al., 2006).

Therefore, the aim of the study was to use existing and historical data sets which include physical, chemical and biological parameters to develop and evaluate an EST that can be used to categorise AMD impacted stream reaches for restoration purposes.

Material and methods

Study area and sampling methodology

The watercourses targeted in this study form part of the Bloubank stream drainage system. This stream is a tributary of the Crocodile River (an upper tributary of the Limpopo River) that drains the north-western portion of the Johannesburg Metropole, Gauteng Province, South Africa (Figure 1). The south-western portion (headwater) of the Bloubank stream system comprises the Riet stream and its tributary, the Tweelopie stream. These drainages receive AMD emanating from defunct flooded mines associated with the West Rand Gold Field (also known as the Western Basin) of the regional Witwatersrand Gold Field. The Witwatersrand forms the watershed that marks the continental divide between the westerly flowing Vaal River system to the south and the easterly flowing Limpopo River system to the north. It also hosts highly urbanised areas interspersed with the footprints of historical gold and uranium mining activities. The opencast and underground gold and uranium mines have left behind large pits and areas of mine residues (tailings dams, slimes dams and rock dumps) distributed in a ~2 km wide zone stretching ~98 km from east to west along the watershed. The ~45 Mm^3 of void created by underground mining in the Western Basin (Krige and Van Biljon, 2006) started filling with water following the cessation of mining and pumping in 1998, culminating in the manifestation of AMD on the surface in late-August 2002. The initial drainage rate of 4 to 8 Ml d^{-1} slowly increased to a relatively constant ~25 Ml d^{-1} by mid-2008 as the hydrostatic head in the flooded mine void continued to build. The establishment of containment structures and a high density sludge (HDS) treatment plant with a capacity of

~15 Ml d^{-1} attempted to control the AMD issuing from various point sources (shafts and boreholes). Although successful in curbing the release of raw mine water into the environment, the treated and neutralised mine water still carried concentrations of sulphate >2500 mg l^{-1} and manganese >10 mg l^{-1}, respectively, whilst maintaining a near-neutral pH in the downstream receiving stream reaches. More recently, such as during the abnormally wet 2009-'10 and 2010-'11 summer seasons, the combined discharge of treated/neutralised and raw mine water has on occasion exceeded 60 Ml d^{-1} (Hobbs and Cobbing, 2007), with acidic raw mine water (pH ~3) comprising ~75 % of this volume. The main result of these circumstances has been the manifestation further downstream of acidic surface water (pH ~4) containing consistently higher (>50 mg l^{-1}) manganese concentrations. The ecological sensitivity of the receiving environment relates to its association with the Krugersdorp Game Reserve (KGR) and, still further downstream, the Cradle of Humankind World Heritage Site (COH WHS). The selection of the six survey sites and the reference site was based on water chemistry (e.g. pH, conductivity and metal concentrations) and similar habitat characteristics (e.g. stream bank stability, substrate type and geology) as basis for determining the impact of AMD (Figure 1). Table 1, presents the four sampling surveys over a period of one year and the main features of the survey sites. Site 1 is located ~500 m downstream of the AMD drainage sources, while site 2 and the reference site are situated in the Krugersdorp Game Reserve. Site 3 is located just before the confluence of the Tweelopie stream and its main stem, the Riet stream, while sites 4, 5 and 6 are located further downstream on the Bloubank stream. The discharge of two karst springs, one located between sites 4 and 5 yielding 100 to 150 l s^{-1}, and the other between sites 5 and 6 yielding 300 l s^{-1}, contribute to the increase in discharge observed at sites 5 and 6 respectively (Table 1).

Development of the ecotoxicological screening tool (EST)

Development of the EST entailed aggregating the components of various previous approaches including (a) length of river/stream affected (Jarvis and Younger, 2000), (b) substrate quality and habitat assessment (Gray, 1997), (c) water column pH, turbidity, total Fe and total Al (Jarvis and Younger, 2000), (d) *Daphnia magna* survival test (Thursby et al., 1997; Oberholster et al., 2010), and (e) periphyton biomass (chl-*a* mg m^{-2}) (Niyogi et al., 1999; Bray, 2007; Bray et al., 2008; Sode, 1983; Oberholster, 2011). The EST development occurred in two phases. In the first phase, the impact of mine water on the receiving watercourse was assessed on the basis of physicochemical parameters. The parameters, listed in what is considered by Jarvis and Younger (2000), to be a decreasing order of importance, are (1) length in metres of stream affected, (2) substrate quality and habitat assessment in terms of metal precipitate thickness, and (3) water column turbidity, pH, total Fe and total Al as mg l^{-1}. In phase 2, epilithic filamentous green algae biomass (chl-*a* mg m^{-2} as surrogate for algae biomass), dominant filamentous green algae and diatomswere used (Table 2) together with the *D. magna* 48 hour acute toxicity test. Inclusion of the latter follows recent recognition of the popularity and value of bioassays for laboratory test validation and field extrapolation. Zooplankton community biomass typically declines at a pH <4.8, which makes this test ideal for detecting adverse effects of AMD (Kalff, 2002).

The application of a weight of evidence type approach yielding a single cumulative value out of 100 points characterised the environmental conditions at each sampling site. The higher the score, the less environmentally impacted the survey site. The reference site returns a value of 100 %, while AMD impacted sites range from 0 to 70 %. A ranking was then developed whereby sites that scored 71 to 100 % (largely natural with a few modifications) and 41 to 70 % (modified) were not considered for future restoration (Table 2). Survey sites scoring ≤40 %

were flagged as stressed to severely stressed, and present as priority candidates for future restoration. The EST framework is summarised in Table 2.

Metals, trace metals,chlorophyll-*a* and algal assemblage

At each selected survey site (1 m^2) the presence of epilithic filamentous algae were first defined with the naked eye, since these types of algae have a distinct structure (Sheath and Cole, 1992). The percentage cover of filamentous algae was estimated using the method of Sheath and Burkholder (1985). If present, an area of substrate surface (5 cm in diameter) was isolated for epilithic filamentous algae sampling using a syringe extended with a tygon tube (Douglas, 1958; Hauer and Lamberti, 2006). Epilithic filamentous algae samples were collected at each site on four sampling occasions during high and low flows (June 2011 to May 2012), and combined in a composite sample for each survey site. Epilithic algae abundance in the samples was evaluated by counting the presence of each species (as cells in a filament or equal number of individual cells). In the case of diatom sampling, stones were collected from the submerged part (10-50 cm depth) of the river bank at each sampling site. The attached diatoms were removed by brushing an area of 5 cm^2 of each stone and the material was resuspended in 200 ml deionised water. An aliquot of 50 ml was fixed with formaldehyde at a final concentration of 4 % (v/v) for microscopic examination to identify algal species. In the case of sand and silt samples containing benthic diatoms, the sediment was cleared of organic matter 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 were identified using a compound microscope at 1250 x magnification (Taylor et al., 2007, Van Vuuren et al., 2006). The samples were sedimented in an algae chamber and were analyzed using the strip-count method (APHA, 1992). The Berger-Parker dominance index (Berger and Parker, 1970) was used to measure the evenness or dominance of the algae at each sampling site.The samples were placed on ice and transported to the laboratory in cooler boxes for analysis of chlorophyll *a* (chl-*a*) according to Porra et al. (1989). A PerkinElmer™ Lambda 25 spectrophotometer was used for absorbance determination. On return to the laboratory, water samples from the different survey sites were filtered through 0.45 µm Gelman glassfibre filters and preserved in nitric acid, after which total Al and Fe were determined by ICP-OES. The instrument was calibrated using internal standards.

pH and flow features

The pH and electrical conductivity at each survey site were measured *in situ* using a Hach sension™ 156 portable multiparameter (Loveland, USA), while turbidity was measured *in situ* using a Hach 2100P Turbidimeter (Loveland, USA). Flow was measured on the basis of synoptic discharge measurements using an OTT™ C20 current meter with OTT™ Z400 signal counter set and impellor # 1-239627 (diameter 125 mm, pitch 0.25 m) mounted on a 20 mm diameter rod to determine velocity. Care was taken to select a cross-section that provided as 'clean' and 'neat' a profile as possible. The accuracy of flow measurements reported in Table 1 ranges from ±5-10 % for the smaller discharges (<20 Ml d^{-1}) to ±15-20 % for the higher discharges (>20 Ml d^{-1}).

Stream bottom substrate, canopy cover and stream bank stability

The substrate type of each survey site (i.e. percentage of cobbles, pebbles, gravel, sand and silt) and in-stream substrate cover (i.e. macrophytes) as well as the riparian canopy cover were determined visually according to the method of Stevenson and Bahls (1999). An assessment of the degree of bank erosion was made to distinguish between AMD adverse effects and other land activities according to Spencer et al. (1998). Scores were allocated to each site using the following categories: 5 = stable (where the banks or edges of the stream are stable and are protected by good vegetation cover); 4 = good (evidence of minor localised erosion without damage to bank structure or vegetation); 3 = moderate (some erosion evident, with minor damage to bank structure and vegetation); 2 = poor (significant areas of erosion evident with little vegetation present); 1 = unstable (extensive erosion evident, where bare, steep and sometimes undercut banks are present). The bank stability assessment indicated whether stress originated from abandoned mined land or outside sources e.g. agriculture activities, following the index of Spencer (1998).

***Daphnia magna* 48 hour toxicity test and data analysis**

Daphnia magna organisms which are indigenous to South African waterbodies with an aged of 24 hours or younger were used for the toxicity tests (Day et al., 1999). To obtain the necessary number of young for a test, adult females bearing embryos in their brood pouches were removed from the stock cultures 24 hours prior to the initiation of the test, and placed in beakers containing moderately hard water (Oberholster et al., 2005) and food suspension (trout chow, alfalfa and yeast). Test organisms were transferred to a small intermediate holding beaker and transferred from there to the test beakers. The test was carried out using survey site water and a control containing *D. magna* cultured water (total volume 150 ml^{-1}). A total of 30 organisms per sample were used in the test (1 set of 3 beakers each for 100 % sample concentration, and 1 set of 3 control beakers).

In the case of the *D. magna* 48 hour toxicity tests, the experiments were repeated three times independently and the results recorded in Excel Spreadsheets. The water from a survey site was considered toxic if the given test endpoint measured as % survival was statistically different from those of test organisms (p <0.05), and at least 20 % lower than the mean test organism response in the negative control sample (Thursby et al., 1997). Statistical differences were analysed by computing the Pearson correlation and a *t*-test using the Jandel Scientific Sigma Plot software. A *p* value of <0.05 was considered significant. A correlation of *r* near zero was regarded as unrelated. Benthic chl-*a* concentrations as surrogate for filamentous algae biomass and algal community assemblage were compared with the physical and chemical variables of the surface water (i.e. water column pH and Al and Fe concentrations).

To determine changes in the algal (diatoms and filamentous green algae) community compositions on a spatial scale, the most appropriate univariate and multivariate statistical analyses were used. Univariate analysis, such as diversity and richness indices, was used to describe the algal species-abundance in relations with the software program PRIMER version 6.0 (Clarke and Gorley, 2006). This included the use of the Shannon diversity index (*H'*) (Shannon, 1948). The problem with diversity indices, e.g. the Shannon diversity index, is that it is difficult to interpret differences in the obtained *H'* score as a result that these indices combining different variables (Ludwig and Reynolds, 1988). Therefore, richness (namely the total number of taxa recorded and Margalef's species richness index (d)) was included in this study to compliment the scores obtained from the Shannon diversity index (Margalef, 1951). Multivariate analysis was used to differentiate between the respective survey sites which reflects certain (dis)similarities between each other (Shaw, 2003). Principle Component Analysis (PCA) biplots were constructed to assess spatial trends of the water quality variables at the survey sites. In the PCA plot the arrows of the corresponding variable point in the direction of the steepest increase, whilst the angles between different arrows indicate correlations. If the angle was acute, then there was a positive correlation. Redundancy Analysis (RDA) plots were used to express the results of the diversity and abundance of the different algae as an ordination pattern to reflect certain (dis)similarities between each other in terms of the changes in the algal community structure, with the different water quality parameters overlaid. These plots were derived from PCA plots, but uses the best-fit data that was estimated from multiple linear regressions between each variable and a second matrix of environmental data with the assistance of the software program CANOCO version 4.5 (Ter Braak and Šmilauer, 2002).

Results

Assessing AMD impacts using the EST

In the study, surface waters and substrate quality showed no clear chemical gradient (high metal concentrations and low pH) within the 6.5 km reach of the Tweelopie stream (sites 1 to 3) to its confluence with the Riet stream (Figures 1 and 2). In this reach, metal precipitate deposition remains high with cobbles cemented to each other with ferric hydroxide coating; thickness of coating complex is up to 4-5 mm. The major pattern in benthic chl-*a* mg m^{-2} and diatom species diversity from site 1 to 6 reflected a generalised gradient of disturbance associated with AMD. A very low biomass of epilithic filamentous green algae (chl-*a* >1.2 mg m^{-2}) and diatom diversity were observed in the first 5 km (sites 1 to 3) compared with sites 4 to 6 downstream and the reference site (chl-*a* >19.2 mg m^{-2}). At survey sites 1 and 2, a positive correlation ($p \leq 0.05$; $r = 0.813$) between the low benthic algae (chl-*a* average of 1 mg.m^{-2}) and the low average pH of 2.85 was observed (Figure 2).

Hence, in the water column, average concentration of Al (3.908 $\mu g l^{-1}$) and Fe (195.263 $\mu g\ l^{-1}$) at sites 1 and 2 were high, which correlated negatively ($p \leq 0.05$; $r = -0.975$) with the low benthic chl-*a* of 1 mg m^{-2} measured. A significant positive correlation ($p \leq 0.05$; $r = 0.943$) was observed between the average pH of 6.95 of sampling sites 5 and 6 and the average chl-*a* of 11.25 mg m^{-2} at these sites. Turbidity of the water column at all survey sites was low (NTU <5) in comparison with the reference site (NTU =3). The highest epilithic filamentous algae biomass of chl-*a* of 13.4 mg m^{-2} was measured during low and high flows at site 5. A decrease of epilithic filamentous algae biomass and diatom diversity between sites 5 and 6 were observed in the study period. The thickest layers of hydroxide precipitates were observed at sites 1 to 3, and reduced significantly to site 4, with no substrate deposits present at sites 5 and 6 and the reference site (Table 2; Figure 2). According to the Berger-Parker dominance index the following algal was dominant at each sampling site during high and low flow regims: sites 1-3 the diatom *Stauroneis kriegerii* (Patrick) (0.273; 0.211; 259), and the filamentous green algal *Klebsormidium* sp. (0.252, 0.261, 0.291); site 4 the diatom *Gomphonema insigne* (Gregory) (0.378) and the filamentous green algal *Mougeotia* sp.(0.374); sites 5 and 6 the diatom *Nitzchia linearis* (Agardh) (0.489, 0.421), and the filamentous green algal *Oedogonium* sp. (5.31), while at the reference site the diatom *Navicula cryptotenella* (Lange-Bertalot) (0.455) and the filamentous green algal *Spirogyra* sp. (4.71).

In the *D. magna* bioassay experiment, the negative control had an average specimen survival rate of 98 %, meaning that we considered in the developed EST a score of 20 % less than the negative score as toxic for the tested specimens. The mortality rate of the *D. magna* test specimens at sites 1 to 3 was 100 %, establishing the extreme toxicity of the water column at these sites to the test specimens (Table 2). The high concentration of Al (3.908 µg l^{-1}) at sites 1 and 2 in the water column, correlated negatively ($p \leq 0.05$; $r = -0.984$) with the percentage survival of *D. magna* in the bioassay experiment. The average survival rates for the *D. magna* specimens exposed to the water from sites 4 and 5 was 34 % and 78 % respectively, while the water from site 6 supported a 92 % survival rate. The average percentage survival rate for *D. magna* specimens at sites 1 to 3 was significantly lower (p >0.05) than the reference site (96 % survival; Figure 2). The outcome of the EST indicated that survey sites 1 and 2 were critically modified while survey sites 3 and 4 were seriously modified (Table 3). The EST score of survey sites 5 and 6 were in agreement with the reference site which was categorised as largely natural with few modifications (Table 3).

From Figure 3 it was evident that sampling sites cluster in terms of their water quality. The ordination plot describes 99.4 % of the variation in the data, with 95.3 % described on the first axis and 4.1 % on the second axis. At sampling sites 5 and 6, as well as the reference site, an increase in pH and benthic chl-*a* concentrations can be seen. In contrast to these largely natural sampling sites, survey sites 1 – 4 showed an increase in electrical conductivity, decrease in pH, as well as an increase in Al and Fe concentrations. Hence, the different sampling sites showed distinct water quality signatures according to their degree of impact. This was in agreement with the different ecological categories derived from the application of the EST (Figure 3).

The influence of water quality parameters on algal community structures

At survey sites 1, 2 and 3, a decrease in algal diversity (H'=1.05, H'=1.08 and H'=1.14 respectively) and richness (d=0.35, d=0.35 and d=0.50, respectively) were observed. The opposite was reported for sampling sites 4, 5 and 6 in terms of diversity (H'=2.60, H'=2.37 and H'=2.18 respectively) and richness (d=2.06, d=1.96 and d=1.87, respectively), as well as at the reference site (H'=2.24 and d=1.90) (Figure 4). Based on the RDA triplot (Figure 5), distinct differences can be seen between the respective sites (based on the changes in algal community structures) according to the changes in the degree of impact at the respective sites. This triplot describes 87.2 % of the variation in the data, with 51.2 % described on the first axis and 36 % on the second axis. These multivariate results were in agreement with the results obtained from the univariate analysis, as well as the different ecological categories derived from the application of the EST. For example, the most noticeable decrease in algal diversity (diatoms and filamentous green algae) can be seen at survey sites 1 and 2. This correlates with the most noticeable decrease in water quality measured at these sites (*e.g.*, pH, electrical conductivity and aluminium and iron concentrations). This in turn was in agreement with the EST ecological category determined for these sites, namely critically modified.

Discussion

According to Karr and Chu (1999), AMD studies that focus on water column chemistry alone may fail to recognise detrimental physical disturbance such as flow regimes. Further, chemical analyses of water column chemistry alone may only give a snapshot view of stream conditions, and not indicate the cumulative effects of AMD. To overcome this deficiency, bioindicators such as algae are often utilised because they play a major role as prime producers in the food web and provide an overall indication of stream health (Hogsden and Harding, 2012). Their abundance in aquatic systems, high level of species richness and wide range of ecological tolerance render algae excellent indicators of stream health. In earlier studies, indices were based either on the use of bioindicators only (e.g. Hill, 2000), or on chemical parameters alone (Jarvis and Younger, 2000) to diagnose stream degradation due to AMD. This study focussed on developing a screening tool that combines both biotic and abiotic variables /parameters.

AMD has damaging effects on aquatic ecosystems and in lotic systems, a decrease in pH leads to a decrease in algal species diversity (Verb and Vis, 2000). The decrease in algal species is often related to a variety of factors (e.g. high levels of metals and low pH values) which, in conjunction with metal precipitation impacting the substrate habitat of benthic algae species (Keating et al., 1996). Although previous studies (Muller 1980; Verb and Vis, 2001) indicated an increase in algae biomass which positively correlated with a decrease in pH, the definitive relationship between low pH and biomass increase is not known. Possible links to a decrease in macroinvertebrate grazing pressure or decrease in algal competition and the alteration in the nutrient cycle have been suggested (Parent et al., 1986; Stokes, 1986). Our study shows that there was a longitudinal relationship between increase in water column pH, filamentous algae biomass (benthic chl-*a* mg m^{-2}) and diatom diversity downstream from the AMD source. This result was not unexpected, because the study sites spanned over a wide pH range (2.6 to 7.8). According to O'Halloran et al. (2008) metal concentrations will most likely be secondary to pH effects, particularly Al and Fe as they precipitate out at a pH >3.5 as observed at down stream sites in our study (Figure 2). The small increase in Al concentrations measured (Figure 2) at sampling site 6 and the reference site in comparison to sampling site 5 can possibly be related to naturally higher Al concentrations in the inflowing spring water between sites 5 and 6 an at the reference site. At sites 1 and 2, the low pH, elevated trace metal concentrations and ferric hydroxide precipitates account for the poor algal assemblage with low benthic chl-*a* mg m^{-2} mass. A study conducted by Anthony (1999), showed that algal biomass was low in most of the AMD affected streams (pH >4.5) sampled on the West Coast of New Zealand. This was attributed to precipitates preventing attachment of algae, and precipitate adsorption onto algal cells inhibiting the process of photosynthesis.

Although other authors have found an increase in algal biomass at low pH values, precipitate deposition was minimal or absent at their study sites (Muller, 1980; Niyogi et al. 1999). A study by Niyogi et al. (1999) showed a strong inverse relationship between deposition of metal oxides and algal biomass. They reported a deposition rate in excess of 1.6 gm^{-2} d^{-1} at a stream confluence, but this steadily decreased with distance from the confluence, reaching an average of 0.6 gm^{-2} d^{-1} after 1 km. They further observed that algal biomass was undetectable at high levels of hydroxide deposition, while chl-*a* concentrations reached 80 mg m^{-2} at the lowest levels of ferric hydroxide precipitation. This result is in agreement with findings of this study, where metal precipitates play a major role in reduced algal biomass at sites 1 and 2. The coating of ferric hydroxide on the benthic substrate represents a major stressor on AMD tolerant epilithic filamentous algae. The findings of Niyogi et al. (2002) in New Zealand streams that algal cover and biomass were very low or absent where precipitate deposition was at its highest, are similarly in agreement with those of this study. It was reported that algal biomass was almost 50 % lower at sites with ferric hydroxide deposition than at sites where there was no deposition (Sode, 1983).

Another environmental driver of importance is flow rates especially in cases where the entire substrate is covered in silty material (i.e., sites 1 to 3). The silty substrate in combination with the flow regime can inhibit colonizing of these surfaces when filamentous algae attachment to the silted substrate is overcome by the drag imposed by an increase in the flow regime (Biggs and Smith, 2002). According to Harding and Boothroyed (2004), precipitation of ferric iron as ferric hydroxide becomes visible at a pH >3.5. However in our study a thick coating of ferric iron as ferric hydroxide was observed at a pH of 2.9.

The low water turbidity measured at the survey sites is in agreement with observations by Hogsden and Harding (2012), who found that streams downstream of abandoned mines appear clear because metals remain in solution under highly acidic conditions. The lower epilithic filamentous green algae biomass and diatom diversity at site 6 in comparison to sites 5 can be related to two possible factors. Firstly, the 100 % canopy cover of riparian vegetation at site 6 could reduce the available light for optimal growth of epilithic filamentous green algae and diatoms at this site. Wade (1994) reported that the absence of riparian shade may cause the proliferation of large vascular plants and filamentous algae. Secondly, the higher river flow at site 6 due to the discharge of the second spring (300 $l\ s^{-1}$) could have a negative influence on the epilithic filamentous green algae. According to Clausen and Biggs (1997), increase in flow rates may cause physical disturbance (shear stress) of periphyton and a decrease in biomass. It cannot be ruled out, however, that other chemical and biological factors such as nutrients and grazing might also contribute to a reduction of the epilithic filamentous green algae biomass at site 6. According to Taylor et al. (2007) the diatom *Nitzchia linearis* that was dominant at sampling sites 5 and 6 is a good indicator of circum-neutral, oxygen water of moderated to high electrolyte content, which was in agreement with our water chemistry data of these two sites. Furthermore, the diatom *Gomphonema insigne* that dominated site 4 favours electrolyte-rich water, while *Stauroneis kriegerii* that dominated sites 1-3 is a good indicator for very low pH AMD (Taylor et al., 2007). In this study the later species also showed a strong correlation with Al, Fe and electrical conductivity according to the RDA triplot. The filamentous green algal *Klebsormidium* sp. correlated positive with the high levels electrical conductivity which was in agreement with a previous study of Valento and Gomes (2007). In their study the authors reported that the acidophilic algal *Klebsormidium* sp which occurred at AMD impacted sites favour high levels of electrical conductivity.

Factors that may have played a role in the zero survival rates of the exposed *D. magna* specimens in the bioassay experiments were the elevated levels of Al concentrations measured in the water column of sites 1 and 2. The Criterion Continuous Concentration (CCC), the estimate of the highest concentration to which aquatic communities can be exposed indefinitely without unacceptable effects, is 87 $\mu g\ l^{-1}$ for Al at a pH in the range 6.5 to 9.0 (US-EPA, 1999). This concentration is much lower than the average Al level (3908 $\mu g\ l^{-1}$) measured at survey sites 1 and 2. Another factor is the very low pH level. According to Alibone and Fair (1981), low pH severely depresses the oxygen uptake rates of *D. magna*. Locke (1991) found that a low pH (4 to 5) was lethal to 50 % or more of the *D. magna* tested. The low pH effects survival, longevity, reproduction, sodium flux, heart rate, growth rate, feeding or filtering rate and respiration rate.

The AMD threat in this study area is complex and can have an adverse effect on the whole ecosystem. Recently the dangers of the AMD polluted water in the natural environment was highlighted by (a) the plight of two hippos living in a lake in the Krugersdorp Game Reserve on the western outskirts of the industrial complex, and (b) a fish mortality event in an off-channel storage dam downstream of the Sterkfontein Caves fossil site (Hobbs and Mills, 2011). The intervention by treatment to correct the pH (neutralised) and to remove metals before discharge to the surface water as well as the ecoloxicological screening tool can contribute significantly to the restoration of impacted sites in the game reserve and in peri urban areas down stream of the AMD distage.

Although there were no naturally acidic systems present in the study area it must be taken in count that. the principal characteristics of AMD are determined by site-specific geological and climatic conditions prevailing at each site. Each AMD “occurrence” therefore needs to be evaluated separately at the site where it occurs. A preferable route towards the assessment of AMD effects on the ecosystem would be to first investigate the specific chemical properties of the AMD and the prevailing environmental factors that influence the production of AMD at the specific site of interest. For example, under some South African conditions, the presence of carbonate-rich rocks such as dolomite and calcite can help to raise the pH of AMD water resulting in less acidic water with altered chemical composition, although still with high TDS values (Harrison, 1958). Therefore before employing the ecoloxicological screening tool in other AMD impacted areas, a detailed investigation to prioritise chemical hazards must be undertaken in the area or catchment that is influenced by AMD.

Conclusion

The ecological screening tool (EST) described in this study was successful in gauging the relative adverse impacts of AMD on the receiving watercourses. The biotic component of the EST established the adverse effect of ferric hydroxide precipitation and low pH values on the filamentous algae biomass and diatom diversity at survey sites 1 and 2 closest to the AMD source. The adverse effect of AMD similarly accounted for the low survival rate of *D. magna* in the laboratory toxicity tests. As a consequence, survey sites 1 to 3 were categorised as seriously to critically modified, and identified as priority candidates for future restoration of their respective stream reaches.

Acknowledgements

The authors express their sincere gratitude to the Council for Scientific and Industrial Research for provision of funding. The authors also want to thank the unknown referees for critically reviewing the manuscript and suggesting useful changes.

Reference

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.

Alibone M.R., Fair P., 1981. The effect of low pH on the respiration of *Daphnia magna* Straus. Hydrobiologia 85, 188-188.

Anthony M.K., 1999. Ecology of streams contaminated by acid mine drainage near Reefton, South Island. MSc thesis. University of Canterbury, Christchurch.

Archibald, C.G., Taylor, J.C., 2007. The assessment of diffuse pollution from acid-mine drainage using an updated and revised diatom assessment procedure as an added value bio-monitoring tool. Water Science and Technology 55, 151-160.

Berger W .H., Parker F.L., 1970. Diversity of planktonic *Foraminifera* in deep sea sediments. Science 168, 1345-1347.

Biggs B.J.F., Smith R.A., 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnology and Oceanography 47, 1175-1186.

Bray, J.P., 2007. The ecology of algal assemblages across a gradient of acid mine drainage stress on the West Coast, South Island, New Zealand. MSc. Thesis, University of Canterbury, Christchurch.

Bray, J.P., Broady, P.A., Niyogi, D.K., Harding J.S., 2008. Periphyton communities in New Zealand streams impacted by acid mine drainage. Marine and Freshwater Research 59, 1084-1091.

Clarke, K.R., Gorley, R.N., 2006. Primer v6: User Manual or Tutorial. PRIMER-E, Plymouth.

Clausen, B, Biggs, B.J.F., 1997. Relationships between benthic biota and hydrological indices in New Zealand streams. Freshwater Biology 38, 327-342.

Day, J.A., Stewart, B.A., De Moor, I.J., Louw, A.E., 1999. Guides to the freshwater invertebrates of southern Africa. Volume 2: Crustacea 1. Notostraca, Anostraca, Conchostraca and Cladocera. WRC Report, No. TT 121/00 Water Research Commission, Pretoria, South Africa.

DeNicola, D.M., Stapleton M.G., 2002. Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. Environmental Pollution 119, 303-315.

Douglas, B., 1958. The ecology of the attached diatoms and other algae in a small stony stream. Journal of. Ecology. 46, 295-322.

DWAF, 2010. Mine water management in the Witwatersrand Gold Fields with special emphasis on acid mine drainage. Report to the Inter-Ministerial Committee on Acid Mine Drainage, Department of Water Affairs, Pretoria, South Africa.

DWAF, 1996. South African Water Quality Guidelines (Second edition). Volume 1. Department of Water Affairs. Pretoria, South Africa.

Gray, N.F., 1997. Environmental impact and remediation of acid mine drainage: a management problem. Environmental Geology 27, 358-361.

Gray, N.F., 1999. Field assessment of acid mine drainage contamination in surface and ground water. Environmental Geology 27, 358-361.

Harding, J.S., Boothroyd I., 2004. Impacts of mining. In: Freshwaters of New Zealand. Eds. Harding, J., Mosley, P., Pearson, C., Sorrell, B. New Zealand Hydrological Society and New Zealand Limnological Society.

Harrison, A.D., 1958. The effects of sulphuric acid pollution on the biology of streams in the Transvaal, South Africa. Verhandlungen der Internationale Vereinigung der Limnologie, 13, 603-610.

Hauer, F.R., Lamberti, G.A. 2006. Methods in stream ecology. Elsevier, Academic Press, Amsterdam, pp.357-377.

Hering, D.., Johnson, R.K., Kramm, S., Schmutz, S., Szoszkiewicz, K., Verdonschot, P.F.M., 2006. Assessment of European streams with diatoms, macrophytes, macroinvertebrates, and fish: a comparative metricbased analysis of organism response to stress. Freshwater Biology 51, 1757-1785.

Hill, B.H., Herlihy A.T., Kaufmann P.R., Stevenson R.J., McCormick F.H., Johnson C.B., 2000. Use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Benthological Society 19, 50-67.

Hobbs, P.J., Cobbing, J.E. 2007. Hydrogeological assessment of acid mine drainage impacts in the West Rand Basin, Gauteng Province. Report no. CSIR/NRE/WR/ER/2007/0097/C, CSIR/THRIP, Pretoria, South Africa, pp. 1-109.

Hobbs, P.J., Mills, P.J., 2011. The Koelenhof Farm fish mortality event of mid-January 2011. Report prepared for the Management Authority. Department of Economic Development. Gauteng Province. South Africa, pp. 1- 19.

Hogsden, K.L., Harding J.S., 2012. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. Freshwater Science 31, 108-120.

Jarvis, A.P., Young, P.L., 2000. Broadening the scope of mine water environmental impact assessment: a UK perspective. Environmental Impact Assessment Review 20, 85-96.

Johnson, R.K., Hering, D., Furse, M.T., Clarke, R.T., 2006. Detection of ecological change using multiple organism groups: metrics and uncertainty. Hydrobiologia 566, 115-137.

Kalff, J., 2002. Limnology: Inland water ecosystems. Upper Saddle River, New Jersey, USA: Prentice-Hall Inc; pp.1-592.

Karr, J.R., Chu, E.W., 1999. Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington DC, pp. 1-206.

Keating, S.T., Clements, C.M., Ostrowski, D., Hanlon, T., 1996. Disinfectant properties of acid mine drainage: Its effects on eneteric bacteria in a sewage-contaminated stream. Journal of Freshwater Ecology 11, 271-282

Krige, G., van Biljon, M., 2006. The impact of mining on the water resources and water-based ecosystems of the Cradle of Humankind World Heritage Site. Paper presented at the Mine Water Symposium, Geological Society of South Africa, Johannesburg

Locke, A., 1991. Zooplankton responses to acidification: a review of laboratory studies. Water, Air, & Soil Pollution 60, 135-148.

Ludwig J.A., Reynolds J.F., 1988. Statistical ecology. John Wiley and Sons, New York.

Margalef R., 1951. Diversidad de especies en las comunidades naturales. Publicaciones del Instituto de Biologia Aplicada de., Barcelona. 6, 59-72.

Muller, P., 1980. Effects of artificial acidification on the growth of periphyton. Canadian Journal of Fisheries and Aquatic Sciences. 37, 355-363.

Niyogi, D.K., Lewis W.M., McKnight D.M., 2002. Effects of stress from mine drainage on diversity, biomass and function of primary producers in mountain streams. Ecosystems 5, 554-567.

Niyogi, D.K., McKnight, D.M., Lewis W.M., 1999. Influences of water and substrate quality for periphyton in a montane stream affected by acid mine drainage. Limnology and Oceanography 44, 804-809.

Oberholster, P.J., 2011. Using epilithic filamentous green algae communities as indicators of water quality in the headwaters of three South African river systems during high and medium flow periods. In: Kattel, G, editor. Zooplankton and Phytoplankton. Chapter 5. USA: Nova Science Publishers Inc; pp. 107-122.

Oberholster, P.J., Botha, A-M., Cloete, T.E., 2005. Using a battery of bioassays, benthic phytoplankton and the AUSRIVAS method to monitor long-term coal tar contaminated sediment in the Cache la Poudre River, Colorado. Water Research 39, 4913-4924.

Oberholster, P.J., Dabrowski, J.M., Ashton, P.J., Aneck-Hahn, N.H., Booyse, D., Botha, A-M., 2010. Risk assessment of pollution in surface waters of the upper Olifants River system: Implications for aquatic ecosystem health and the health of human users of water. Report number: CSIR/NRE/WR/ER/2010/0025/B; pp.1-163.

O`Halloran, K., Cavanagh, J., Harding, J.S., 2008. Response of a New Zealand mayfly (*Deleatidium* spp.) to acid mine drainage: implications for mine remediation. Environmental Toxicology and Chemistry 27, 1135-1140.

Parent, L., Allard, M., Planas, D., Moreau, G., 1986. The effects of short-term and continuous experimental acidification on biomass and productivity of running water periphytic algae. in B.G. Isom and J.M. Bates (eds.), Impact of acid Rain and Deposition on Aquatic Biological Systems, American Society for Testing and Materials, Philadelphia, Philadelphia, PA, U.S.A., pp. 688

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficient and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. Biochimica et Biophysica Acta 975, 384-394.

Shannon, C.E., 1948. A Mathematical Theory of Communication. The Bell System Technical Journal 27, 379-423, 623-656.

Shaw, P.J.A., 2003. Multivariate statistics for environmental science. Arnold Publishers. London.

Sheath, R.G., Burkholder J.M., 1985. Characteristics of softwater streams in Rhode Island. II. Composition and seasonal dynamics of macroalgal communities. Hydrobiologia 128, 109-118.

Sheath, R.G., Cole, K.M., 1992. Biogeography of stream macroalgae in North America. Journal of Phycology 28, 448-460.

Sode, A., 1983. Effect of ferric hydroxide on algae and oxygen consumption by sediment in a Danish stream. Archiv für Hydrobiologie Supplement 65, 134-162.

Spencer, C., Robertson, A.I., Curtis, A., 1998. Development and testing of a rapid appraisal wetland condition index in South-eastern Australia. Journal of Environmental Management 54, 143-159

Stevenson, R.J., Bahls, L.L., 1999. Periphyton protocols. In: Barbour, M.T., Gerritren, J., Snyder, B.D. & Stribling, J.B., editors. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish. 2nd ed. Washington DC: USA EPA 841-B-99-002. United States Environmental Protection Agency; pp. 6-22.

Stevenson, R.J., Pan, Pan Y., 1999. Assessing environmental conditions in rivers and streams with diatoms, In: Stoermer, E.F., Smol, P.J. (Eds.), The Diatoms: Applications for the Environmental and Earth Science, Cambridge University Press, New York, NY, U.S.A., pp. 11-40.

Stevenson, R.J., White, K.D., 1995. A comparison of natural and human determinants of phytoplankton communities in the Kentucky River Basin, USA. Hydrobiologia 297: 201-216.

Stokes, P.M., 1986. Ecological effect of acidification on primary producers in aquatic ecoystems. Water, Air, & Soil Pollution 30, 421-438

Taylor, J.C., Harding, W.R., Archibald, C.G.M., 2007. An illustrated guide to some common diatom species from South Africa. WRC Report, No. TT 282/07. Water Research Commission, Pretoria, South Africa, plates 1-178.

Ter Braak C.J.F., Šmilauer P., 2002. CANOCO reference manual and Canodraw for Windows user's guide: Software for canonical community ordination (Version 4.5). Microcomputer Power, New York, USA.

Thursby, G.B., Heltshe, J., Scott, K.J., 1997. Revised approach to toxicity test acceptability criteria using a statistical performance assessment. Environmental Toxicology and Chemistry 16, 1322–1329.

US EPA., 1999. National recommended water quality criteria-correction. EPA 822-Z-99-001, Office of Water, Washington, DC.

US. Geological survey mineral year book. 2005. The mineral industry of South Africa, p 36.1.

Valente, T.M., Gomes, C.L., 2007. The role of two acidophilic algae as ecological indicators of acid mine drainage sites. Journal of Iberian Geology 33, 283-294.

Van Ho, A., Ward, D.M., Kaplan, J., 2002. Transition metal transport in yeast. Annual Review of Microbiology 56, 237-261.

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.

Verb, R.G., Vis, M.L., 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and non-impacted sites. Journal of the North American Benthological Society 19, 274-288.

Verb, R.G., Vis, M.L., 2001. Macroalgal communities from an acid mine drainage impacted watershed. Aquatic Botany 71, 93-107.

Verb, R.G., Vis, M.L., 2005. Periphyton assemblages as bioindicators of mine-drainage in unglaciated Western Allegheny Plateau lotic systems. Water, Air, & Soil Pollution 161, 227-265.

Wade, P.M., 1994. Management of macrophytic vegetation. The River Handbook. Hydrological and Ecological Principles. Volume 2. (Eds P. Calow and G.E. Petts). Blackwell Scientific Publications, Oxford.

Zajack, J.T., Smucker, N.T., Vis M.L., 2010. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. Ecological Indicators 10, 287-295.