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

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Tables
Figures
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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\textsuperscript{-2}), diatoms and filamentous green algae community structures were employed as bioindicators as well as \textit{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 (≥ 4.0) precipitation of metal hydroxides (e.g. ferric hydroxides (FeOH₃) 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 diatoms were 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-α 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 sedimentoined 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
The samples were placed on ice and transported to the laboratory in cooler boxes for analysis of chlorophyll $a$ ($\text{chl}-a$) 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 µg l$^{-1}$) and Fe (195.263 µg 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 kriegeri* 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 µg 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 µg l$^{-1}$)
measured at survey sites 1 and 2. Another factor is the very low pH level. According to Alibone
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.

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27


