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Influence of oxidation on leaf decomposition in acid mine water

Identification

Student's name:	Bongani Peter Mohasoa
Student number:	1222496
Course:	Masters Environmental Science
Date:	September 2017
Supervisor:	Dr. Darragh Woodford
Co-Supervisor:	Dr. Gavin Snow

DECLARATION

I declare this dissertation is my own, unaided work. It is being submitted for the degree of Master of Environmental Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of Candidate)

Signed at Johannesburg on 9 September 2017.

ABSTRACT

Acidification of freshwater systems by Acid Mine Drainage (AMD) is a persistent risk to aquatic ecosystems in South Africa, particularly in Gauteng and Mpumalanga. From several studies that have been conducted, it is clear that AMD has profound effects on aquatic life and functionality of the ecosystem. One of the ecosystem processes affected by AMD is the decomposition process. It is has been established that AMD-affected streams inhibit the decomposition rate of organic matter. The present study investigated the effect of AMD in the presence of oxygen on organic matter decomposition. The objectives of the study were to compare the decomposition rates of organic matter in AMD oxygen rich and deprived conditions to those found in AMD-free oxygen rich and deprived conditions. A laboratory experiment was carried out over a period of six weeks consisting of four treatments, namely; AMD-free oxygen, AMD oxygen, AMD-free nitrogen and AMD nitrogen. The significance of time effect on overall mass loss was tested using one-way ANOVAs, the results showed that time was not a significant ($p > 0.05$) co-variate of mass loss. Pearson's correlations showed there was no correlation between physico-chemical variables (SO_4, Fe^{2+}, pH, DO, EC and Temperature) of each treatment and time. Factorial ANOVA showed a significant effect ($p < 0.05$) in the interaction between O_2 and AMD treatments but not in the independent effects of O_2 or AMD. Post hoc Tukey HSD test showed that mass loss in organic matter was greater in AMD-free O_2 than AMD O_2. The conclusion was that the decomposition rate of organic matter in AMD-free O_2 was accelerated potentially due to increase in microbial and fungi activities as oxygen increased. In AMD O_2, however, decomposition rate of organic matter was inhibited potentially due to reduced microbial and fungi activities as increased oxygen sustained the acidity. This implies that the organic matter decomposition will be less in AMD contaminated streams and lesser still in AMD contaminated streams rich in oxygen content.

Key-words: anoxic, leaf decomposition, organic matter, oxic, water quality.

Introduction

South Africa is considered to have the world's largest mineral endowment (Carroll, 2012), the mining industry has played a principal role in the country's socio-economic development over years and continues to be the most important earner of foreign exchange for the economy (Fedderke and Pirouz, 2002). Despite the impressive economic outputs, the sector also carries several environmental challenges including generation of acid mine water, the most significant and expensive environmental pollution in mining. According to DWA (2010), the closure of mining operations and the subsequent cessation of underground mine water pumping led to flooding of mine voids and eventual overflow of acid mine water onto the surface. AMD is not only a legacy matter, groundwater contamination has been noticed from active mines (Roux, 2011; Vermeulen *et al.,* 2011).

AMD is generally characterized by high concentrations of dissolved iron, aluminium, manganese and sulfate and pH can drop below 2.5 (Campaner *et al.,* 2014). AMD occurs when mineral pyrite (commonly found in coal and gold deposits) comes into contact with oxygenated water. Pyrite undergoes oxidation in two phases; the first phase generates sulfuric acid and ferrous sulfate, and the second phase generates orange-red iron (III) oxide-hydroxide and additional sulfuric acid (McCarthy, 2011).

Tutu *et al.* (2008) stated that the gold tailing dumps in the Witwatersrand gold fields are a major contributor of AMD formation and heavy metal pollution. AMD is decanting at an estimated volume of 20 ML/day into the Tweeloopies Spruit near Randfontein (Western Basin). It was estimated that a further 60 ML/day would decant near Boksburg (Central Basin) by 2013 and an additional 120 ML/day near Springs (Eastern Basin) by 2014 (Bologo *et al.,* 2012). The mines near Westonaria and Carletonville generate 160 ML/day and the coal mines in Mpumalanga generate a further 200 ML/day (Bologo *et al.,* 2012). According to Grobbelaar *et al.* (2004), 360 ML/day may be generated after closure of the entire Mpumalanga Coalfields.

From a number of studies that have been conducted, it is evident that AMD is a multi-factor pollutant. When released into water bodies, it pollutes drinking water and may render available water resources unfit for other users such as agriculture (Warhusrt and Noronha, 2000; Sarnoski, 2002; Garrido *et al.,* 2009; Ochieng *et al.,* 2010). Kimmel (1983) indicated that acid water is toxic to most aquatic organisms, thus contamination by AMD can cause loss of aquatic life. Fish is one of the aquatic organisms threatened by AMD; numerous studies have shown that fish are severely impacted at pH 4.5 to 5.5 (Cooper and Wagner, 1973; Barry *et al.,* 2000; Farag *et al.,* 2003), further investigations revealed that in most streams there is complete loss of fish below pH 4.5 (Cooper and Wagner, 1973). Acidification of groundwater is another great concern, as many people and animals rely on its availability for survival. Moreno *et al.* (2001) stated that AMD poses a threat to humans as it carries heavy metal contaminants that tend to accumulate in the tissues and organs and therefore causes diseases. Ingestion of AMD polluted water may lead to inhibited growth, birth defects, skin lesions, lower reproduction rates and increased risk to cancer (Lewis and Clark, 1996; Alder and Rascher, 2007).

On the subject of the impacts of acidification to stream ecosystem, most studies have concentrated on analysing the pH tolerance of particular taxa (Hamalainen and Huttunen, 1996; Davy-Bowker *et al.,* 2003). The response of macro-invertebrate communities is a well-documented field of research (Sutcliffe and Carrick, 1973; Sutcliffe and Hildrew, 1989; Peterson and Eeckhaute, 1992; Tixier and Guerold, 2005). There are also several studies on impacts of stream acidification on organic matter decomposition and implications to functionality of the ecosystem. Decomposition rate is a function of water chemistry (Carlisle and Clements, 2005; Bergfur *et al.,* 2007) and water temperature (Webster and Benfield, 1986). Research conducted by (Carpenter *et al.,* 1983; Mulholland *et al.,* 1987; Dangles *et al.,* 2004; Jenkins *et al.,* 2013) revealed that decomposition rate of organic matter is inhibited in acidified streams and thus mass loss is very low. In contrast, an unpublished laboratory experiment by (Ms K Olsen 2016, pers. comm.) indicated that organic matter in AMD had a net gain in mass instead of losing it, this was attributed to coating of the decomposing material by iron precipitate which formed a secondary layer.

The impacts of AMD-affected streams on organic matter decomposition has indirect effects on the functionality of an ecosystem. Decomposition is considered to be one of the most important ecosystem processes, which enables nutrient cycling by converting organic matter to stable forms and is a pathway for energy flow (Karavin *et al.,* 2016). Therefore, when organic matter decomposition is impaired by acidification, nutrient availability becomes limited and thereby sinking secondary production (Krueger and Waters, 1983; Griffith and Perry, 1994; Pretty *et al.,* 2005; Woodward *et al.,* 2005). This is one of the reasons AMD contamination is undesirable.

Oxygen also influences the decomposition rate as it supports microbial activity and respiration (Amon and Benner, 1996). Under aerobic condition, the decomposition rate of organic matter occurs at a faster rate when compared to anaerobic conditions (Couteaux *et al.,* 1995). Within the AMD context, extended exposure to oxygen stimulates the generation of additional sulfuric acid and the precipitation of ions (second phase). Thus, AMD in the presence of high oxygen content could further aggravate the effects of AMD on decomposition rate as it increases the precipitation rate of ions that coat the leaves (Johnson and Hallberg, 2005). It is now established that AMD-affected streams suppress organic matter decomposition; however, the impact of the presence of oxygen in AMD-affected streams on organic matter decomposition is not well documented. It is expected that exposure to oxygen will accelerate the decomposition rate except in the presence of AMD.

Kepenyes *et al.* (1983) indicated that oxygen comes into water through diffusion process and surface-water agitation resulting from turbulence of fast-moving water which propels mixing of atmospheric air and water. In an artificial setup, surface-water agitation is usually through aeration by compressed air diffusers. According to Murphy (2008), fast-moving streams generally have high dissolved oxygen (DO) concertation than slow-moving waters. Given the influence of oxidation on AMD, it expected that fast-moving streams with high DO will stimulate generation of additional sulfuric acid and precipitation of ions (AMD second phase) and thus decelerating decomposition rate of organic matter.

Microorganisms (fungi and bacteria) also play a significant role in organic matter decomposition. A group of fungal decomposers, the aquatic hyphomycetes, is generally accepted to be more important in the early stages of decomposition

(Thompson and Barlocher, 1989). However, at pH 4 and 3 fungi are almost completely absent (McKinley and Vestal, 1982). According to (Hendrey *et al.,* 1976; Minshall and Minshall, *1978*; Rao *et al.,* 1984; Traaen, 1980), microbial numbers associated with decomposing organic matter are minimal at pH below 5. Therefore, with reduced fungi and bacteria activity, the decomposition rate is expected to decelerate in acidified streams.

This research tested the hypothesis that the decomposition rate of organic matter in AMD oxygen rich conditions will be higher than oxygen deprived conditions but significantly lower than AMD-free treatments. A study by (Ms. K Nyembe 2015, pers.comm.) examined the effects of synthetic AMD on leaf decomposition under oxic and anoxic conditions over a period of four weeks. The results of the study were inconclusive as there was no variation between the various treatments. The study attributed the inconsistencies to the continuous precipitation and dissolving of iron compounds, due to the use of synthesised AMD that was unbuffered and difficult to maintain at a stable pH. This research advanced the previous study by using genuine coal mine AMD from the Olifants River catchment, Mpumalanga, and also extended the time period to six weeks. The approach of using real AMD allowed minimal changes in pH simulating the natural process, and the extended time period allowed adequate time for the breakdown of organic matter. This enabled a clear comparison of decomposition rates of organic matter in AMD oxygen rich and deprived conditions to those found in AMD-free oxygen rich and deprived conditions.

Materials and Methods

Experimental design and protocol

The study was conducted in the Aquatics Laboratory, Oppenheimer Life Science Building at the University of Witwatersrand. Figure 1 illustrates the experimental design. Coal mine AMD was provided by the University of Witwatersrand School of Chemical Engineering which had sourced it from the Olifants River catchment. AMD-free water was also collected from the upper Olifants River so that water can have analogous natural geochemistry. Wild guava *(Psidium guajava)* leaves were collected from trees along the riparian zone of the Blyde River, an Olifants River tributary. These leaves were specifically preferred as they were large, fairly uniform in size, and thus making the experiment a reasonable analogue of local ecological processes. Four 85 L containers were grouped into two pairs. In the first pair, containers were filled with AMD, one was sealed and purged with nitrogen gas five minutes before lifting the container lid and after placing back the container lid to minimize contact with atmospheric oxygen when recovering samples, and one was oxygenated using an air pipe connected to air stones.

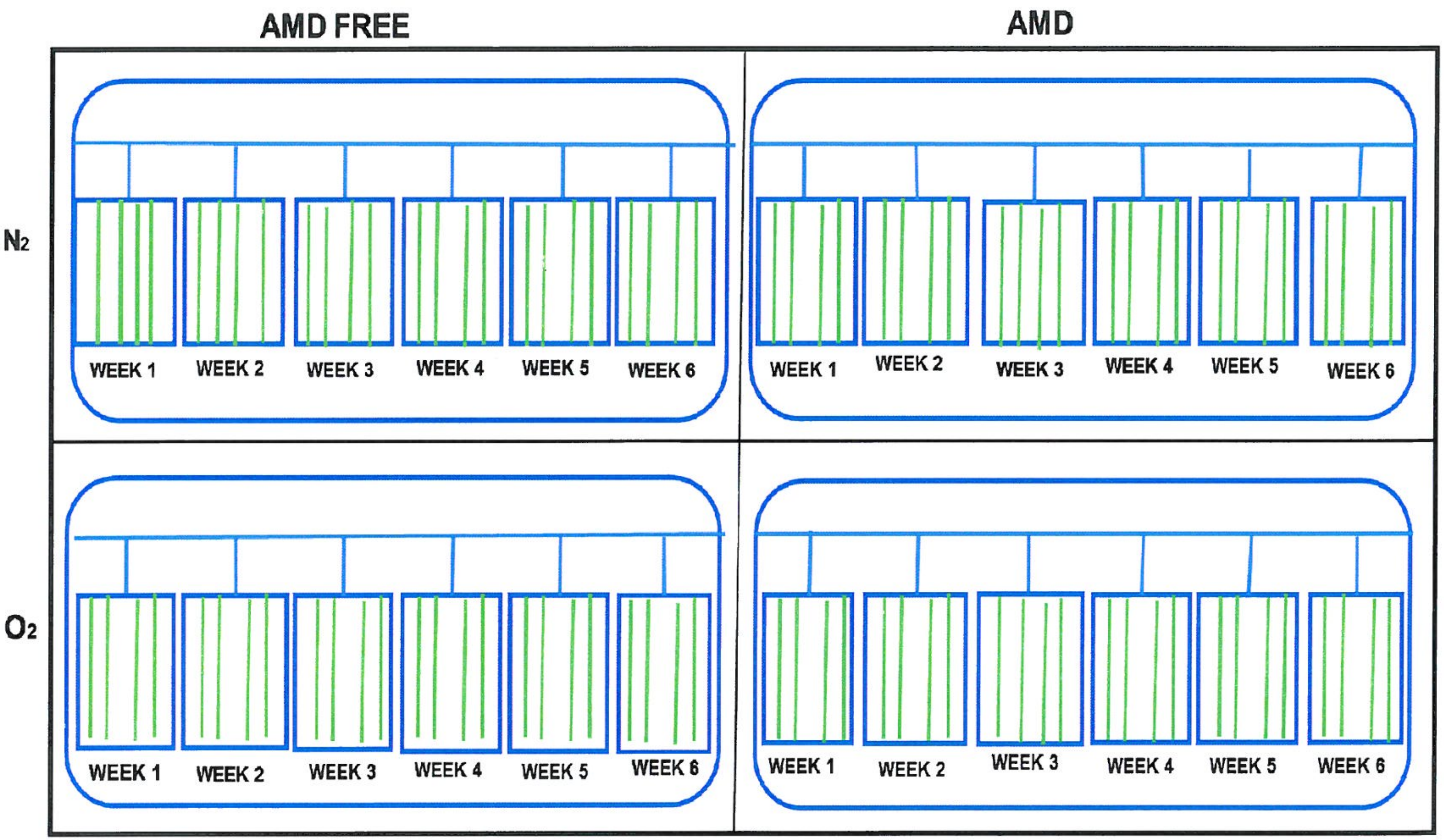

Figure 1: Illustration of the experimental design. Each vertical green line represents a replicate leaf in a bag, suspended in the treatment water.

In the second pair, containers were filled with AMD-free water, one was sealed and purged with nitrogen gas five minutes before lifting the container lid and after placing back the container lid to minimize contact with atmospheric oxygen when recovering samples, and one was oxygenated using an air pipe connected to air stones.

Leaf decomposition

Undamaged wild guava leaf samples collected from the Blyde River were dried at 40 °C in a drying oven two weeks prior to the experiment, weighed (g) and enclosed into 96 mesh bags (85mm x 170mm, 1.295g), one leaf per bag. The bags were incubated in each treatment of the four containers, 24 bags per treatment. The bags were secured in the treatments with plastic-coated wires knotted to suspended rods in the containers. Six replicate bags were recovered from each treatment on a weekly a basis for a period of six weeks. The bags were oven dried for 24 hours at 50 °C, the leaves were removed from the bags then weighed to obtain net mass loss (g).

Water chemistry

The physico-chemical variables; DO (mg/L), temperature (°C), electrical conductivity (EC) (µS/m) and pH of each treatment were measured twice a week over the course of the experiment using a YSI Professional Plus Multiparameter Water Quality Meter (Xylem Inc., New York, USA). Concentrations of sulfate ions (SO_4^{2-}) (mg/L) and ferrous ions (Fe^{2+}) (mg/L) were measured with a HACH Colorimeter (Bitang Instrument, Jakarta, Indonesia) using the SulfaVer 4 (USEPA accepted for reporting wastewater analysis) and FerroVer (USEPA approved for reporting water analysis) methods respectively.

Data analyses

Statistical analyses was performed using Statistica version 12 software, an analytics package for multivariate statistics. All mass loss data were log (x+1) transformed in order to meet statistical assumptions that ANOVA data follows normal distribution. Pearson's correlations were run to measure the strength of the relationship between physico-chemical variables and time across all treatments, using significance level of 0.05.

Shapiro-Wilk tests were run to test for normality of the variables before using Person's correlation. The effect of time as a covariate to the experiment was assessed using One-way ANOVAs. Factorial ANOVA was conducted on all treatment replicates to test for independent effects of O_2 and AMD, and their interactive effects on leaf mass loss. Finally, Post hoc Tukey Honest Significant Difference (HSD) tests were carried out to assess the differences among means of the treatments to provide specific information on which treatments were significantly different from each other.

Results

Water Chemistry

Table 1 provides descriptive statistics for the physico-chemical variables of the treatments. Pearson's correlation provided an indication of the strength of association between the individual physico-chemical variables and time, which then provided an understanding of factors that may have contributed to mass loss. The output showed there were no correlations between any of the physico-chemical variables (across all treatments) and time, ($p > 0.05$). This indicates that time of measurement during the experiment did not have a statistically significant effect on the key physico-chemical variables of the experimental system. Temperature appears to have remained constant throughout the experiment between the four treatments. However, Pearson's correlation showed a significant correlation ($p < 0.05$) between temperature and DO. This can be attributed to temperature variation between day and night. In the AMD Free O_2 treatment, a negative correlation between DO and temperature indicated that dissolution of DO increased as temperature decreased ($r = -0.6510$, $p = 0.012$). In the AMD O_2 treatment, a negative correlation between DO and temperature indicated that dissolution of DO increased as temperature decreased ($r = -0.5854$, $p = 0.028$). In the AMD Free N_2 treatment, the negative correlation between pH and EC indicated that as pH increased EC decreased ($r = -0.595$ $p = 0.025$). Again, in the AMD Free N_2 treatment, the negative correlation between temperature and pH indicated that as temperature decreased, pH increased ($r = -0.542$, $p = 0.045$). In the AMD N_2 treatment, a strong correlation between EC and temperature indicated an increase in EC as temperature increased ($r = 0.865$, $p = 0.001$).

Ion Precipitation

In AMD O_2 treatment in the fifth and sixth week precipitation of insoluable ferrous hydroxide was noticed on the leaves.

Table 1: Descriptive statistics of physico-chemical variables of the four diferent treatments (mean and standard deviation).

Treatment	AMD-free O2		AMD O_2		AMD-free N_2		AMD N_2	
Variables	M	SD	M	SD	M	SD	M	SD
SO_4	0.000	0.000	74.643	3.795	0.000	0.000	80.000	0.000
Fe^{2+}	0.000	0.000	3.300	0.000	0.000	0.000	3.257	0.581
pH	6.743	0.690	2.739	0.107	6.263	0.626	2.659	0.206
DO	5.361	0.796	5.411	1.016	3.241	1.086	3.533	0.659
EC	812.071	95.586	4838.5	215.24	815.143	67.233	4796.357	137.94
Temperature	19.478	1.451	19.693	1.445	19.457	1.649	19.579	0.683

Notes: The concentrations of treatment variables pH and EC in AMD-free samples fall within the freshwater catergory as defined in the South African Water Quality Standards (DWAF, 1996), EC < 1,000mg/L and pH > 3.0.

Mass loss over time

One-way ANOVAs showed that time effect on the overall mass loss in each treatment was not significant ($p > 0.05$), therefore it was concluded that time is not a covariate of mass loss and that a factorial block design could be used to compare all replicate leaves. The AMD-free O_2 treatment had the greatest mass loss reaching a peak of 0.2 g (18.71%) (Figure 2 and Annexure A). In the other treatments there was a fluctuation (increase and decrease) in mass loss over time. AMD O_2 had the lowest mass loss as the highest peak was at 0.075 g; whereas, in other treatments mass loss exceeded 0.075 g repeatedly.

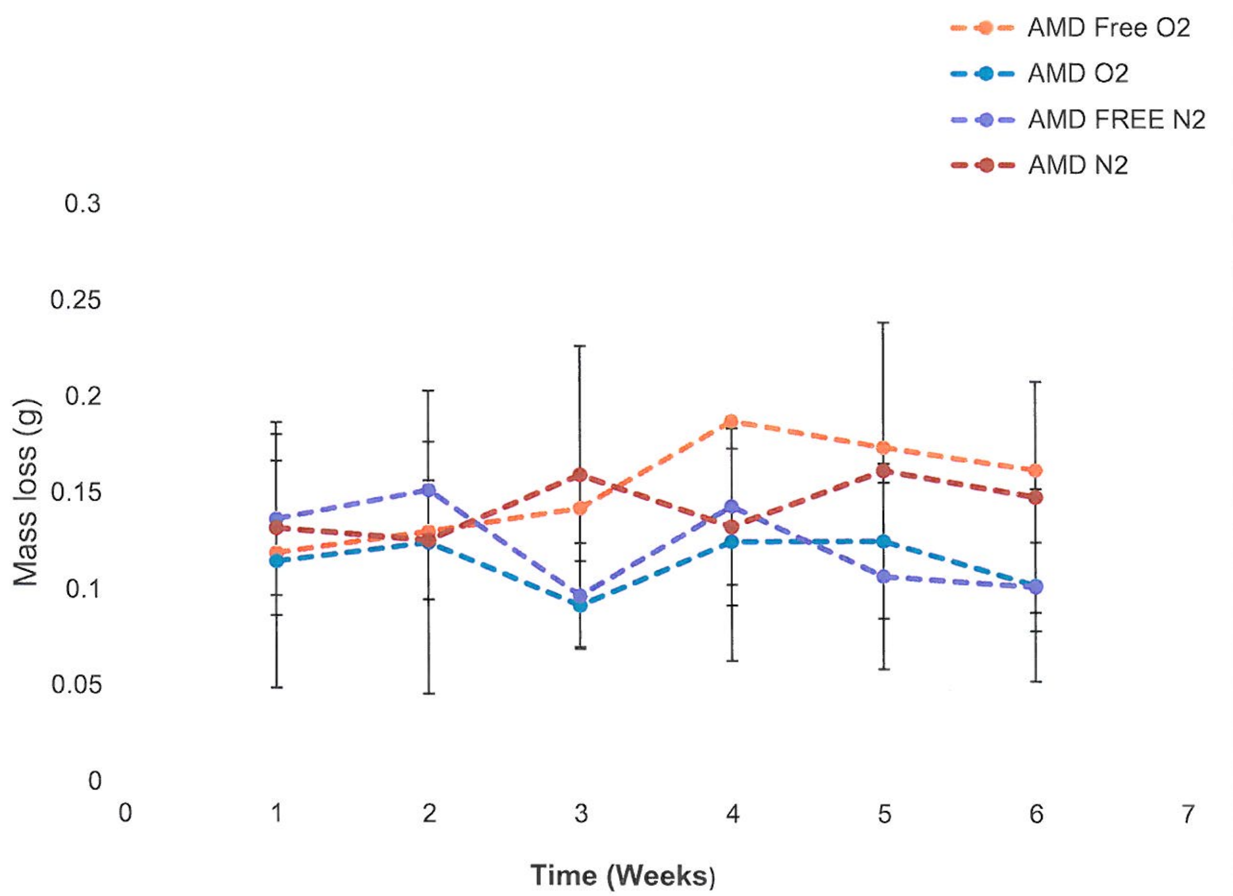

Figure 2: Mean (± standard deviation) mass loss of leaves over time among the four treatments

Examining mass loss in different treatments

Leaf starting weights did not differ significantly among weekly removal treatments (one way ANOVA: $F = 0.909$, $df = 5.90$, $p = 0.408$), thus weight loss in grams was an unbiased measure of decomposition over time. Factorial ANOVA showed that the independent effects (O_2, N_2, AMD and AMD-free) had no significant effect on mass loss; however, the interaction effect (O_2 and AMD) was significant on mass loss (Table 2). Post hoc Tukey HSD test showed that there was a statistically significant difference in mass loss between AMD Free O_2 treatment and AMD O_2 treatment ($p < 0.05$), as well as between AMD Free O_2 treatment and AMD Free N_2 ($p < 0.05$). The Post hoc Tukey HSD test revealed that AMD O_2 treatment lost significantly less mass than AMD Free O_2 treatment.

Table 2: Factorial Anova univariate test for significance in combination of treatments

Effects	df	F	p
Intercept	1	400.592	0.001
O_2	1	0.004	0.950
AMD	1	2.104	0.150
O_2*AMD	1	13.915	0.001
Error	92		

Table 3: Tukey HSD test for significance in the mean differences between the four treatments.

TREATMENT (GROUP A)		TREATMENT (GROUP B)		Mean Difference (A-B)	Standard Error	Significance
Cell No.	Treatment	Cell No.	Treatment			
1	AMD-free O_2	2	AMD O_2	-2.237*	0.103	0.002
		3	AMD-free N_2	-2.146*	0.103	0.042
		4	AMD N_2	-1.997	0.103	0.709
2	AMD O_2	1	AMD-free O_2	-1.898*	0.103	0.002
		3	AMD-free N_2	-2.146	0.103	0.761
		4	AMD N_2	-1.997	0.103	0.053
3	AMD-free N_2	1	AMD-free O_2	-1.898*	0.103	0.042
		2	AMD O_2	-2.237	0.103	0.761
		4	AMD N_2	-1.997	0.103	0.377
4	AMD N_2	1	AMD-free O_2	-1.898	0.103	0.709
		2	AMD O_2	-2.237	0.103	0.053
		3	AMD-free N_2	-2.146	0.103	0.377

* The mean difference is significant at level 0.05

Discussion

This study sought to determine the effects of AMD and how the availability of oxygen associated with AMD could affect the decomposition process. The experiment was designed to test the decomposotion rates of organic matter in AMD oxygen rich and deprived conditions and compare the decompositon rates to those found in AMD-free oxygen rich and deprived conditions. The results as illustrated by Figure 3 showed that the experiment had two separate processes working in opposition to each other, thus the interaction effect (O_2 and AMD) was significant on mass loss in organic matter ($p < 0.01$).

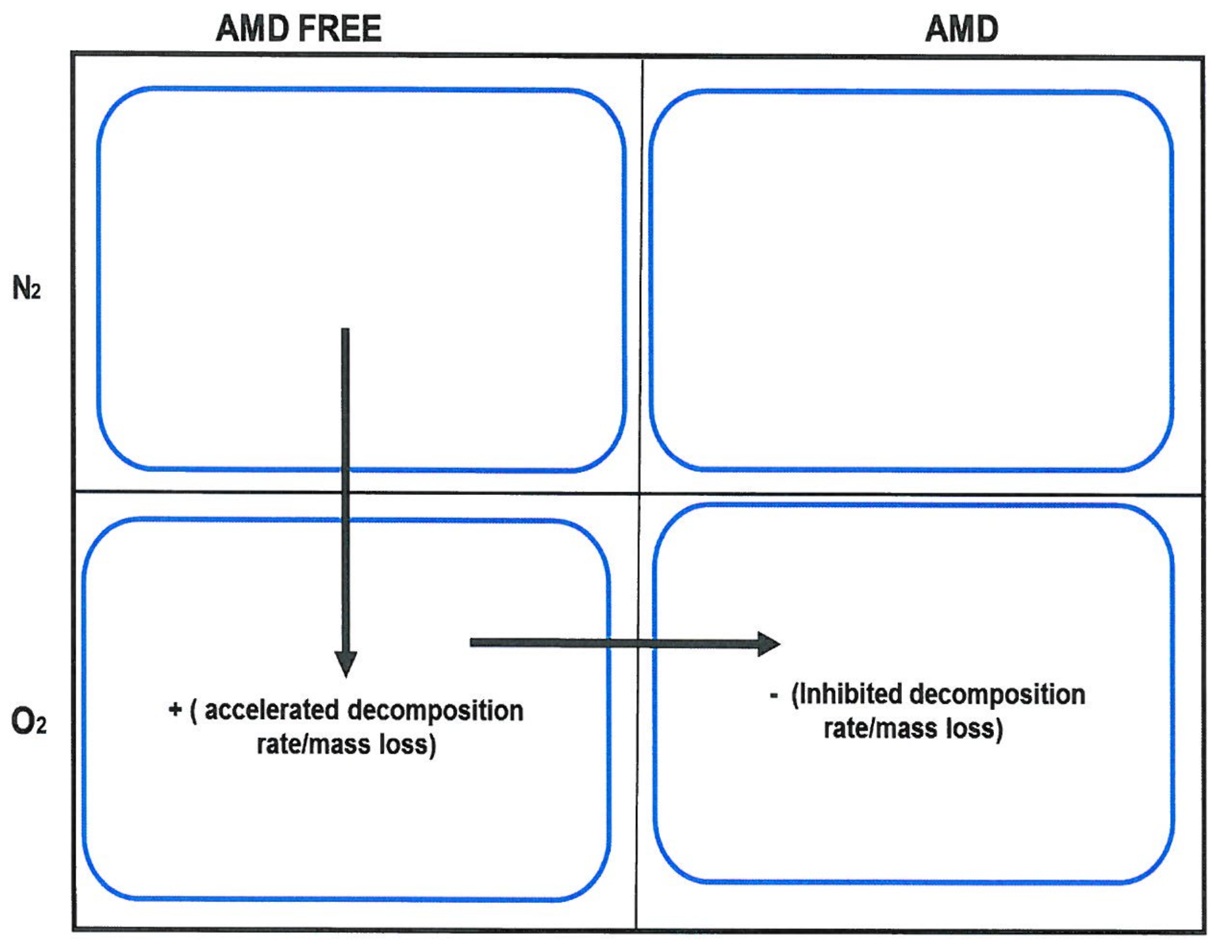

Figure 3: The interaction effect (O_2 and AMD) on decomposition rate of organic matter among the four treatments.

As hypothesided, the results showed that in the AMD-free treatments addition of O_2 accelerated the decomposition rate of organic matter therefore the AMD-free O_2 treatment had a greater mass loss in organic matter. The results further supported the notion that in the AMD treatments the addition of O_2 will inhibit the decomposition rate of organic matter, therefore the AMD O_2 treatment had less mass loss in organic matter relative to the AMD-free treatments. The results confirmed observation made in various other studies. Carpenter *et al.* (1983) compared the decomposition rates of organic matter between freshwater, dilute AMD and severe AMD reservoirs of pH 6.3, 5.7 and 3.7 respectively. The results showed that the rate of decomposition in organic matter was suppresed in AMD contaminated resevoirs, the degree of suppression varied significantly depending on the pH, decomposition was slower with decreasing pH. In freshwater, decomposition rate was twice that of severely AMD contaminated resevoirs. Mulholland *et al.* (1987) study indicated that the rate of mass loss in organic matter was significantly reduced in acidified streams with pH < 5.7 compared to streams with pH 6.4. A study by Dangles *et al.* (2004) revelead that the decomposition rate was 10 times slower in streams with pH < 5 compared to those with pH 7. In addition, Jenkin *et al.* (2013) showed that cellulolytic decomposition rate increased as stream acidification was reduced as pH increased. It was expected that AMD independently will have a significant effect on the mass loss in organic matter; however, the results showed an insignifcant effect. The reason for this outcome is not entirely clear but it is possible that due to constant temperature and O_2 levels within the experiment the independent effect of AMD was maintained below detectable levels throughout the experiment's duration.

The significant difference in mass loss of organic matter between AMD O_2 treatment and AMD-free O_2 is not suprising given the difference in water chemistry between the two treatments and its influence on decomposion rates. Correlation analyses provided an indication of the physico-chemical variables that most probably had the largest influence on the decomposition rates. Noting the negative correlation between DO and temperature in the AMD O_2 treatment, cooler temperature allowed for higher saturation of DO (USEPA, 1986) which then supported second reaction in AMD which is oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Branam *et al.*, 2011). In severely AMD affected streams wherein pH is below 3.5, ferric iron remains moslty in solution and only precipitates into insoluble ferric hydroxide at a significantly low rate (Hem and Cropper, 1959; Stumm and

Lee, 1961; Brock and Gustafson, 1976). In the AMD O_2 treatment the average pH was 2.739, thus ferric iron remained dissolved for the most part of the experiment and only precipitated later in the experiment.

The oxidation reaction takes place at a pH of approximately 3.5, which is too low to support most aquatic life (Cravotta III and Kirby, 2004). At a low pH, microbial numbers, respiration rates and bacterial production are generally low (Minshall and Minshall, 1978; Rao *et al.*, 1984; Mulholland *et al.*, 1987). Moreover, fungi decrease rapidly at pH 4 and almost completely absent at pH 3. In this experiment, the mean pH was 2.74, which is likely to have negatively impacted on the microbial and fungi activity in AMD O_2 treatment resulting in the lower mass loss of organic matter. Furthermore, it has been proven in various studies that deposition of metal hydroxides tend to form coating on leaves which reduces substrate accessibility and limit microbial colonization (Gray and Ward, 1983; Siefert and Mutz, 2001; Schlief, 2004; Schlief and Mutz, 2005), thus inhibiting decomposition rate. It is likely that in the AMD O_2 treatment decomposition rate was inhibited by iron (III) hydroxide coating the leaves.

In the AMD Free O_2 treatment, negative correlation between DO and temperature allowed for higher saturation of DO as temperature was cooler (USEPA, 1986), creating suitable conditions for microbial and fungi activity. Thus the AMD-free O_2 treatment had more mass loss of organic matter than that of AMD O_2 treatment as determined by the decomposition rate, which was faster in the AMD-free O_2 treatment compared to the than AMD O_2 treatment. In AMD-free N_2 treatment, there was a negative correlation between pH and EC/temperature, a lower EC and cooler temperature promoted a more neutral pH (Leveling, 2002). It has been established that streams/reservoirs with a neutral pH increase the decomposition rate of organic matter (Carpenter *et al.,* 1983; Mulholland *et al.,* 1987; Dangles *et al.,* 2004; Jenkins *et al.,* 2013), in this experiment the AMD-free N_2 treatment had an average pH of 6.26 (neutral) which enabled significant mass loss in organic matter. Furthermore, in deprived oxygen conditions it is likely that bacteria more so than fungi played an important role in decomposition of lignin (Leitner *et al.,* 2012).

In general decomposition rate is higher in aerobic conditions than anaerobic conditions (Webster and Benfield, 1986); however, there are several studies that are conflicting the assumption. A study by Godshalk and Wetzel (1978) showed a slower decomposition rate of macrophytes under unaerated conditions. Reed (1979) showed that during summer decomposition rate was slower at a greater depth in a lake and this was ascribed to low DO concentration. Novak *et al.* (1975) and Bastardo (1979) studies showed that there is no difference in decomposition rates under aerated or unaerated conditions. Whereas Nichols and Keeney (1973) found faster decomposition rate in low DO conditions. In this experiment, aerated condition had a higher decomposition rate that unaerated conditions which supports the general assumption and studies by Godshalk and Wetzel (1978) and Reed (1979). In AMD N_2 treatment, although unaerated, the negative correlation between DO and temperature indicated that the cooler temperature allowed higher saturation of DO (USEPA, 1986). However, the treatment did not yield significant results as expected and the reason for this outcome is not clear.

Future work/Limitations

It has been proven that exposure to oxygen stimulates generation of AMD and further exposure lowers the pH and produces ion precipation. Bucknell University (2012) states that ion precipitation coats the stream floor, this may support the results of the unpublished laboratory experiment conducted by (Ms K Olsen 2016, pers. comm.) that indicated AMD treatment had a net gain in mass of organic matter instead of losing it. In constast, the results of the present study indicated that despite ion precipitation in the AMD O_2 treatment, there was mass loss in organic matter. The two studies did not take into consideration the conflation of mass loss and gain due to coating by ion precipitation. In advancing this research in future, experiments should assess the effects of AMD ion precipitation on the decomposition of organic matter at pH levels of around 3.5.

The non signifcance of the time effect on breakdown of organic matter was unexpected despite the fact that the experiment was carried out over six weeks period, with an intention of advancing the previous experiment by (Ms. K Nyembe 2016, pers.comm.) carried over four weeks. According to Boyd (2016), the constituents of organic matter do not breakdown at the same rate. Materials such as proteins and fats decompose sooner (within weeks) than cellulose and lignin which may persist for up to 12 months. This obsevation can be confirmed as the leaves were mostly intact when recovered but slightly disintegrated, thus partially decomposed. To advance this study in future, the experiment may need to be carried out over 12 months in order to have complete leaf breakdown. An additional source of unmeasured bias may have been dissoved nutrients. Different concentrations of alternative food sources among the treamtents may have caused a difference in leaf decompostion rates. The role of alternative dissolved Nitrogen and Phosphorus sources in mediating decomopsition rates of organic matter under AMD conditions needs further investigation in future experiments.

Synthesis and application

The results of the study provided an insight on how the decomposition process may unfold in AMD contaminated rivers, in particular the Olifants River catchment. The breakdown of organic matter in different sections of the river will differ depending on the pH of AMD water and its oxygen content. In the upper Olifants where AMD-free water was collected, decomposition rate is likely to be higher than reaches downstream of AMD inputs. Consequently, the mass loss in organic matter is likely to be greater upstream of sources of AMD inflow. Moreover, in the reaches of the Olifants that are affected by AMD, sections of the river rich in oxygen content are likely to have a higher acidity therefore suppressing decomposition rate.

One of the reasons AMD is undesirable in aquatic ecosystems is that it causes significant alterations to the structure and function of benthic communities and food chain. Several studies have shown that the abundance, richness and diversity of benthic communities (such as fish, invertebrates, microbes and algae) consistently decrease with increasing stream aciditiy. A study by Cooper and Wagner (1973) revealed that in most streams there is complete loss of fish below pH 4.5. If fish are present in AMD contaminated streams, their richness and abundance are always low compare to freshwater stream (Sullivan and Gray, 1992; Rutherford and Mellow, 1994; Besser *et al.*, 2001). A study conducted by Schorr and Baker (2006) showed that pH accounted for >70% of the variation in fish species richness and abundance. With invertebrate communities, Winterbourn (1998) and Cherry *et al.* (2001) indicated that very few invertebrate species remain in severely AMD contaminated streams and in some cases they are totally absent (Soucek *et al.*, 2003). At a low pH, tolerant invertebrate predators tend to dominate (Gerhardt *et al.,* 2004). In the case of microbe communities, a study by Lear *et al.* (2009) showed less diversity in bacterial communities at $pH < 3.5$. At a low pH, Fe-oxidising bacteria which use ferric iron for metabolism dominate (Baker and Banfield, 2003). Algal species richness and diversity is also affected by pH. A study by Verb and Vis (2005) showed that periphyton community composition declined with decreasing pH; however, AMD tolerant species such as diatoms and filamentous green algae dominated (Douglas *et al.,* 1998; Bray *et al.,* 2008). Hogsden and Harding (2012) indicated that a combination of these factors cause significant changes to food webs as basal resources are reduced or absent and breakdown of organic matter by microbes is slower. The study concluded the following; structurally, loss in species diversity abridges food webs as interaction of species is restricted, and functionally, energy corridors are destabilized as various trophic levels are disturbed.

Over and above the importance of species richness and diversity to maintain a healthy aquatic ecosystem, decomposition of organic matter as a means of nutrient cycling is another important process to maintain a healthy aquactic ecosystem. Molles (2005) indicated that the amount of nutrients made available to fuel primary production depends on the rate of mineralisation that is dependent on the decomposition rate; reduced decomposition rates will yield lower loads of nutrients. According to Newbold *et al.* (1982) and Peterson *et al.* (2001) this could disturb nutrient rentention and energy flow through food webs. Meyer (1987) stated that disturbance in these processes can adversely impact the downstream ecosystems and have consequences for society. Given the description of how AMD contaminated streams could affect the aquatic life and ecosystem processes, coupled with the fact that water is a limited resource in South Africa (Basson *et al.,* 2004), it is important that Government and mining industry implements remediation measures with emphasis on overflowing AMD water as exposure to atmospheric oxygen could escalate the negative impacts.

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Annexure A: Raw data on leaf mass over time between the four treatments

		AMD-free O2				AMD O2				AMD-free N2				AMD N2			
Weeks	Samples	Pre-treatment Mass (g)	Post-treatment Mass (g)	Mass Loss (g)	Mass Loss (%)	Pre-treatment Mass (g)	Post-treatment Mass (g)	Mass Loss (g)	Mass Loss (%)	Pre-treatment Mass (g)	Post-treatment Mass (g)	Mass Loss (g)	Mass Loss (%)	Pre-treatment Mass (g)	Post-treatment Mass (g)	Mass Loss (g)	Mass Loss (%)
1	A	1.006	0.906	0.1	9.94	0.946	0.858	0.088	9.30	0.751	0.654	0.097	12.92	0.854	0.675	0.179	20.96
	B	0.934	0.823	0.111	11.03	0.952	0.865	0.087	9.14	0.787	0.672	0.115	14.61	0.833	0.71	0.123	14.77
	C	0.94	0.803	0.137	13.62	0.867	0.654	0.213	24.57	0.73	0.605	0.125	17.12	0.852	0.757	0.095	11.15
	D	0.954	0.825	0.129	12.82	0.697	0.626	0.071	10.19	0.928	0.718	0.21	22.63	0.757	0.626	0.131	17.31
2	E	0.944	0.792	0.152	15.11	0.917	0.809	0.108	11.78	0.865	0.691	0.174	20.12	0.987	0.876	0.111	11.25
	F	1.008	0.87	0.138	13.72	0.954	0.856	0.098	10.27	0.958	0.832	0.126	13.15	0.993	0.822	0.171	17.22
	G	0.829	0.719	0.11	10.93	1.079	0.841	0.238	22.06	0.909	0.736	0.173	19.03	0.959	0.857	0.102	10.64
	H	0.882	0.76	0.122	12.13	0.932	0.877	0.055	5.90	0.979	0.844	0.135	13.79	0.797	0.677	0.12	15.06
3	I	0.978	0.831	0.147	14.61	0.785	0.68	0.105	13.38	0.656	0.533	0.123	18.75	0.821	0.743	0.078	9.50
	J	0.883	0.734	0.149	14.81	0.919	0.807	0.112	12.19	0.767	0.661	0.106	13.82	0.917	0.681	0.236	25.74
	K	0.911	0.767	0.144	14.31	0.924	0.833	0.091	9.85	0.762	0.661	0.101	13.25	0.892	0.75	0.142	15.92
	L	0.822	0.692	0.13	12.92	0.935	0.875	0.06	6.42	0.805	0.746	0.059	7.33	0.938	0.754	0.184	19.62
4	M	0.954	0.775	0.179	17.79	0.976	0.805	0.171	17.52	0.964	0.792	0.172	17.84	0.997	0.901	0.096	9.63
	N	0.661	0.52	0.141	14.02	0.947	0.854	0.093	9.82	0.972	0.787	0.185	19.03	0.732	0.607	0.125	17.08
	O	0.809	0.607	0.202	20.08	0.594	0.492	0.102	17.17	0.913	0.809	0.104	11.39	0.879	0.688	0.191	21.73
	P	0.955	0.724	0.231	22.96	0.852	0.716	0.136	15.96	0.942	0.828	0.114	12.10	0.953	0.833	0.12	12.59

	Q	0.822	0.635	0.187	18.59	0.923	0.813	0.11	11.92	0.958	0.86	0.098	10.23	0.832	0.699	0.133	15.99
5	R	0.619	0.457	0.162	16.10	0.806	0.688	0.118	14.64	0.809	0.752	0.057	7.05	0.867	0.774	0.093	10.73
	S	0.825	0.665	0.16	15.90	0.861	0.719	0.142	16.49	0.913	0.812	0.101	11.06	0.967	0.695	0.272	28.13
	T	0.881	0.692	0.189	18.79	0.934	0.801	0.133	14.24	0.847	0.673	0.174	20.54	0.796	0.644	0.152	19.10
6	U	0.884	0.704	0.18	17.89	0.97	0.879	0.091	9.38	0.788	0.716	0.072	9.14	0.787	0.643	0.144	18.30
	V	0.933	0.777	0.156	15.51	0.631	0.516	0.115	18.23	0.925	0.819	0.106	11.46	0.865	0.796	0.069	7.98
	W	0.973	0.84	0.133	13.22	0.893	0.744	0.149	16.69	0.961	0.833	0.128	13.32	0.94	0.77	0.17	18.09
	X	0.953	0.771	0.182	18.09	0.514	0.458	0.056	10.89	0.783	0.681	0.102	13.03	0.891	0.679	0.212	23.79