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Passive treatment of acid mine drainage using an integration of neutralisation and a constructed aerobic wetland

By

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A thesis submitted to University of South Africa, College of Agriculture and Environmental Science in the Department of Environmental Sciences, in fulfilment of the requirements for the degree of Doctor of Philosophy in environmental sciences

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DECLARATION

I **Nguegang Beauclair** solemnly declare that the thesis titled **″Hybrid treatment of acid mine drainage using an integration of neutralisation and constructed wetland″** is my own personal research work and has not previously been submitted for the purpose of obtaining any academic qualification to this or any other university. The research in the thesis, except where otherwise indicated by means of complete references is my original research work.

SIGNATURE………………………………………………………………………………..

Mr NGUEGANG BEAUCLAIR

DATE………………………………………………………………………………………...

DEDICATION

I dedicate this work to my family and specifically to the following people:

1 My mum Pauline Demanou
2 My dad Maurice Kenfack
3 My childhood teacher, Mr Dieunedort Thomas Anouleko
4 My Step mum Prudencia Florence Asongfack
5 My step dad, Mr Victor Ngemasong Djoumetsa
6 My son Skelly Cedric Ngoune Tankoua Nguegang
7 To the entire Kana Yallow family and Lotsa at large

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Published papers

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Papers under review

Paper 2:

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Paper 3:

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Paper 4:

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Book Chapter

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ABBREVIATIONS AND ACCRONYMS

Abbr	Meaning
AGP	Acid generating potential
AG	Analytical grade
ALD	Anoxic limestone drains
AMD	Acid mine drainage
ANP	Acid neutralisation Potential
APC	Aerobic polishing cell
APHA	American public health association
ASL	Alkaline sulphidogenic liqua
ATR	Attenuated total reflectance
BBC	British Broadcasting Corporation
BCF	Bio-concentration factor
BDL	Below Detection Limit
CEC	Capacity exchange capacity
CSIR	Council for Scientific and Industrial Research
CNN	Cable News Network
CW	Constructed wetland
DEA	Department of Environmental Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
ED	Electrodialysis
EDS	Energy-Dispersive Spectroscopy
EC	Electrical Conductivity
EPA	Agency Protection Agency
FB	Focused Beam
FTIR	Fourier-transform infrared spectroscopy

FTW	Floating wetland
FWS-CW	Free water surface- constructed wetland
GDP	Gross Domestic Products
HLR	Hydraulic loading rate
HR	High Resolution
HRT	Hydraulic retention time
IC	Ion chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Atomic Emission Spectroscopy
I-MC	Inter-Ministerial committee
ILR	Inflow loading rate
LC	Lethal concentration
LD	Lethal dosage
LMIC	Low and Middle Income Countries
MD	Membrane distillation
LSB	Limestone leach bed
MF	Micro Filtration
NEMA	National environmental management act
NF	Nano Filtration
NIST	National Institute Standards and Technology
OFAT	One – factor-at –a- time
OLR	Outflow loading rate
OLC	Open limestone channel
PEL	Probable effect level
PHREEQC	pH Redox Equilibrium (in C language)
PRB	Permeable reactive barriers
PZC	Point of zero charge
QA	Quality assurance
QC	Quality control
RE	Removal efficiency

RO	Reverse osmosis
RSA	Republic of South Africa
SANS	South African National Standard
SAPS	Successive alkalinity producing system
SLB	Slag leach beds
SSHF-CW	Subsurface horizontal flow- constructed wetland
SSVF-CW	Subsurface vertical flow-constructed wetland
SRB	Sulphate reducing bioreactor
SEM	Scanning electron microscopy
TDS	Total dissolved solid
TF	Translocation factor
TI	Tolerance index
TSS	Total suspended solid
UF	Ultrafiltration
UN	United Nations
VFW	Vertical floating wetland
WHO	World health organization

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ABSTRACT

Water pollution and environmental degradation caused by acid mine drainage (AMD) is an ever more pronounced matter in environmental pollution. This is imputed to the nature and magnitude of its effects on the environment and its resources. Acid mine drainage is generated from the hydro-geo-chemical oxidation of sulphides bearing minerals such as arsenopyrite, marcasite. Acid mine drainage is prevalently rich in Aluminum, iron, Manganese and sulphate with traces of toxic and hazardous chemical components such as Copper, Nickel, Zinc, Lead, Cobalt, Arsenic, Chrome, and nuclides. As a legal requirement, these components must be removed from acid mine drainage before they could contaminate the environment. A number of passive and active treatment technologies have been developed to curtail the impacts of acid mine drainage. However, they have their own drawbacks and advantages. In light of that, technologies have been migrating towards an integrated approach. This study was therefore motivated with the aim of using an integration of neutralisation and staged hybrid constructed wetland to treat acid mine drainage from active and disused mines. It integrated neutralisation upstream and staged hybrid constructed wetland downstream to polish the water. To achieve this, two batches of experiments were conducted: the first batch consisted to assess the performance of three different types of constructed wetland operating individually for the treatment of acid mine drainage while the second batch assessed a hybrid approach integrating neutralisation of acid mine drainage using cryptocrystalline magnesite and bioremediation using staged hybrid constructed wetland. The present study consisted of seven chapters including: (1) Introduction and background information, (2) Literature review, (3) materials and methods, (4) passive remediation of acid mine drainage using phytoremediation: partitioning of inorganics contaminants between plant, substrate and external factors, (5) the treatment of acid mine drainage using vertically flowing wetland: insight into fate of chemicals species, (6) the assessment of the performance of subsurface horizontally flowing constructed-wetland for treatment of acid mine drainage, (7) The treatment of acid mine drainage using a combination of cryptocrystalline magnesite and a staged hybrid constructed wetland equipped with *Vetiveria zizanioides*.

In the first part the study, the root of AMD were identified and described, the scope and extent to which previous studies have investigated the problems, the existing gaps and attempts to address them.

In the second part, the origins and formation process of AMD were explored and explained as well as its associated environmental, socio economic and public health impact. This second part also explored the existing technologies for AMD treatment, their limitation as well as description of constructed wetland and properties of some plants used as wetland macrophytes.

The third part of the study described succinctly how study was conducted, study design, samples collection and analysis; it also outlined ethical consideration, risks and limitation of the study as well as wetland maintenance plan.

In the fourth part, the passive remediation of AMD using free water surface constructed wetland equipped with *Vetiveria zizanioides* was assessed with focus on partitioning of inorganic contaminant between plant, substrate and external factors. Water quality parameters were monitored by chemical analysis of samples every 24 hours for 30 days retention time. The average result of five days period was recorded. The results showed net removal of pollutants with Fe registering the highest removal percentage of 90%, followed by Zn (73%), SO_4^{2-} (67%), Mn (58%), Cu (34%), Al (31%) and Ni (12%) with huge quantity of metals and sulphate retained in the substrate. The total dissolved solid (TDS) decreased from 3880 to 1400 mg/l and electrical conductivity (EC) decreased from 5 to 2 mS/cm while pH rose from 2.6 to 3.1. The finding further revealed that *Vetiveria zizanioides* was tolerant in AMD water while metals removed were partitioned in the following order: substrate ≥ plant ≥ external factors of which, the substrate contributed 77.23% (Mn), 72.01% (Al), 69.91% (Zn), 66.51% (Ni), 60% (Cu) and 56.56% (Fe). The plant contributed Fe (40.42%) > Cu (36.66%) > Ni (30.09%) > Zn (27.89%) > Al (22.11%) > Mn (20.58%), and the external factor contributed 5.88% (Al), 3.4% (Ni), 3.34% (Cu), 3.02% (Fe), 2.19% (Mn), and 2.2% (Zn). Overall the Free Water Surface-Constructed Wetland equipped with *Vetiveria zizanioides* improved the quality of AMD but did not meet the water quality standard required by Department of Water and Sanitation (DWS) and Department of Environmental Affairs (DEA) for effluents discharge.

In the fifth part, AMD was treated using subsurface vertically flowing wetland equipped with *Vetiveria zizanioides* with focus on the fate of chemical species. The experiment lasted for 30 days and leachates parameters were monitored by chemical analysis of samples every 24 hours. The results revealed a net reduction of sulphate from 3137 mg/L to 1406 mg/L (55.18%), net removal of metals with removal efficiency ranged in the order: Fe (71.25%) > Zn (70.40%) > Al (68.93%)> Mn (62%) > SO_4^{2-}(55.18%) > Ni (35%) > Cu (18.83%). The results further revealed that *Vetiveria zizanioides* was tolerant to acid mine water with tolerant index of 1.031. Substrate played a huge role in metals removal with contribution faction ranged from 65.12% for nickel to 81.39% for zinc whereas the accumulation of heavy metals by *Vetiveria zizanioides* accounted only for small faction (10.46% to 30.57%) and external factors contribution accounted for a minor faction for Cu (0.36%), Al (1.13%) and Fe (1.3%) and small faction for Mn (8.1%), Zn (8.12%) and Ni (24.26%) of the overall heavy metals removal by the wetland. The vertically flowing wetland planted with *Vetiveria zizanioides* slightly improved the quality of mine water.

In the sixth part of the study, AMD was treated using subsurface horizontal flowing wetland equipped with *Vetiveria zizanioides*. Leachates parameters were monitored daily and the average result of five days period was recorded for 30 days retention time. The results showed a slight increase of pH by 1.4 and net reduction of EC, TDS and SO_4^{2-} at 47.20%, 46% and 33.04% respectively. There was a net removal of metals with Zn registering the highest removal percentage of 77.75%, followed by Fe (75.36%), Mn (67.48%), Al (55.05%), Ni (44.01%) and Cu (11.36%). This system significantly improved the quality of AMD. However, the water quality guidelines as set by the Department of Environmental Affairs and Department of Water and Sanitation were not met.

In the seventh part, the potential of hybrid technology in treating AMD was assessed. Feed AMD was treated with magnesite at the ration of 1:100 (one gram of magnesite and 100 ml of AMD water). The contact of magnesite with AMD water for an optimal time of one hour led to an increase in pH from 2.6 to 9.8 and a net reduction of EC, TDS, metals and sulphate. The product water was further treated by a bioremediation process using staged hybrid constructed wetland and the finding revealed that pH was further increased from 9.8 to 10.4 within 30 days retention time, TDS was reduced from 1552 to 780.3, EC

was reduced from 2.1 to 0.7 mS/cm, sulphate was reduced from 1354.8 to 608.7 mg/L; Al was reduced from 12.64 to 0.71 mg/L; Cu was reduced from 0.84 to 0.11 mg/L; Fe was reduced from 17.05 to 0.66 mg/L; Mn was reduced from 1.48 to 0.28 mg/L; Ni was reduced from 0.47 to 0.09 mg/L and Zn was reduced from 0.68 to 0.14 mg/L. Overall, the removal efficiency of hybrid technology (neutralisation and bioremediation) was 86%, 79.88%, 80.59%, 99.5%, 97.38%, 99.8%, 99.24%, 97.7% and 98.36% for EC, TDS, sulphate, Al, Cu, Fe, Mn, Ni and Zn respectively and a pH increment of 8.8. The hybrid technology (neutralisation and staged hybrid constructed wetland) removed contaminants to below South African legal requirement for effluent discharge and water use. As such it can be concluded that neutralisation with magnesite and bioremediation using staged hybrid wetland planted with *Vetiveria zizanioides* has a synergetic potential in AMD treatment.

Keywords: acid mine drainage; hybrid treatment; cryptocrystalline magnesite; constructed wetland; *Vetiveria zizanioides*; chemical species; bioaccumulation.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Mining exploration and minerals exploitation is an important wealth- generating industry. However, the effects of mining industry on the environment are diverse and varied in degree of severity. In addition to deforestation and land excavation, mining industry also generates metal-rich acid solution known as acid and metalliferous drainage (AMD) or acid rock drainage (ARD) which is jeopardizing the environment and its suitability to sustain life. Acid mine drainage is usually produced when pyrite (FeS_2) reacts with oxygen or water to form an acidic solution rich in metals (iron, aluminum, manganese) and ion sulphate (Johnson and Hallberg, 2005; Simate and Ndlovu, 2014; Nleya *et al*., 2016). As results of elevated iron concentration and low pH (< 3), there is proliferation of iron-reducing bacteria such as *Thiobacillus ferrooxidans* and sulphate reducing bacteria (SRB) such as *Deulfovibrio desulfuricans* which accelerate the oxidation of ferrous iron to ferric ion (Equation 1.1). Ferric ion can later cause the oxidation of pyrites thereby leading to more acidity (Equation 1.2) (Dos Santos *et al*., 2016)

$$\mathrm{Fe^{2+}} + \frac{1}{4}\mathrm{O_2} + \mathrm{H^+} - \mathrm{bacteria} \rightarrow \frac{1}{2}\mathrm{H_2O} + \mathrm{Fe^{3+}} \quad \text{(Eqn 1.1)}$$

$$\mathrm{FeS_2} + 14\mathrm{Fe^{3+}} + 8\mathrm{H_2O} \rightarrow 15\mathrm{Fe^{2+}} + 2\,\mathrm{SO_4^{2-}} + 16\mathrm{H^+} \quad \text{(Eqn 1.2)}$$

The generation of AMD is also affected by numerous aspects such as the sulphur content, hydrology and geology of the surrounding mining area (Campaner *et al*., 2014; Dold, 2014; Yucel and Baba, 2016). Overall, AMD is distinguished by acidic pH (usually less than 5), elevated concentration of sulphate, and toxic metals such as iron, aluminum, manganese and trace metals species such as arsenic, cobalt, copper, nickel and zinc. The release of such acidic wastewater with elevated concentration of metals and sulphate is responsible to severe detrimental effects on the environment and human health (Roychowdhuryb *et al*., 2015; Africa ground water Atlas, 2019; Moeng, 2019)

Acid mine drainage also contains radioactive substances like uranium and its damaging effect are a cause of concern in AMD polluted sites (Coetzee *et al.*, 2006; CSIR, 2013; Moeng, 2019). Acid mine drainage alters the chemical composition of surface and ground water thereby rending it unsuitable to use for various purposes (Madzivire *et al.*, 2010; Masindi *et al.*, 2015; Africa ground water Atlas, 2019). Acid mine drainage also has deleterious effects on the economy because regions experiencing AMD pollution have their biosphere contaminated and such situation requires a huge treatment cost. Furthermore, biosphere pollution leads to the decline in fish of economy value and reduction of touristic activity (Sangita *et al*., 2010). Water is considered as “mining’s most common casualty” because the process of ore extraction consumes huge volume of potable fresh water and simultaneously generates by-products which pollute water resources (Ochieng et al., 2010; Bwapwa, 2017). Despite the growing concern worldwide about environmental legacy of mining industry, littles measure are undertook to mitigate environmental problems associated with mining activities. Water pollution from mining industry is significant environment problem in South Africa (Bwapwa, 2017; Africa Ground Water Atlas, 2019; Masindi *et al*., 2019). As one of the major minerals producers in the world and with limited water resources, South Africa’s available fresh water resources are under serious threat due to pollution by mining activities and others industries. Cobbing. (2008) reported that as a direct consequence of mining activities, AMD water has been flowing into streams on the West Rand (Randfontein area) since 2002 while according to (Naidoo, 2013; 2017), toxic AMD water is reported to negatively affect the aquatic ecosystem in the Tweelopiespruit and Robinson Lake near Randfontein on the West Rand basin. The overall environmental impact of AMD is the deterioration of surface and ground water resources thereby leading to a negative impact on the domestic, industrial and agricultural use **(**Gitari *et al.*, 2010; Grande *et al.*, 2014; Rimstidt and Vaughan, 2014; Bwapwa, 2017). Access to water resources is globally considered as prerequisite for economic and social development (U.S.A.I.D, 2009; Itumeleng and Pramod, 2011; Haddis *et al.*, 2014), but it follows that AMD pollution is putting water resources under threat and countries with scarce water resources such as South Africa are more concerned. Recent research conducted by the Department of Water and Sanitation (DWS) has predicted that South Africa will face potable water shortage if nothing is done to curtail the water resources pollution by AMD. Due to its physicochemical characteristics and associated environmental impact, it is therefore advised to minimise or avoid AMD in any way possible (Sangita *et al*., 2010; Bwapwa, 2017; Kefeni *et al*., 2017).

The reduction of environmental effects of AMD has attracted attention globally while in South Africa, the issue of AMD is reported by media and debates carried out in parliament. Inter-ministerial committee on AMD (I-MC AMD, 2010) suggested that the Witwatersrand basin should be seen as national emergency in respect to AMD pollution (CSIR, 2009, 2013). Various technologies have been developed to prevent AMD formation and one way to prevent AMD formation is the exclusion pyrite-bearing waste from the system (Kuyucak, 2002) or co-disposal of pyritic material with some alkaline material such as limestone (Johnson and Hallberg, 2005). This technology is the most common practice to prevent AMD production from mine waste while the neutralisation potential of the soil type is also used to reduce acidity by mixing pyritic waste with alkaline amendment such as limestone (Skousen and Ziemkiewicz, 2000). Due to the fact that bacteria play a vital role in the AMD formation process, chemical agent helping to prevent the formation of bacteria such as anionic surfactants have been used as liquid adjustment to prevent AMD formation for a limited period (Park *et al.*, 2019) while alkaline material can be used to prevent generation of AMD stabilizing the oxidized acid-generation tailings (Elghali *et al.*, 2019). Currently, several AMD prevention and remediation technologies are operating in various AMD-polluted sites worldwide. Prevention technologies consist to inhibit the formation of AMD generating reactions by controlling the source whereas remediation technologies consist to treat the already generated AMD prior to their discharge into the receiving environment.

In order to remediate AMD, various treatment technologies have been developed and are currently operating worldwide and they include: Active, passive and hybrid technologies (Muhammad *et al.*, 2015; Nleya *et al.*, 2016). Precisely, active technologies include: precipitation (MacIngova and Luptakova, 2012; Buzzi *et al.*, 2013; Bortnikova *et* *al.*, 2020), ion-exchange (Khan, 2014; Zewail and Yousef, 2015), adsorption (Orakwue *et al.*, 2016; RoyChowdhury, Sarkar and Datta, 2019b; Shabalala and Ekolu, 2019), coagulation (Mamelkina *et al.*, 2017; Nariyan et al., 2017; Mwewa *et al.*, 2019; Song *et al.*, 2021), Neutralization (Masindi *et al.*, 2015; Igarashi *et al.*, 2020) while passive technologies include bio-sorption (Ouakibi *et al.*, 2014; Park and Lee, 2017; Burman *et al.*, 2018), constructed wetland (Rodríguez-Galán *et al.*, 2019; Singh and Chakraborty, 2020; Nguegang *et al.*, 2021; Chen *et al.*, 2021), membrane technology (Aguiar *et al.*, 2018; Lopez *et al.*, 2018; Agboola, 2019), phytoremediation (Kiiskila *et al.*, 2017, 2019; RoyChowdhury *et al.*, 2020) an integrated technology (Masindi, 2017; Masindi *et al.*, 2017), hybrid technology (Oberholster *et al.*, 2018; RoyChowdhur *et al.*, 2019a; dos Santos *et al.*, 2020). However, the aforementioned technologies present some inconveniences: they are not cost effective, selective in pollutant removal, inability to meet the guidelines standard for effluent discharge set by World Health Organization (WHO) and the generation of a huge volume of sludge which needs to be properly disposed off thereby incurring additional cost (Johnson and Hallberg, 2005; Simate and Ndlovu, 2014; Seervi *et al.*, 2017). Furthermore, the aforementioned technologies are designed to operate for a limited time frame. Following the stated limitations, it is therefore necessary to concept a cost effective and environmental friendly technology for AMD treatment.

Due to its high capacity to neutralize AMD water and remove toxic inorganic pollutant, magnesite has been used to increase the pH of AMD water and remove inorganic contaminants (Masindi *et al.*, 2015; Masindi, 2016) while literatures have shown that constructed wetlands are efficient in improving the quality of AMD (Prihatini *et al.*, 2016; Sheoran, 2017; Nguegang *et al.*, 2021; Chen *et al.*, 2021). Furthermore, *Vetiveria zizanioides* which is perennial bunch grass of the family of poaceae with roots grows downward 2 metres to 4 metres (Truong and Tran, 2014; Truong and Danh, 2015) is tolerant to a wide range of extreme harsh conditions such as AMD (Bakhshoodeh *et al.*, 2016; Kiiskila *et al.*, 2017, 2019, 2020; Bahri *et al.*, 2021; Nguegang *et al.*, 2021).

In order to respond to the challenges faced by current technologies, the mining industry, government, and scientific community in environmental field are in constant seek of a cost effective and environmentally friendly technology to treat AMD. This present research was motivated by the need to investigate and design a hybrid technology consisting of active treatment (neutralisation) and phytoremediation (staged hybrid constructed wetland equipped with *Vetiveria zizanioides* for the treatment of AMD water.

1.2 Problem statement

The outflow of acidic water from active and disused mines is source of significant and devastating environmental effects (Tutu and Cukrowska, 2008; Sangita *et al.*, 2010; Nleya *et al.*, 2016; Bwapwa, 2017; Kefeni *et al.*, 2017). To date, AMD is the single largest non-point source of pollution (Macías *et al.*, 2017) and in the Witwatersrand basin, an average volume of 350 ML of AMD is been released daily from active and abandoned mines (CSIR, 2009). This volume accounts for 10% of potable fresh water supplied on daily basis by rand water Ltd to municipalities in Gauteng provinces and surrounding areas (CSIR, 2009). AMD water is characterized by acidic pH with toxic chemical species which change the physicochemical quality of the receiving streams leading to partial or complete change of the stream ecosystem (Jennings *et al.*, 2008; Sangita *et al.*, 2010; Bwapwa, 2017). For instance, due to its physico chemicals characteristics, AMD has the ability to modify the ecosystem and create an unbalance in the life form species of surrounding area (McCarthy, 2011; Lemeiruti, 2015; Nordstrom *et al.*, 2015; Bwapwa, 2017). Thisani *et al.* (2020) pointed out that AMD polluted streams may have pH between 2.0 to 4.5 depending on the host rocks, and notable levels of toxic species that can impair most forms of aquatic life. Other pollutants and toxic metals such as arsenic, cyanide may also be present in AMD water at elevated concentration (Sheoran, 2017). The impact of AMD on water quality also include physical effect such as turbidity, soil erosion and smothering of the stream substrate (Sangita *et al.*, 2010; Bwapwa, 2017; Thisani *et al.*, 2020). In addition to soil, surface and ground water pollution, AMD is also source of severe public health problem. For instance, radioactive substance and its devastating consequences constitute a serious public health problem in AMD polluted regions (Coetzee *et al.*, 2006; CSIR, 2013; Moeng, 2019). Following the environmental and public health problems associated with AMD, it is therefore required to treat this harsh mining wastewater to meet the water quality guidelines standard for effluent discharge as set by the world health organization.

Various treatment technologies including active, passive and hybrid technologies have been developed for the treatment of acid mine drainage. However many drawback such as selective pollutant removal (Masindi *et al.*, 2015), generation of sludge (Simate and Ndlovu, 2014; Nleya *et al*., 2016; Kefeni *et al*., 2017) cost effectiveness and inability of their application at large scale are rendering these technologies ineffective for long-term solution to AMD. The quest for cost-effective, environmentally friendly and long-term solutions is on-going. Emerging treatment technologies such as phytoremediation or their integration with others active technologies to obtain a synergy could have long-term success in remediating AMD (RoyChowdhury *et al*., 2019a). Such environmental, public health and economic problems associated with AMD are a real source of motivation to investigate an emerging treatment technology such as combination of neutralisation and staged constructed wetlands in order to determine precisely the potential of the above cited hybrid approach in remediating acid mine drainage.

1.3 Motivation

South Africa is one of the largest mining countries in the world and as direct consequences; the country is facing the problem of AMD and its ramifications. As a result of AMD pollution, stream and soil in mining producing area are facing devastating consequences that can last for decades or even centuries (Bwapwa, 2017; Dzwairo and Mujuru, 2017; Moeng, 2019). These consequences include pollution of South Africa's water resources, destruction of food crops, threatening human health, and the destruction of wildlife and eco-systems, infrastructure and heritage sites **(**McCarthy, 2011; CSIR, 2013; Bwapwa, 2017; Dzwairo and Mujuru, 2017; Moeng, 2019). Currently millions of litres of AMD are still flowing from abandoned mines into streams around Johannesburg thereby leading to devastating consequences for human communities and the environment.

Many studies aimed to develop an efficient technology for the remediation of AMD were carried out. However, due to the multiple drawback such as inability to meet guidelines standard for effluent discharge as set by the WHO, selective pollutant removal and the release of highly polluted toxic sludge associated with the existing technologies, the development of a low cost, environmentally friendly and sustainable technology such as neutralisation and constructed wetland can be considered as an alternative. This technology is applicable at large scale and has been regarded as possible solution to long-term remediation of AMD because it may provide a continuous and effective solution to a growing AMD problem hence it requires low investment, has low energy dissipation, does not generate toxic sludge, produces high-quality effluent and easy to operate. Constructed wetlands are engineered systems that utilize natural process which involve macrophytes, solar energy and microorganisms to assist in waste water treatment (Vymazal, 2010; 2011).

From literature, it follows that *Vetiveria zizanioides* is able to clean up metal, metalloid, radionuclide-contaminated water and soil (Suelee *et al.*, 2017; Yu *et al.*, 2017; Ghadiri *et al.*, 2018; RoyChowdhury *et al.*, 2020) whereas magnesite is able to increase the pH of AMD from 2.5 to 10 (Masindi *et al.*, 2015; Masindi, 2016). Hybrid approach consisting of neutralisation and staged hybrid constructed wetlands can be more effective in treating AMD since the specific advantage of neutralisation and each type of wetland will be exploited in order to enhance the efficiency of the overall systems. Since AMD is self-renewing process, to protect the environment and safeguard the public health to ensure sustainable development during and after mining operations, it is necessary to develop a low cost, self-renewing treatment technology such as combination of neutralisation and staged hybrid constructed wetland equipped with acidic and metals tolerant plants species such as *Vetiveria zizanioides* to remediate acid mine drainage.

1.4 Hypothesis

The integration of neutralisation and staged hybrid constructed wetlands equipped with *Vetiveria zizanioides* can neutralise AMD, attenuate the enshrined chemical species to required standard guidelines for effluent discharge as set by the World Health Organisation.

1.5 Aim and objectives

1.5.1 Aim

The overall aim of this study was to develop a hybrid system for the treatment of AMD using the integration of neutralisation by magnesite and staged hybrid constructed wetland equipped with *Vetiveria zizanioides*

1.5.2 Objectives

In order to achieve the above mentioned aim, the following sets of objectives were set.

- To determine the physico-chemical properties of raw water, AMD, activated magnesite, soil substrate, and plant fractions, before and after the reactions.
- To design and construct three types of wetland, i.e. free water surface constructed wetland (FWS-CW), subsurface vertical flow constructed wetland (SSVF-CW) and subsurface horizontal flow constructed wetland (SSHF-CW).
- To assess the efficacy of the designed wetlands on the attenuation of chemical species from acid mine drainage and point-out the underpinning chemistry.
- To assess the performance and synergistic effects of integrating activated magnesite (at 1 g: 100 mL S:L ratios) and staged hybrid constructed wetlands (equipped with *Vetiveria zizanioides*) on the treatment of acid mine drainage.
- To employ PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model to gain insights on speciation and mechanisms governing the removal of pollutants from AMD including chemistries thereof.
- To assess the system performance by comparing the quality of product water with the DEA and DWS guidelines and requirements for wastewater discharge.

1.6 Significance of study

Mining by-products are generated during and after mine closure and a significant part of that waste is AMD water. Following the characteristics of AMD and its associated environmental effects, it is therefore necessary to treat such harsh wastewater in order to reduce pollutants concentration to acceptable level and if possible recover drinking water from AMD. Various AMD treatment plants are in operation worldwide. However, the remediation approach of AMD using a hybrid technology consisting of neutralization and staged hybrid constructed wetland is yet to be investigated. It was then important that this hybrid approach be investigated in order to determine precisely to which extent neutralisation and staged hybrid constructed wetlands can be effective in AMD treatment. It is hoped that this hybrid technology will be environmentally friendly, cost effective, long-term solution, ability to meet the guidelines standard for effluent discharge and applicable at large scale for AMD treatment.

1.7 Novelty of the thesis

Various technologies have been explored for the treatment of AMD, but according to the author’s knowledge, the use of activated magnesite as a neutralising agent and staged hybrid constructed wetlands using compost soil as substrate and equipped with *Vetiveria zizanioides* as a phytoremediation agent operating on an integrated fashion for the treatment of AMD is yet to be explored. As such, this is the first study in design and execution to assess the feasibility of integrating activated magnesite and staged hybrid constructed wetland equipped with *Vetiveria zizanioides* in a hybrid fashion for the treatment of AMD and point-out the underpinning chemistries.

1.8 Thesis structure

This thesis in divided into eight chapters, and each chapter is explaining different aspects of the study investigated. Below are the summary of each chapter

CHAPTER ONE: Introduction

A brief background of the AMD and its associated environmental problems are highlighted together with different solution to the problem. The magnitude of AMD worldwide and in South Africa in particular and the need for a cost effective and environmentally friendly solution in order to ensure sustainable development. Aim, objectives, research questions, hypothesis and significance of the study are outlined and briefly discussed.

CHAPTER TWO: Literature review

This chapter explores the formation of AMD, its characteristics and associated environmental problems, exciting treatment methods and their limitation, future research perspectives. The description of constructed wetland as well as the properties of plant used as wetland macrophytes.

CHAPTER THREE: Materials and methods

This chapter succinctly describes the methodology and materials used. It clearly describes sampling and samples analysis processes and instruments used. It also outlines ethical consideration, risks and limitations associated with the project.

CHAPTER FOUR: Passive remediation of acid mine drainage using phytoremediation: Partitioning of inorganic contaminants between the substrate, plants, and external factors

This chapter describes the process of passive remediation of AMD with free water surface constructed wetland using *Vetiveria zizanioides.* The parameters of concerns were pH, electrical conductivity (EC), sulphate and heavy metals (Al, Cu, Fe, Mn, Ni and Zn). This section also investigated the role of substrate and external factors in metals removal and the accumulation of metals by Vetiver grass.

CHAPTER FIVE: The Treatment of Acid Mine Drainage Using Vertically Flowing Wetland: Insights into the Fate of Chemical Species

This section studied the fate of heavy metals (Al, Cu, Fe, Mn, Ni, Zn) and sulphate in subsurface vertical flow constructed wetland treating acid mine drainage as well as their accumulation and transfer of heavy metals from roots to shoot of the plants and substrate contribution in metals removal.

CHAPTER SIX: Assessing the performance of horizontally flowing subsurface wetland for the treatment of acid mine drainage

This section investigated the performance of subsurface horizontal flow constructed wetland (SSHF-CW) in treating acid mine drainage. The parameters of concerns were: pH, TDS, EC, sulphate and metals (Al, Cu, Fe, Mn, Ni and Zn). The chemistry of treated water and metals contents of substrate and plants were determined. The response of *Vetiveria zizanioides* grass to acidic water by means of tolerance index was calculated. The contribution of each wetland component as well as external factors to metals removal was also reported in this section.

CHAPTER SEVEN: A hybrid approach towards the treatment of acid mine drainage using the integration of neutralisation and phytoremediation

This section investigated the hybrid approach by neutralizing AMD using magnesite and further polished the pre-treated AMD water using staged constructed wetland. Parameters of concerns were: pH, EC, TDS, sulphate, heavy metals (Al, Cu, Fe, Mn, Ni and Zn). The contribution of each step was investigated as well as the process of metals precipitation and fate of pollutants in staged hybrid wetland.

CHAPTER EIGHT: General conclusions and recommendations

The chapter presents a general conclusion of the study project and also makes some recommendation for further research

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CHAPTER TWO

LITERATURE REVIEW

This chapter explores and explains the origin of AMD, the formation process, its sources, associated environmental, socio economic and public health impacts. This chapter also explores the existing treatment technologies and their limitation and the description of constructed wetland as well as the properties of some plants used as wetland macrophytes.

2.1 Introduction

Acid mine drainage (AMD) is an unavoidable by-product of mining industry flowing from actives and abandoned mines which can remain the sources of AMD generation for decades or even centuries after the mine closure (Macías *et al.*, 2017; Moodley *et al.*, 2018). Depending of the area and geohydrological factors, AMD contains elevated concentration of total dissolved solids, sulphate ion major toxic chemical species including iron, manganese and aluminum, in addition to traces of toxic chemical species such as cadmium, copper, nickel, zinc and cobalt, and radioactive substances (Tutu and Cukrowska, 2008; Masindi *et al*., 2018a; Sun *et al.*, 2020). This contaminated water is very significant to the environment since it alters the pH thereby increasing the quantity of dissolved metals in the receiving water body (Hogsden and Harding, 2012; Masindi e*t al*., 2017). Acid mine drainage contains minerals which can settle at the bottom of receiving water bodies and thus creating an unbalance in the quality of the aquatic ecosystems hence forcing tolerant species to adapt new environmental conditions (Sangita *et al*., 2010; Candeias *et al.*, 2014). In addition to negative impact on aquatic ecosystem, AMD also affects the quality of surface water intended for different defined uses. Streams or rivers contaminated by AMD usually demonstrate a reddish or red-brown colour often called ‘’yellow boy’’ due to the precipitation of iron leading to more detrimental effect on aquatic ecosystem and human health (Roychowdhury *et al*., 2015; Shim *et al.*, 2015; Park *et al.*, 2019).

The problem of AMD is not only limited on its environmental impacts but rather to high volume of AMD generated throughout the year in countries with well-developed mining industries including USA, Australia, Canada, South Africa and Chile (Park *et al.*, 2019). For instance, coal and gold mines located in the western part of South Africa produce around 400 000 mega liters of AMD annually (Hobbs and Cobbing, 2007; Tutu *et al*., 2008; Masindi *et al*., 2019) thereby pointing out the nature and extend of unpleasant problem. This huge volume is demonstrative of AMD problem in South Africa and others countries with well-developed mining industry. Acid mine drainage is a little documented global crisis and according to the United Nations (UN), AMD is the world second largest environmental problem after global warming. If nothing is done to contain acid mine drainage, the world will face chronic pollution as a result of mining activities since it is an important revenue-generating industry. This situation has triggered a prompt response from the government and water management agencies in countries with well-developed mining industry thereby accentuating the need for better management of such acidic water (Costello, 2003; Hobbs and Cobbing, 2007; Moeng, 2019). Various treatment technologies (active and passive) have developed to remediate AMD and associated problems

AMD has traditionally been treated using two different technologies: Active treatment and passive treatment. Of which, active methods involve the use of chemical to raise the pH and precipitate metals whereas passive methods involve the use of naturally occurring chemical or synthetic compounds and biological reactions that assist in AMD treatment to take place in the controlled environment (Costello, 2003; Seervi *et al.*, 2017; Saha *et al*., 2019). However, following the drawback associated with the aforementioned technologies, mining companies and others researchers involved in environmental pollution are investigating the association of many different technologies including their hybrid generation to tackle acid mine drainage and its problems. The hybrid system consists of an association of two or more different technologies in order to use individual advantages of each method to achieve higher treatment efficiency (Dold, 2014; Herrera-Melián *et al.*, 2020).

This chapter succinctly describes and discusses different technologies developed for the treatment of AMD, their advantages, their limitations and proposes future perspectives and avenues. The ultimate goal is to demonstrate the feasibility of fostering sustainability in AMD treatment. It will also unpack success and failures of individual technology and its environmental footprints. The sustainability and economic viability of each approach will be highlighted. Future research avenues and focus will also be discussed in detail. This will aid in guiding future research initiatives and curtail potential environmental impacts.

2.2 Acid mine drainage

Acid mine drainage is a metal and sulphate rich by-products flowing from actives and abandoned mines. It is the most significant environmental problem associated with mining industry (Grande *et al.*, 2014; Kefeni *et al.*, 2017). Acid mine drainage is formed following the oxidation of sulphides mineral ores initially exposed to the environment as a result of intensive mining activities and in some major construction work (Costello, 2003; Johnson and Hallberg, 2005; Simate and Ndlovu, 2014). Depending of the area and geohydrological factors, may contain high levels of total dissolved solids, sulphate ion, Al^{3+}, Fe^{2+}, Mn^{2+}, in addition to traces of toxic heavy metals such as As^{3+}, Ni^{2+}, Cu^{2+}, Cd^{2+} and Co^{2+}, and radioactive elements (Pope *et al.*, 2010; Sun *et al.*, 2020)**.** This contaminated water is source of severe environmental damages and public health problems if it spreads and flow into streams and rivers adjacent to its vicinity (Shim *et al.*, 2015; Galhardi and Bonotto, 2016)

2.3 Acid mine drainage formation

Acid mine drainage is formed when a sulphide rich bearing minerals (eg, FeS_2, CuS, Cu_2S, $CuFeS_2$, NiS, ZnS and PbS (Simate and Ndlovu, 2014) commonly found in coal (Akinwekomi *et al*., 2017), copper (Simate and Ndlovu, 2014) and gold (Masindi *et al.*, 2015) and others mining deposits are exposed to atmospheric oxygen and water. Exposure of sulphides bearing minerals can either be instinctively initiated or occur as a result of mining activities and in some cases resulting from major construction work (Johnson and Hallberg, 2005; Akcil and Koldas, 2006; Potgieter-Vermaak *et al.*, 2006;

Jennings *et al.*, 2008) pyrite (FeS_2) being in the forefront of AMD formation with pyrite oxidation in the presence of water and oxygen (Equation 2.1) while the contribution of others sulphide minerals in AMD formation is very limited (Nordstrom et al., 2015a).

$$4FeS_2 + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_3 + 8H_2SO_4 \qquad \text{(Eqn 2.1)}$$

In fact, when pyrite is in contact with oxygen and water, the oxidation reaction of pyrite results to the release of hydrogen ion (H+), sulphate ions (SO_4^{2-}) and soluble metal cations as summarized by the following Equation 2.2 (Jennings *et al.*, 2008).

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2F_e^{2+} + 4SO_4^{2-} + 4H^+ \qquad \text{(Eqn 2.2)}$$

This oxidation process takes place in unperturbed rock when enough oxygen is disintegrated in the water; it leads to the reaction of soluble ferrous ions with oxygen to form ferric ions as described in the Equation 2.3.

$$2Fe^{2+} + O_2 + 4H^+ \rightarrow 2Fe^{3+} + 2H_2O \qquad \text{(Eqn 2.3)}$$

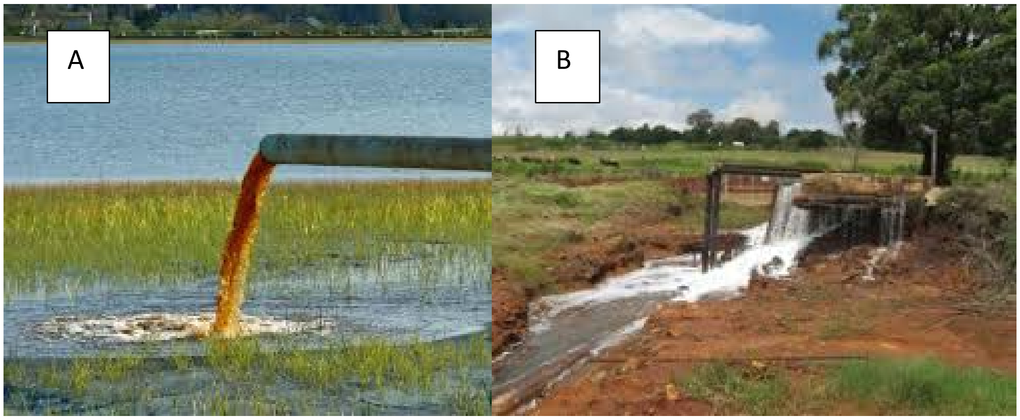
If pyrite is in contact with ferric iron, it is dissolved and then oxidized by the reduction of ferric iron which produces more acid (Equation 2.4). In the meantime, ferric iron (Fe^{3+}) is accelerated into hydrated iron hydroxide [$Fe(OH)_3$] (Equation 2.5):

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{3+} + 2SO_4^{2-} + 16H^+ \qquad \text{(Eqn 2.4)}$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \qquad \text{(Eqn 2.5)}$$

The equations above clearly illustrate that waste water released by mining industries contains hydrogen ions, sulphate ions, ferrous and ferric ions which contribute to lowering the pH values ($pH < 4$) and elevated concentration of metals and sulphate. At $pH \geq 3$, ferric ion reacts with water to generate hydrogen ion and hydrated ion hydroxide. At $pH < 3$, ferric hydroxide dissolves into ferric ions confirming the fact that pH determines the precipitation of ferric hydroxide and formation of ferric ion as reported by Tutu et al. (2008). These reactions occur spontaneously and are mediated by microorganisms (Zhao *et al.*, 2012; Candeias *et al.*, 2014; Amos *et al.*, 2015) and are also influenced by the interactions between microbiological diversity, mineralogical and geochemical composition of site where the ores is being extracted (Dold, 2014; Singer *et al.*, 2018).

These hydrogen ions (H^+) released by the reactions above lowers the pH and maintains metals/minerals soluble in AMD water (Simate and Ndlovu, 2014; Amos *et al.*, 2015) affect the physicochemical quality of receiving water bodies thereby leading to an unbalance in the aquatic ecosystem (**Figure 2.1**).



A: Point of discharge **B: Point of discharge**

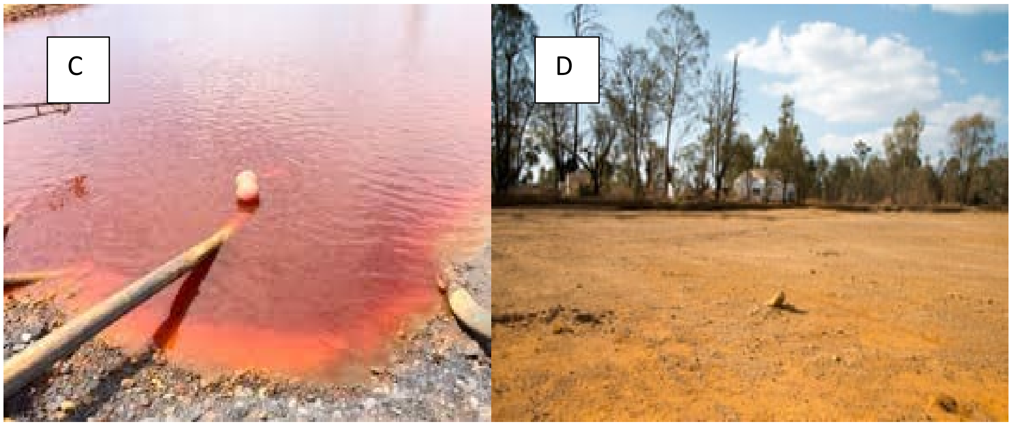


Figure 2.1 (A-D): (A) and (B) are point of AMD discharge. The water looks colourless but after oxidation with atmospheric air, the water turns red (C) and (D) landscape corroded by acid mine drainage

Once formed, AMD can find his way on water bodies through open mining activities, seepage from mine residues deposits, mining water loss, and sewage and storm water reticulation effluents (Sheoran *et al.*, 2010; Masindi, 2017a; Park *et al.*, 2019).

2.4 Roles of different influencing factors on acid mine drainage formation

The process of AMD formation is complex controlled by microbiological activities, water and oxygen as well as the depositional environment, lithology and mineralogy of mine site. The ability of a mining activity or associated waste to generate AMD is influenced by bacterially mediated process, oxygen and water.

2.4.1 Role of bacteria in acid mine drainage generation

Microbial activity plays a huge role in AMD formation process. The contact of bacteria with sulphide minerals is a catalyst agent in the process of AMD formation and bacteria such as and *Thiobacillus thioxidans* are ubiquitous and can be found everywhere including mine tailings and abandoned mines (Wang *et al.*, 2018; Chaix *et al.*, 2019)**.** Due to their potentiality to quickly oxidize reduced forms of iron and sulphur, these bacteria play an important role in AMD formation process (Natarajan, 2008a). The reduced form of iron and sulphur lead to the generation of sulphuric acidic solution**.** The bio-oxidation of sulphide minerals can be explained by direct and indirect process (Natarajan, 2008b; Wang *et al.*, 2018). The direct process occurs due to contact between oxygen and sulphide minerals mediated by bacteria as illustrated in the following reaction: (Equation 2.6)

$$FeS_2 + H_2O + \frac{7}{2} O_2 \xrightarrow{bacteria} Fe^{2+} + 2\,SO_4^{2-} + 2H^+ \qquad \text{(Eqn 2.6)}$$

The indirect process occurs following the action of ferric iron produced by ion-oxidizing bacteria following Equations (2.7) and (2.8) below.

$$FeS_2 + 8H_2O + 14\,Fe^{3+} \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \qquad \text{(Enq 2.7)}$$

$$Fe^{2+} + \frac{7}{2} O_2 + 14H^+ \rightarrow Fe^{3+} + 7H_2O \qquad \text{(Eqn 2.8)}$$

The overall reaction can be written as follows: (Equation 2.9)

$$2FeS_2 + 7O_2 + 2H_2O \xrightarrow{bacteria} 2FeSO_4^{2-} + 2HSO_4^{2-} \qquad \text{(Eqn 2.9)}$$

Bacteria also take part in ore processing by producing enzymes for industrial process (Baker and Banfield, 2003). Bacteria are vital to catalyze ferrous ion to ferric ion during

AMD formation. According to Akcil and Koldas. (2006), two types of the bacteria (*Metallogenium* and sulphur oxidizing bacteria *Thiobacillus ferrooxidans*) catalyze the reaction above. The direct process of pyrite oxidation contribute less to AMD formation and the main pathway in the AMD formation involves the indirect bacterial oxidation of available of available ferrous ion to ferric ion which oxidizes FeS_2 to generate further Fe^{2+} and acidity in a cyclic process (Sahoo *et al.*, 2013). The **Table 2.1** below presents some common sulphur oxidizing bacteria involved in AMD formation.

Table 2.1: Common sulphur oxidizing bacteria involved in AMD formation (Sheoran et al., 2011; Zhao et al., 2012)

Reference: **(USEPA, 2002**)

Microorganism	pH	Temp (◦ C)	Aerobic	Nutrition
Thiobacillusthioparus	4.5-10	10-37	+++	Autotrophic
T. ferrooxidans	0.5-6.0	12-25	+++	Autotrophic
T. thiooxidans	0.5-6.0	10-37	+++	Autotrophic
T.neapolitanus	3.0-8.5	8-37	+++	Autotrophic
T.denitrificans	4.0-9.5	10-37	++/- -	Autotrophic
T. novellus	5.0-9.2	25-35	+++	Autotrophic
T. perometabis	2.8-6.8	25-35	+++	Autotrophic
T. intermedius	1.9- 7.0	25-35	+++	Autotrophic
Sulfolobus acidocalderius	2.0- 5.0	55-85	+++	Autotrophic
Desulfovibrio desulfuricans	5.0-9.0	10- 45	+++	Heterotrophic

2.4.2 Role of oxygen in acid mine drainage generation

Mine tailings have higher oxygen content since they are in contact with atmospheric oxygen where various chemical reactions are occurring because atmospheric oxygen acts as driver for oxidation reactions (Macías *et al.*, 2017). The chemical reaction rate is conditioned by the concentration of oxygen and the oxidation of sulphide is substantially reduced when the concentration of oxygen is less than one percent (< 1%) (Macías *et al.*, 2017). The temperature also plays a huge role and according to Akcil and Koldas. (2006), higher temperatures promotes higher chemical reaction rate due to the exothermic nature of oxidation reactions whereas some sulphide such as framboidal, pyrite, marcasite and pyrrhotite are easily oxidized. In essence, the physical structure of sulphide mineral also contribute to the reaction rate since crystalline sulphides less exposed to oxygen are less oxydated compared to sulphides more exposed to atmospheric oxygen (Akcil and Koldas, 2006).

2.4.3 Role of water in acid mine drainage generation

Water also plays a double role the process of AMD formation because water acts both as a reactant and medium for bacteria in the oxidation processes (Macías *et al.*, 2017). Others factors influencing the generation of AMD are: Type of contaminants, type of sulphide minerals, type of carbonate minerals as illustrated in the **Figure 2.2** below. However, the physico chemical characteristic of AMD formed is highly depending of the contribution percentage of each contributing factor.

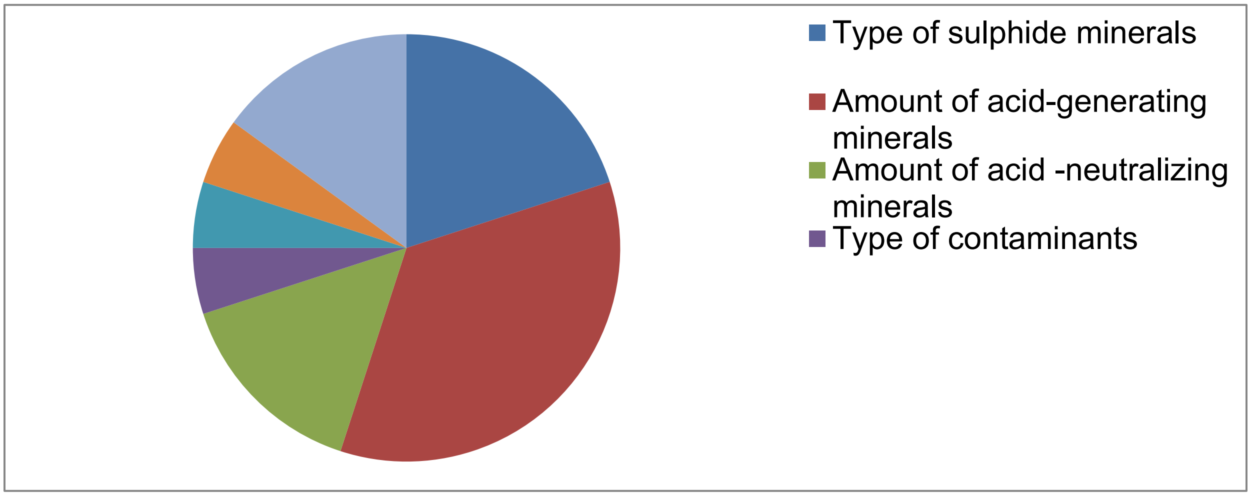


Figure 2.2: Factors influencing the formation of acid mine drainage

2.5 Types of mine drainage

Mine drainage is by-products of mining industry generated during mining operation and in some cases after the cessation of mining activities. It is the results of chemicals reaction that occurs during operational phase of mining and in some cases after mining activities have ceased. Dietz and Dempsey. (2002) classified mine drainage in two broad types named acid mine drainage (AMD) and neutral/alkaline mine drainage (NAMD).

2.5.1 Acid mine drainage

Acid mine drainage is the most documented since it is seen as the most severe environmental hazard resulting from mining industry (Sangita *et al*., 2010; Roychowdhury *et al*., 2015)**.** It is formed when an iron sulphide bearing minerals (pyrite) is in contact with water and air and the reaction that follows leads to the production of sulphuric acid and dissolved ions to form a net acidic solution (Pozo-Antonio *et al.*, 2014; Aguiar *et al.*, 2018; Skousen *et al*., 2019).Acid mine drainage seems to retain attention worldwide and well documented compared to neutral/alkaline mine drainage which is not really documented.

2.5.2 Neutral/alkaline mine drainage

Neutral/alkaline mine drainage is formed following the exposition of already generated AMD to oxygen and moisture (Equeenuddin *et al.*, 2010; Cravotta and Brady, 2015; Nordstrom *et al*., 2015a). Once exposed to oxygen and moisture, if the AMD contains sufficient carbonate buffering, this carbonate will be dissolved as illustrated in the Equation 2.10

$$CaCO_3 \quad + \quad H^+ \qquad \leftrightarrow \quad Ca^{2+} \quad + \quad HCO_3^- \qquad \text{(Eqn 2.10)}$$

The dissolution of carbonate minerals will increase the pH of AMD and the resulting product is neutral/alkaline mine drainage (NAMD). Neutral/alkaline drainage with pH (6-7) and moderate concentration of trace metal such as Arsenic and Zinc and is less documented compared to AMD (Uster *et al.*, 2014) but is also harmful to the environment (Doulati Ardejani *et al.*, 2013, Doulati Ardejani *et al.*, 2014; Uster *et al.*, 2014)**.**

2.6 Physico-chemical properties of acid mine drainage

Once generated, AMD is characterized by low pH, high concentration of major metals such Iron, Aluminum, Manganes and low concentration of trace metals such arsenic, nickel, copper, zinc, elevated electrical conductivity and high concentration of total dissolved solid (TDS) and very high concentration of sulphate (Gaikwad and Gupta, 2008). However the physico-chemical the physicochemical properties chemical properties of AMD vary according to the nature of mine and the geophysical characteristics of the underlying geology combined to the water quality, local climatic conditions and type of bacteria involved in the generation process dictate of the final AMD (Sheoran *et al*., 2010; Amos *et al.*, 2015; Nordstrom *et al*., 2015b)**.**

2.6.1 Physical properties

Colour is among the most visible physical properties of AMD and it depends of the nature of dissolved chemical species (Dold, 2014). For instance, the greenish colour of AMD is associated to presence of copper thereby suggest AMD from copper mine (Simate and Ndlovu, 2014). The bluish colour of AMD is associated to ferrous ion, white colour of AMD is associated to the presence of ion aluminum while black colour of AMD is associated to the presence of ion magnesium (Mg^{2+}) (Shim *et al.*, 2015). However, the colour of most AMD is dictated with the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) leading to an AMD reddish in colour (Tutu and Cukrowska, 2008). In most cases, the formation of ferric iron sediment leads to the orange-yellow colour commonly known as yellow-boys (Amos *et al.*, 2015; Akinwekomi *et al.*, 2020). Overall, AMD transmits its colour to the receiving water bodies thereby leading to the change of aesthetic quality of water and aquatic ecosystem of receiving water. In addition, hardness, total dissolved solid (TDS) and total suspended solid (TSS) of receiving waterbodies are also affected as results of AMD pollution (USEPA, 2017).

2.6.2 Chemical properties

The chemical property of AMD depends of the chemicals species present with pH playing a huge role in chemical species since low pH (2-4) is suitable to the oxidation of most metals thereby releasing ions and increase the sulphate concentration whereas alkaline pH is suitable for the precipitation of metals through reduction reaction leading to low metals concentration in AMD (Masindi *et al.*, 2015). Generally, typical AMD has very low pH (≤ 4) and contains high concentration of Al (70-500 mg/L) (Masindi *et al.*, 2017), Ca (50-450 mg/L), Mn (20-100 mg/L) and Mg (50-500 mg/L) very high concentration of Fe (300-8000 mg/L) (Petrilakova *et al.*, 2014) and sulphate (1000-80000 mg/L) (Petrilakova *et al.*, 2014) and low concentration of trace metals such as Cu, Ni, Pb, Zn (Madzivire *et al.*, 2010; Trumm, 2010). However, metal concentration in AMD water is highly dependent of pH level. As the pH increases, the concentration of chemical species decrease (Masindi *et al.*, 2015) and AMD is progressively changing to NAMD. As such, drinking water can be reclaimed and minerals of economic values recovered from AMD. However, it is important to bear in mind that radioactive substance may also be present in AMD from gold mine (Masindi and Tekere, 2020). The high concentration of sulphate, magnesium and calcium found in NAMD may be attributed to NAMD pH level above the precipitation ability of most metals in water (Akinwekomi *et al.*, 2020). For instance, the study of (Gitari *et al.*, 2008) on physico chemical properties of AMD revealed very low pH (~2), higher concentration of Iron (6000 mg/L) and very higher level of sulphate (24 000mg/L), significant concentration of Mn, Mg, Al, Cu, Pb, Na, Ni, Ca, and others trace metals. These characteristic of AMD (very low pH, higher level of iron and sulphate) render the water unfit for domestic use, agricultures, industries, or any kind of commercial activities (Alegbe *et al.*, 2019) thereby leading to huge impact of AMD on the environment.

2.7 Environmental impacts of acid mine drainage

Once generated, AMD becomes social, economic and especially environmental problem (Talukdar *et al.*, 2016). Released into the environment untreated, toxic pollutants present in AMD water become readily available to all types of life forms (Masindi and Tekere, 2020) thereby leading to devastating consequences on different environmental compartments (Simate and Ndlovu, 2014; Zhang *et al.*, 2020). The problem of AMD is not limited to the local area surrounding the source, but may be extended to distances since the untreated acidic water discharged to the neighboring stream can be transported for a long distance leaving receiving streams devoid of most living creatures (Watmough et al., 2007; Luís *et al.*, 2009; Malar *et al.*, 2016). Acid mine drainage polluted streams may have pH as low as 2.0 to 4.5, levels toxic to most forms of aquatic life (Luís *et al.*, 2009;

Gonah, 2016). As results of low pH due to AMD pollution, metals such as Al, Fe and Mn become soluble in water thereby increasing in concentration to the level toxic to most aquatic ecosystems life form (Sangita *et al*., 2010; Gonah, 2016; Bwapwa, 2017)**.** The negative effects of acid mine drainage in fact are not only limited to the environment since it is also a threat to human and other living being as it contains non-biodegradable toxic chemicals species and thus tends to accumulate in living organisms including man thereby causing a significant public health problem such as skin lesion, hyperpigmentation, cancer, respiratory, pulmonary, gastrointestinal and cardiovascular failure in addition to nerve damage (CSIR, 2013; Moeng, 2019)**.** For instance, the study of Coetzee *et al.* (2006) in the Witwatersrand basin has revealed that the presence of radioactive substances as result of AMD pollution may increase the risk of cancer in human being. As such, the toxic effects of AMD water can be tested using organisms such as Daphnia and Zebra fish placed in ‘’test chambers’’ full of AMD water where the lethal concentration (LC) and lethal dosage (LD) is measured using whole effluent toxicity (WET) method (Singh *et al*., 2017; Bownik, 2020) among others.

Depending of the area and geohydrologicals factors, If the AMD producing mine is located in water permeable ground, water with low pH infiltrates into the groundwater and expand over a wide surface area through ground water movement and finally finds his way in well and bore wells and can be consumed by human (Coetzee *et al.*, 2006; Moeng, 2019). The issue of AMD in South Africa is drawing activists and media worldwide including CNN, Sky News, Reuters and BBC (Name and Sheridan, 2014). However, their target is more on environmental effects of AMD and especially on surface water since those impacts are more visible and the impacts on human health seem to be neglected since they are long term effects and less visible. South Africa is a semi-arid country with very limited freshwater resources which are further threatened by pollution emanating from human activities and especially mining industries (Adewumi *et al*., 2010; McCarthy, 2011; Naidoo, 2017)**.** In the near future, South Africa could face a threat to its water security if the issue of pollution and especially from mining industry is not fully addressed since the AMD is severely contaminating surface and groundwater in the Witwatersrand basin (Akcil and Koldas, 2006; Africa, 2019) leading to an impairment of ground and surface water due to high level of toxic pollutants (Bwapwa, 2017; Moeng, 2019).

The effects of AMD on the economy should not be neglected. Acid mine drainage has the potential to damage infrastructure and equipment due to its corrosive nature (Johnson and Hallberg, 2005; Sangita *et al*., 2010; Sun *et al.*, 2020). The effects of AMD in streams are disastrous since it causes ecological harm to downstream areas (Naidu *et al.*, 2019). As consequences the treatment cost may be incurred by mining companies in the form of abating pollution, water treatment and reclamation costs. However, in case where mining companies go defunct and not able to tackle environmental and public health problem associated with mining activities, it belongs to the government to take such action and this will likely has a negative effect in the gross domestic product (GDP) of a country. This situation can lead to the recession of the economy as results of rise in unemployment. In order to prevent environment, public health and socioeconomic problems associated with AMD, it is necessary to treat such acidic and much polluted water prior to the discharge into the environment. Since AMD is generated by actives and abandoned mines, its environmental effects and associated public health problems can persist for decades or even centuries if nothing is done to mitigate those impacts (CSIR, 2013; Africa ground water atlas, 2019)**.** In that regards, numerous pollution abatement and treatment technologies have been developed. Currently, the hybrid approach is been investigated as means to improve the treatment efficiency and reduce environmental impacts of acid mine drainage.

2.8 Acid mine drainage abatement

Acid mine drainage abatement is a set of methods applied to reduce or prevent AMD formation from both actives and abandoned mines. The main objective of AMD abatement is to prevent the formation of large volume of AMD by limiting the exposed mine area and tailing to contact water, oxygen and microorganisms which are the main catalyzers behind AMD formation (Sahoo *et al.*, 2013; Sánchez-Andrea *et al.*, 2014; Kefeni e*t al*., 2017; Park *et al.*, 2019) or through the elimination of one of the catalyzers (Pozo-Antonio *et al.*, 2014; Kefeni *et al*., 2017). However, the abovementioned technologies are not a long-term solution since their efficiency decrease over time and the continuous generation of

AMD becomes unavoidable thereby proving that it is practically difficult or even impossible to inhibit the formation of AMD at source. It is therefore important that more research be conducted to develop environmentally friendly technologies to treat the already generated acid mine drainage as discussed below.

2.9 Treatment technologies of acid mine drainage

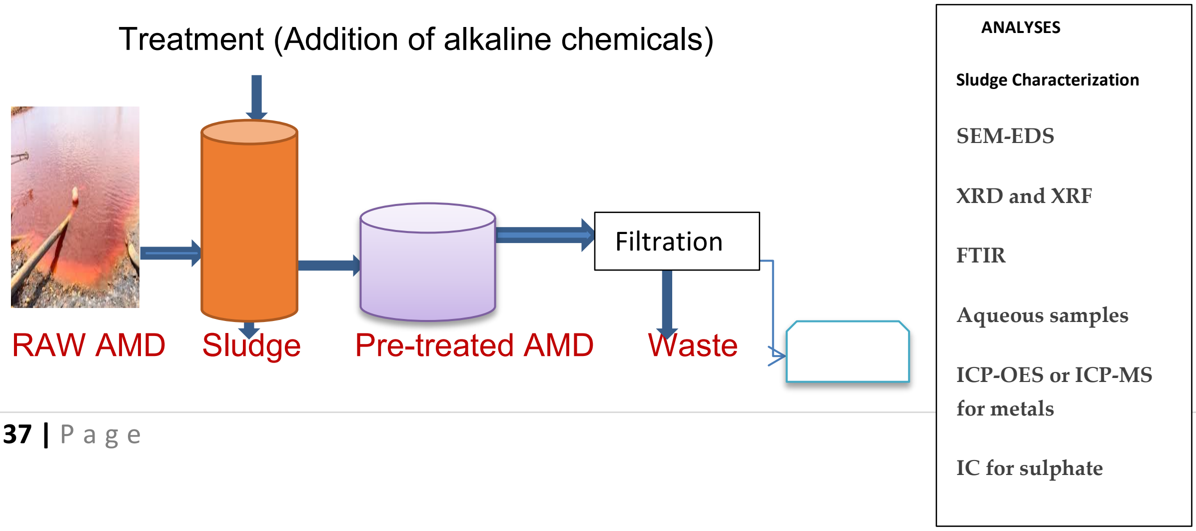
Acid mine drainage treatment technologies are a set of methods used to treat AMD or to remediate it when it is already released into the environment. The objectives of AMD treatment include raising the pH and precipitate metals (Sánchez-Andrea *et al.*, 2014; Masindi *et al.*, 2015; Akinwekomi *et al.*, 2017) , recovery and reuse of mine water within the mining operations (Sánchez-Andrea *et al.*, 2014; Akinwekomi *et al.*, 2017) production of potable drinking water (Masindi, 2017b; Akinwekomi *et al.*, 2020) as well as protection of human health (Africa ground water atlas, 2019; Moeng, 2019) and in some cases, minerals of great economics values can be recovered from AMD treatment (Nleya *et al.*, 2016; Akinwekomi *et al.*, 2020**)**. To this end, various treatment technologies have been developed to tackle acid mine drainage. However following the limitations of already developed technologies, researchers and environmental engineers are still in quest to develop a cost effective, long-term sustainability and environmentally friendly technology.

2.9.1 Active treatment

Active treatment method is the use of alkaline chemicals to raise the pH and precipitate metals. Active treatment falls into two main categories: (i) fixed plant and (ii) in-situ. (Johnson and Hallberg, 2005; Jennings *et al.*, 2008**)** stated that active treatment approaches fall into two main categories: The fixed plant is a conventional active treatment plant built next to the AMD production site and the AMD is pumped from production sites to the treatment plant where reagents and others chemicals are added to raise the pH. Treated AMD and sludge are then collected and discharged separately. An in-situ active treatment approach consists to use portable land-based or water-based systems to treat a site or stream polluted by acid mine drainage. This approach is efficient to treat AMD at both low and high flow rate. However, in-situ treatment approaches generate toxic sludge which may be transported and deposited downstream of the treatment site leading to another environmental problem to be solved (Rambabu *et al.*, 2020). Various potable active approaches are currently applied for AMD treatment and they include neutral-mill dosing system (NDS), calibrated reagent applicating blender (CRAB), aqua-fix system (AFS), hydro-active limestone treatment (HALT) (Jennings *et al.*, 2008). Both fixed plant and in-situ treatment approaches uses chemicals as their actives agents to raise the pH and precipitate metals. A wide range of alkaline chemicals including Limestone ($CaCO_3$), hydrated lime [$Ca(OH)_2$], caustic soda (NaOH), soda ash (Na_2CO_3), calcium oxide (CaO), anhydrous ammonia (NH_3), magnesium oxide (MgO), cryptocrystalline magnesite ($MgCO_3$), calcined magnesite (MgO) and hydrated magnesite ($Mg(OH)_2$), periclase and brucite are the most alkaline chemicals used during active treatment of AMD. The effectiveness of each chemical depends on factors such as the site specificity (seasonal variation), daily AMD load and metal concentration (Skousen and Ziemkiewicz, 2005; Zipper *et al.*, 2018; Skousen *et al.*, 2019). Lime neutralization, carbonate neutralization and ion exchange are the most widely active technologies used to treat AMD (Johnson and Hallberg, 2005; Jennings *et al.*, 2008; Skousen *et al.*, 2019). In South Africa, Reverse osmosis is another active treatment technology that is currently being used by Anglo American PLC (Name and Sheridan, 2014; Akinwekomi *et al.*, 2017) while magnesite is currently being used to neutralize AMD water and precipitate metals and remove inorganic pollutants (Masindi *et al.*, 2015; Masindi, 2016).

2.9.1.1 Neutralization and acid mine drainage treatment

The most common method for AMD treatment is neutralization technology which consists to the addition of alkaline reagents/chemicals to increase the pH and precipitate heavy metals. Through this technology, lands contaminated by AMD can be reclaimed by addition of alkaline chemicals to neutralise the acidity or by addition of non-polluted top soil and planting vegetation to stabilize the soil while AMD water can be neutralized by addition and stirring of alkaline reagents to AMD water for a given period. (Masindi *et al.*, 2015) assessed the potential of magnesite cryptocrystalline to neutralize AMD and attenuate inorganic contaminants. Magnesite and AMD water were mixed at 1:100 (1g:100 ml) ratio and stirred for one hour. The finding revealed net increase of pH and attenuation of inorganic contaminants however with release of highly contaminated sludge. Beside magnesite, others chemical such as lime are also widely used to neutralize AMD water. Pyrbot *et al*. (2019) investigated the potential of open limestone channel (OLC) to neutralize AMD water in East Jaintia Hills District, Meghalaya, India. The finding revealed that the OLC was able to raise the pH of AMD water from 4.31-6.57 promoting the appearance of many aquatic life forms which could not support the acidity of the river due to AMD pollution. Active technologies present some advantages including: increase of pH, precipitation of metals, less surface area to implant the treatment plant. Furthermore, they are suitable for high water discharge and high acidity. However, active technologies present some drawbacks: consumption of high quantity of alkaline chemicals which are not always available, ineffectiveness to remove metal ions at low concentration thereby requiring an oxidation step. Production of huge volume of sludge which requires additional cost for handling and disposal (Crini and Lichtfouse, 2019). Active treatment technologies are effective in treating AMD but they are not cost effective since they incur high capital to establish the system and also high operational cost for the overall life time of the system. For instance, Costello. (2003) stated that the annual maintenance cost of an active AMD treatment system in Australia is around USD 64000. Active treatment technologies generate a huge volume of toxic sludge (Ochieng *et al*., 2010; Masindi, 2016) thereby leading to a new environmental problem to be solved. Following the drawback of active treatment technologies of AMD, their feasibilities are difficult or even impossible and especially for developing and emerging countries. They also require constant monitoring and maintenance by experts and do not provide a long-term solution to acid mine drainage treatment thereby leading to the need by the researchers to investigate others treatment technologies such as passive treatment. A typical AMD active treatment is shown in **Figure 2.3**.



Clean water

Figure 2.3: Schematic illustration of a typical active acid mine drainage treatment plant

However, neutralisation technology can be associated with passive technology such as phytoremediation using suitable plant species such as *Vetiveria zizanioides* to decontaminate highly polluted sludge.

2.9.2 Passive treatment

Passive technology is an alternative approach to active technology and it is been investigated for the treatment of AMD (Costello, 2003; Johnson and Hallberg, 2005; Jennings *et al.*, 2008; Zipper *et al.*, 2018; Skousen *et al*., 2019). They have been used for decades to treat acid mine drainage of varying compositions and different pH levels (Jennings *et al.*, 2008; Akinwekomi *et al.*, 2017) and from abandoned mines (Masindi *et al*., 2018b). Passive technologies treat the effluent by adjusting themselves into an automatic renew system where constant monitoring is not necessary (Ochieng *et al.*, 2010; Skousen *et al.*, 2017)**.** They are amongst the most used AMD treatment technologies and utilize naturally occurring energy sources such as vegetation and microbial metabolic energy. Passive treatment can become a sustainable solution to AMD in the future if more researches are conducted to investigate their full potential in AMD treatment (Jennings *et al.*, 2008; Zipper *et al.*, 2018). They consist mainly of wetland and emerging passive treatment technology such as phytoremediation (RoyChowdhury *et al*., 2019). Passive treatments are relatively new and involve the use of naturally occurring biological and chemical processes to treat AMD water in a self-chemicals and biological reactions to treat AMD in a self-controlled medium.

2.9.2.1 Wetlands

Wetlands are complex ecosystems consisting of water and sediments supporting macrophytes and have the potential to naturally remove pollutants from water via a range of physico-chemical reactions, microbial and plant-mediated processes (Vymazal, 2011; Lizama Allende *et al*., 2012b; Sultana, 2014). Wetlands have been used for centuries as treatment technologies for wastewater (Groudev *et al.*, 2002; Matthies *et al*., 2010; Prihatini *et al*., 2016)**.** Compared to conventional technologies, wetlands appear to be more attractive as they are environmentally friendly and cost effective. Furthermore, wetlands do not require a specific skill for their maintenance and they can remain effective for close to 15 years if properly designed and installed (Sheoran, 2017; Palihakkara *et al.*, 2018). However, the efficiency of wetland in treating harsh waste water such as AMD is hardly depending of the ability of plant species to tolerate AMD waste water (Kiiskila *et al.*, 2019, 2020; Nguegang *et al.*, 2021). A typical wetland usually has three components which are: Vegetation or macrophytes, media or substrate and microorganisms. These three components interact and operate simultaneously making constructed wetland complex bioreactors. Each of these three components is described below.

1: Vegetation

The vegetation is dominated by macrophytes which play an important role in constructed wetland. They stabilize the temperature, reduce wind speed and limit the re-suspension of nutrients and sludge, supply surface for periphyton and bacteria and help in providing the optimal conditions for various biological, physico-chemical processes within a constructed wetland thereby improving the treatment efficiency (Shelef *et al*., 2013) . The selection of plants to be used in constructed wetland should consider many factors such as climate, the type of waste water and the tolerance ability of the plant to the wastewater to be treated (Kadlec and Wallace, 2008).

Macrophytes provide the structure that stimulate pollutants removal processes (Kadlec and Wallace, 2008) , thereby seen as major component of constructed wetland. For metals removal, plants can act directly or indirectly. Many studies have shown that pollutants direct uptake by macrophytes is often not the principal pollutants removal mechanism (García-Ávila *et al.*, 2019; García-Ávila, 2020). In constructed wetland, macrophytes however play an indirect but important role because they can:

1- Foster the growth of metal-oxidising bacteria by oxygen transfer into the rhizosphere **(**Shelef *et al*., 2013).

2- Provide organic matter as a carbon source for sulfate-reducing bacteria (SRB) and metal-oxidising bacteria (Shelef *et al*., 2013) .

3- Provide a surface for microorganisms within the water column (Türker *et al.*, 2016).

4- Provide their roots as a surface for iron plaque, which is mostly iron hydroxides precipitates. This plaque can be highly reactive with different metals and metalloids, bonding As, Mn, Zn, Cu and Pb (Kröpfelová *et al.*, 2009). **Table 2.2** summarizes the main characteristics of some plants species commonly used in constructed wetland.

Table 2.2: Some plants species suitable for FWS and SSF wetlands (Kadlec and Wallace, 2008)

Common name	Scientific name	Types of wetland	Geographical distribution	pH range	Salinity	Comments
Cattail (broadleaf)	Typha latifolia	FWS, SSVF and SSHF	Across north America	5.5-7.5	Less than 0.5 ppt	Used as a food source by aquatic mammals
Cattail (narrowleaf)	Typha angustifolia	FWS, SSVF and SSHF	Across north America	3.7-8.5	Less than 0.5 ppt	Tolerates low pH

Common reed	Phragmites australis	FWS, SSVF and SSHF	Worldwide	3.7-9.0	Up to 20 ppt	Used mostly in Europe
Green bulrush	Schoenoplectus (Scirpus) atrovirens	SSVF and SSHF	North and Central America	-------	Fresh	Low maintenance in SSVF and SSHF plant
River bulrush	Schoenoplectus (Scirpus) fluviatilis	SSVF and SSHF	North and Central America: New Mexico, California	-------	Fresh, less than 0.5 ppt	Low maintenance in SSVF and SSHF plant

2: Media

Gravel, grits and sand are the most common media used in constructed wetlands. However, soils and other substrates have also been used for the removal of different pollutants. Media provide support to plants growth and also serves as energy source for biogeochemical reactions. Media play filtration role by trapping suspended materials in the pore spaces between the media particles. Media are also a place of sedimentation of various suspended particles from the waste water. In an aerobic wetland, media convert ammonia to nitrate (nitrification) and further converts nitrate to gaz (denitrification) in case of anaerobic wetland (Lizama Allende *et al*., 2012a). Media also play a major role in pollutants reduction since they absorb pollutants such as heavy metals thereby facilitate their accumulation by the plants roots.

3: Microorganisms

Microorganisms play vital roles in the wetland functioning as both primary producers and decomposers. Inorganic contaminants removal in constructed wetlands is primary due to microbial activity (Faulwetter *et al.*, 2009). Microorganisms are often attached to plants roots, and their metabolism is important in the removal of inorganics pollutants *(Lizama Allende et al.*, 2012b). In wetland, most microorganisms are found at four level, substrate, decaying solid, organic matter and solid surface of plant (Kadlec and Wallace, 2008)**.**

2.9.2.2 Wetlands and acid mine drainage treatment

Wetlands have been used as alternative solution to mitigate the harmful effects of active technology. In that regard, Sheoran and Sheoran. (2006), investigated the potential of natural wetland equipped with three plants species (*Typha angustifolia*, *Desmostachyabipinnata* and *Scharum Bengalese*) in treating AMD and the findings revealed that the system was efficient in removing metals, sulphate and reducing total hardness. From these results, it follows that natural wetlands can provide a sustainable solution for the treatment of mine water as long as the hydraulic loading rate (HLR) of water do not overwhelm their capacity, though periodic maintenance is recommended. However, it is unsure about all components of natural wetland which contributed to improve the quality of AMD and it emerges that a proper wetland should be designed and constructed with known components in order to assess with accuracy the extent of the system in improving AMD water quality. Sheoran. (2017) assessed the potential of free water surface constructed wetland (FWS-CW) equipped with emergent macrophyte *Desmostachya bipinnata* in treating AMD. The researcher used a mixture of (soil + goat manure + wood shaving) as substrate and the experiment lasted for 168 hours (7 days). The results showed that within 24 hours of retention time, there was a significant increase of pH from 2.93 to 7.22 and alkalinity from zero mg/L to 204.30 mg/L as $CaCO_3$, while electric conductivity decreased and increased likely due to the recirculation of treated water. Turbidity decreased by 30.14 to 66.65% sulphate reduced by 21.52 to 28.09%, acidity reduced by 88.89 to 100%, hardness reduction was 18.18 to 26.07%. The removal efficiency of metal was 95.51% for Fe, 91.72% for Cu, 78.21% for Zn, 90.12% for Pb, 72.33% for Co, 63.76% Ni and 76.44% for Mn respectively. The study further revealed that the mixture of substrate was very significant in the efficiency of the wetland since it accelerated sulphate reduction which led to the rise of pH and metals precipitation thereby facilitating their accumulation by the plants. The substrate in this study may play a role of neutralizer which is similar to magnesite in the study of Masindi *et al.* (2015). Macrophytes play a crucial role in FWS-CW since they remove heavy metals from acid mine drainage by two major mechanisms: Phytoextraction and rizhofiltration. In phytoextraction, plants accumulate metals from wetland substrate and store them in their roots/shoot whereas in rhizofiltration, plants concentrate metals in their root zones (Padmavathiamma and Li, 2007). Free water system wetlands are more efficient in removing iron, aluminum, manganese but they cannot handle typical AMD effluents and their efficiency in treating AMD depends of the factors such as seasonal variation, acidity, metals load and metals concentration (Sheoran, 2017; Pat-Espadas *et al.*, 2018). In order to deal with the above mentioned disadvantages, free water system wetlands are amended by changing the water flow regime to obtain subsurface constructed wetland.

Similarly to free water surface constructed wetland, subsurface horizontal flow constructed wetland also neutralizes AMD, and reduces pollutants concentration. Prihatini *et al*. (2016) assessed the performance of SSHF-CW with *Purun tikus* aquatic plant in removing soluble-Fe and raising pH of AMD. The experiment consisted of batch flow system and the continuous flow system. The results revealed that the batch flow system was more efficient (89%) in removing concentration than the continuous system (81.44%). However, the study further revealed that in the continuous flow system the waste water quality standard was met in the fifth days while in the batch experiment, it took longer (21 days). In the continuous system, the pH rose from 3 to 4.97 within four days while in the batch experiment, the pH rose from 2.5 to 3.42 within 15 days. The finding of this study clearly revealed that SSHF-CW with *Purun tikus* is capable of raising the pH and remove iron from AMD with better performance with the continuous flow system. Given that the system was able to neutralize the AMD, in addition to iron, others metals were also precipitated and sulphate reduced and therefore can be considered efficient for the treatment of AMD as long as plant species used is tolerant to acidic wastewater since SSVF-CW is more efficient in removing Al, Mn, Zn and Ni (Vesper and Smilley, 2010; Vymazal, 2010; Zhang *et al*., 2010; Andreo-Martínez *et al.*, 2017; Sheoran, 2017; Tatoulis *et al.*, 2017; Pat-Espadas *et al.*, 2018). Subsurface horizontal flow constructed wetland present several advantages such as reliable low cost, aerobic post treatment solution and provide high and consistent levels of treatment efficiency when they are well designed (Kadlec and Wallace, 2008; Andreo-Martínez *et al*., 2016). Despite its efficiency in treating AMD, the major inconvenience of SSHF-CW is clogging which results to the flow of water at the surface thereby compromising the efficiency of the system (Kröpfelová et al., 2009; Singh and Chakraborty, 2020). However, this problem can be solved by pre-treating the waste water and continuous maintenance of the system or by modifying the regime flow of water to obtain subsurface vertical flow wetland.

Demchak et al. (2001) reported that four vertical flow wetland with limestone as substrates were able to significantly reduce acidity of AMD while Prihatini et al. (2016) reported that *Purun tikus* was able to increase the removal efficiency of subsurface vertical flow wetland treating AMD from 15.53% to 78.94%. From this result, it follows that SSVF-CW can be used to treat AMD but the macrophytes must be a metals accumulating plant since in the above mentioned study, plant play a significant role by increasing the efficiency of the wetland in treating AMD by 63.41%. Macrophytes appear to be a very important component of wetland since they provide the structure that stimulates pollutants removal processes (Kadlec and Wallace, 2008) thereby seeing as major component of constructed wetland.

Different types of constructed wetland can be associated to form a mega system or hybrid wetland operating simultaneously in order to achieve better treatment efficiency by exploiting the advantage and particularity of each type of wetland (Vymazal, 2010, 2011; Pare *et al.*, 2011; Wang *et al.*, 2017; Herrera-Melián *et al.*, 2020). Most hybrid systems of wetland combine vertical flow and horizontal flow (Sayadi *et al.*, 2012; Ventura *et al.*, 2019, 2021; Herrera-Melián *et al.*, 2020). However, free water system can also be inserted to improve the efficiency of the system (Vymazal, 2011). In fact constructed wetlands are adapted from natural wetlands and they have the potential to improve water quality (USEPA, 2017).

Lesage *et al.* (2007) assessed the potential of a hybrid system (SSVF + SSHF) to accumulate heavy metals waste water. The system consisted of two SSVF ranged in parallel followed by two SSHF also ranged in parallel and the plant used was *P. australis* and the metals considered for the project were: Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The waste water percolated through the SSVF and channeled by the pipes to the SSHF. Leachate were collected at the external bottom of each wetland using installed valves and analysis was done using ICP-OES and the results revealed that metals were more removed in the SSVF-CW with exception of Mn and Ni which were more removed in the SSHF-CW. This study in fact was focused on waste water treatment but since the aim was to assess the ability of the hybrid approach to remove metals from waste water, it can also be applied for the treatment of acid mine drainage since the studies of (Guo *et al.*, 2015; Tercero *et al.*, 2017; Nagy *et al.*, 2020) have shown that *P. australis* is efficient in term of metals accumulation from acid mine drainage.

Prihatini et al. (2016) investigated iron removal from acid mine drainage using a hybrid constructed wetland with *Eleocharis dulcis*. The system consisted of subsurface vertical flow constructed wetland (SSVF-CW) and subsurface horizontal flow constructed wetland (SSHF-CW) in South Kalimantan, Indonesia. The results revealed that iron removal was more efficient in SSVF-CW than SSHF-CW. The researcher did not determine the contribution of wetland substrate in iron removal since the investigation was limited only to metal concentration in plants tissues. Substrate plays a crucial role in wetland performance such as pollutant sedimentation and cannot be left aside when assessing the performance of constructed wetland. Furthermore the treatment of AMD shouldn't be limited to one parameter. Other important parameters such as pH and sulphate concentration should be considered when treating AMD.

Constructed wetlands (CWs) appear as cost effective and environmental friendly technology in AMD water treatment. However, they present some drawbacks such as the reduction of removal efficiency of metals when the pH of the AMD rises (pH > 4.2) (Woulds and Ngwenya, 2004; Sheoran, 2017). Generally the removal efficiency is less than 34% at alkaline conditions (Sheoran and Sheoran, 2006). Plants play an important role in the CW including the accumulation of heavy metals; the selection of plants species to be used in CW system is crucial since it should be done taking into account the characteristics of AMD water to be treated (Türker *et al.*, 2016). Another disadvantage of CW is the high concentrations of toxic metals which can become toxic to the plants and microorganisms thereby reducing the ability of the plants to uptake nutrients and non-toxic metals (Rengel *et al.*, 2015; Dubey *et al.*, 2018). Constructed wetlands require minimum maintenance and as such, they can be installed in remote area or abandoned mine sites where it is impossible to install an active treatment plant. However they require large land spaces and the cost of installation is affected by the retention time, the treatment goal, and media type, number of cells, source and availability of substrate to be used. Analyzing the performance of each types of wetland, it follows that for metals removal, the efficiency varies according to the type of wetland. For instance, the study of Pat-Espadas *et al.* (2018) revealed that free water system constructed wetland is more efficient to remove iron with removal efficiency up to 92% whereas the studies of Sheoran. (2017) revealed that both SSVF-CW and SSHF-CW are more efficient to remove Al, Mn, Ni and Zn.

2.9.2.3 Emerging passive treatment technologies: phytoremediation

Phytoremediation is the use of selected plants species to decontaminate polluted soils and aquatic system (Chekroun and Baghour, 2013). The plants used in phytoremediation can be either aquatic or terrestrial. Plants accumulate heavy metals in different concentrations, but significant differences in metal accumulation exist between and within plant species (Mellem, 2005). The phytoremediation process involves three mechanisms: (1) phyto-extraction which is a process of removing heavy metals from soil and water and especially heavy metals with high density, (2) phyto-stabilization which reduces the mobility of heavy metals in soil and water thereby facilitating their accumulation by plant, (3) rhizo-filtration or (phyto-filtration) which is the absorption of contaminants into plants roots (Tiyasha and Shaktibala, 2013; Abubakar *et al*., 2014; Galal *et al.*, 2018). Phytoremediation can be used to treat both polluted soil and water (Andreazza *et al.*, 2015).

Groudev *et al.* (2001) in Curilo, Bulgaria reported that *Typha. sp* and some algae related to the genera *pediastrum Eudorina, Volvox, Melosira* and *Scenedesmus* growing in an AMD polluted sites were accumulating heavy metals by means of natural phytoremediation. The finding revealed that metals were more stocked mainly in their roots systems for *Typha sp* while *pediastrum Eudorina, Volvox, Melosira and Scenedesmus* were efficient accumulators of metals. This study clearly demonstrated that AMD can be treated by phytoremediation using acid tolerant plants and algae. However, high concentration of metals in AMD can be toxic to plants thereby compromising the efficiency of the wetland system.

Sakiah. (2006) assessed the performances of two grass species (*Vetiver* and *Eragrotis curvula*) to grow in acid mine drainage polluted area and their potentialities to extract metals. Two grass species (*Vetiver* and *Eragrotis curvula*) were planted in an AMD polluted soil and potty soil without AMD for control purposes. After two months, it was noted that Vetiver growing normally in both soil while *Eragrotis curvula* showed delay growth in acid mine drainage soil and potty soil. Vetiver was harvested separated into roots, stem and leaves followed by the determination of metal concentration by ICP-MS methods and the results revealed high concentration of As, Cd, Cr, Pb, Hg and Zn in the roots. The study revealed that Vetiver grass could be a good candidate to treat AMD using phytoremediation technology. However, the researcher considered only roots and others parts of the plants (stem and leaves were not considered). The researcher could have determined metals concentration on shoot and calculate the bio concentration factor (BCF) in order to find out if Vetiver is efficient to be used in phytoremediation of AMD polluted sites since BCF higher than 1 (BCF > 1) indicates the ability of plant species to uptake metals (Zhan *et al*., 2010; Andreazza *et al.*, 2015; Tawde and Bhalerao., 2017; Kafil *et al.*, 2019)**.** The researcher could have determined metals concentration on *Eragrotis curvula* because the fact that it showed delay growth could be the results of high concentration of metals accumulated since Abagale *et al.* (2013) reported that high concentration of metals such as lead, manganese and iron was toxic to plants.

In some cases, plants growing in AMD polluted sites may by means of natural process remediate acid mine waste water. Miguel et al. (2017) reported that *Calamagrostis ligulata* (proceae) and *Juncusimbricatus* (juncaceae) plants species growing in two natural sites polluted by AMD (Mesapta wetland and Hrancapeti wetland) in central Peru were accumulating heavy metals thereby treating AMD polluted sites. The results further revealed that both plants have high potential to accumulate high concentration of Fe, Zn, As and Al but low concentration of Pb and Cd.

Herniwanti et al. (2013**)** investigated the best type of local plants for phytoremediation of AMD. The objectives of their study were to investigate five different species of local plants growing in mining area and assess their ability to accumulate metals from contaminated sites. The potential of the following plants: *Purun rat (Eleocharisdulcis*), *Rumput Umbrella* (*Cyperusoderatus*), Water plants (*Hydrillavercilata*), Kale water (*Ipoema aquatic*) and Kayapu (*Pistiastratatiotes*) were investigated. The results revealed that the above cited plants associated with organic matter were able to accumulate metals (Fe, Mn) thereby increasing the pH of the medium. The finding revealed that *Ipomea aquatica* was a good aquatic plant to repair acidity while *Purun rat* is good to accumulate Fe and *Pistia stratatiotes* is good to accumulate Mn and other plants did not show any phytoremediation ability. The finding of this research clearly showed that some plants growing in the vicinity of area polluted by AMD can be used for phytoremediation of AMD. The researchers considered only two metals (Fe and Mn) for their study and did not evaluate which plant could be able to accumulate many metals. Acid mine drainage is composed of metals such as Cd, Cu, Ni, Pb, Zn, Fe, Al, Cr Mn and Mg (Herniwanti *et al.*, 2014)**.** To consider a plant as a potential candidate for phytoremediation of AMD, the plant should be able to accumulate many heavy metals. Reporting and compare to South Africa context where many active and abandoned mines are generating AMD, it follows that plants species growing in South Africa such as *Vetiveria zizanioides* can also be investigated for their potential to accumulate metals from AMD.

Pérez-Sirvent *et al.* (2017) carried out a study in Sierra Minera, Spain to determine the feasibility of three aquatic macrophytes (*P.australis, Jucuns* effuses and *Iris pseudacorus* for bioremediation of AMD using aerobic wetlands. The objective of their study was to construct two potty wetlands for each plant (one with AMD and one without AMD for control purposes). After one year, plants were harvested from both wetland and separated into roots, stems and leaves carefully washed with water, air dried following by the determination of metals (As, Cd, Pb, Fe and Mn) concentration at each part of the plants and wetland soil. The results revealed that metals were more accumulated in roots than other parts of the plants. The bio concentration factor (BCF) and the transfer factor (TF) were also determined for each plant and it revealed each plant species showed high BCF and low TF indicating that these plants species can be used for bioremediation of acid mine drainage using an aerobic wetland. The researchers did not specify which plant was more efficient in accumulating heavy metals.

Ag Masayuki et al., (2017) studied the potential of aquatic macrophytes *Eleocharis acicularis* to accumulate heavy metals from acid mine drainage in south west Japan. Their study aimed to determine heavy metal accumulation in *E. acicularis* and the influence of silicon on metal uptake by plant. For their methodology, two aerobic wetlands were constructed using the seedling of experimental plant and AMD from mine. The level of metal (Fe, Cu, Zn, Pb, As) in AMD was determined and fertiliser (silicon) was added at the bottom of plant in wetland 1 while no addition of silicon was made in wetland 2. After six months, it was realized that *E. acicularis* adapted well to the mine drainage water with a good growth rate within both wetlands. Plants from both wetlands were harvested separated into roots and leaves; air dried and followed by the determination of metals by a particle-induced X-ray emission (PIXE) method. The results showed that metals were more accumulated in the roots than leaves however with higher accumulation of metal observed in wetland 1 where silicon was applied to fertilize plants. This study project clearly demonstrated the potential of *E. acicularis* to remove metals from AMD and the positive impact of silicon which precipitates heavy metals and accelerate their accumulation by plants. The researchers could have extended their research by determining the quantity of metals moved from each wetland by determining the bioaccumulation factor (BCF) which according to Coakley *et al.* (2019), it is an index of the ability of the plant to accumulate a particular metal depending of its concentration in the soil. The potentiality of the plant to translocate metals from roots to aerial parts of the plants could also be calculated by the researchers using the translocation factor (TF) because it indicates the ability of the plant to translocate metals from the roots to the aerial part of the plants (Galal *et al.*, 2018; Kafil *et al.*, 2019; Usman *et al.*, 2019). The phytoremediation use solar energy and does not require constant maintenance and various metals can be removed at once. It is cost effective and environmental friendly technology and very suitable for emerging and developing countries. It is easily applied in situ without excavation of large amount of contaminated soil thereby leaving the topsoil usable for others purposes once treated (Hossein *et al*., 2016). Phytoremediation can be applied to wide range of inorganic and organic contaminants. However, phytoremediation is limited by the deep of plants roots, depends of the climatic and seasonal conditions (only applicable when the climatic conditions are favorable for the plants growth). It becomes ineffective when plants are disturbed either by pests or diseases (Alkorta *et al.*, 2004; Koptsik, 2014).

2.9.3 Other commonly used passive treatment technologies

2.9.3.1 Anaerobic Sulphate-Reducing Bioreactors (biological treatment)

Anaerobic sulphate-reducing bacteria (ASRB) are types of bacteria facilitating the conversion of sulphate to sulphide with the sulphides reacting with heavy metals to precipitate. These bacteria treat AMD in anaerobic conditions which combine a heterogeneous layer of organic-rich material and limestone added to an additional layer of limestone under the organic layer which provides extra alkalinity and also supports the underlying drainage channels. This type of passive treatment technology is widely used and involves sulphate reducing bacteria such as *Desulfovibrio, Desulfomicrobium, Desulfobacter* and *Desulfotomaculum* to remediate AMD which flows vertically via the organic layer into the limestone bed and finally to the drainage system. The organic layer serves as the substrate where sulphate reducing bacteria reduce SO_4^{2-} to H_2S and oxidize organic matter (CH_2O) to bicarbonate ions (CHO_3^-) as shown in the Equation 2.11.

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \qquad \text{(Eqn2.11)}$$

Sulphate reducing bacteria technology reduces acidity thereby leading to the precipitation of metals. For instance, the study of Sun *et al.* (2020) revealed that Zinc were removed in a controlled bio-mineralisation as ZnS (s) in sulfidogenic bioreactors and iron as schwertmannite by microbial iron oxidation and removed as ferric hydroxide precipitate. The sulphate–reducing bioreactors help to reduce acidity, metal and sulphate concentration of AMD water and improve the overall water quality. Sulphate reducing bioreactor is promising in term of AMD treatment since it can completely remove zinc and reduce the mobility of all metals in AMD (Castillo *et al.*, 2012**)**. Furthermore, it is cost effective. However, SRB is applicable only to abandoned mines producing AMD and his efficiency depends on various factors such as the available surface area, hydraulic retention time and initial sulphate concentration in AMD (Neculita *et al.*, 2007).

2.9.3.2 Anoxic limestone drains (ALD)

They are layers of coarse limestone aggregate placed carefully under a constructed drainage lime (Genty *et al.*, 2012). In ALD treatment process, the reaction of limestone with AMD water produces dioxide of carbon (CO2) which raises the overall alkalinity (LaBar et al., 2008). The optimal performance of the ALD is attained when iron and aluminum are completely removed and the pH become neutral (pH 6 to 8) because under more acidic conditions, metals like Fe and Al precipitate as hydroxides and form coats limestone (Johnson and Hallberg, 2005). Anoxic limestone drains is very limited in treating AMD since it cannot raise the pH of AMD up to 8 (Seervi *et al.*, 2017) . Furthermore, ALD technology also requires pre-treatment step to remove oxygen from AMD (Ordonez *et al*., 1999) or be applied as part of the hybrid passive treatment system in corporation with the aerobic or anaerobic wetlands (Johnson and Hallberg, 2005; Skousen *et al*., 2019).

2.9.3.3 Vertical flow wetlands (VFW)

They are also called permeable reactive barriers (PRB) where AMD water percolates through an organic layer to a limestone bed and finally into the drainage system where ferric ion is reduced to ferrous iron. Sulphate reduction and Fe sulphide precipitation can take place in this system (Johnson and Hallberg, 2005; Skousen *et al*., 2019). A series of drainage pipes placed below the limestone layer carry the water to aerobic ponds where ferrous ions are oxidized and precipitated.

2.9.3.4 Limestone leach beds (LSB)

Limestone leach bed method is conceived to treat AMD water by allowing the liquid to be retained in the limestone layer for a specific period. The limestone layer can be replaced when necessary and the alkalinity in this system can reach 75 mg/L (Skousen and Ziemkiewicz, 2005)**.**

Passive treatment technologies are commonly used to treat AMD, however, the installation of the passive treatment plant depends on several factors such as characteristics of wastewater, availability of land in the case of wetland, flow rate, local topography and environment conditions (Moodley *et al.*, 2018). Many treatment technologies operating individually have been developed to treat AMD but the expected results are yet to be obtained. As such the integration of two or more different technologies also called hybrid approach is getting popularity.

2.9.4 Hybrid approach in acid mine drainage treatment

A hybrid system by definition is a way of working or doing something composed of elements of two or many separate systems (Abdulla *et al.*, 2016; Herrera-Melián *et al.*, 2020)**.** In water engineering, it consists to the combination of two or three distinct and different approaches to treat waste water. It exhibits both continuous and discrete dynamic and it is more efficient in wastewater treatment than single system because of the combination of advantages of each system (Abdulla *et al.*, 2016). In that regard, different types of hybrid system have been developed to treat acid mine drainage. Park *et al.* (2013) investigated the combination of active treatment (neutralization of AMD using calcium oxide (CaO) and magenesium (MgO) and a pipe inserted microalgae reactor (PIMR). The AMD water was firstly treated by raising the pH using CaO and MgO which led to precipitation of metals. The pre-treated AMD water was then polished by a bioremediation method which consisted to load the pre-treated AMD into a pipe inserted microalgae reactor with (*Nephroselmis sp*). The combination led to the neutralization of AMD and efficient removal of metals by accumulation. However this hybrid system is limited only to two parameters, (pH and metals). Metals such as iron contributes to the growth of *Nephroselmis sp* since the growth of *Nelphroselmis* reduced when the concentration of iron decreased (Rout and Sahoo, 2015). For effective treatment of acid mine drainage, sulphate, electrical conductivity and total dissolved solid should be considered since their levels AMD water is generally above the WHO guideline for industrial effluent discharge. Furthermore this method leads to the generation of a huge volume of sludge containing metals in case of a large volume of AMD treated. The management of sludge is another environmental problem emanating from the treatment of AMD water. As such, it is needful to assess and investigate a long time environmental friendly solution for acid mine drainage in order to assure sustainable development for present and future generations.

Oberholster *et al.* (2018) investigated the efficiency of a combination of active and biological technology for sulphate removal in AMD. For active treatment, they used NaOH to increase the pH of AMD from 2.6 to 5 and for their biological treatment, they used selected macro algae to accumulate sulphate, Mg, Ca and P from mine water previously treated. AMD samples were collected from a decanting mine in Calorina, Mpumalanga province, South Africa. pH and EC were measured in situ using a Hach sension 156 portable multiparameter probe (USA) and the determination of S, Mg, Ca and P concentration were done following the standard method for examination of water and waste water (APHA, 2002). Three different types of macro algae (*Microspora tumidula, Oedogonium crassum and Klebsormidium klebsii*) were cultured and exposed in triplicate to previously treated AMD water for 192 h and the results revealed that *M. tumidula* accumulated sulphate and phosphate at pH 5 and Ca at pH level 7 whereas *O. Crassum* showed the greatest accumulation of Mg and Ca at pH 7. Overall, the bioaccumulation of sulphate by the macroalgae was ranked in the order *M. tumidula* > *O. crassum* > *K. klebsii*. No bioaccumulation of Ca was reported with *K. klebsii* while the accumulation of Mg in all macroalgae decreased significantly with the decrease of pH. The authors limited their investigation to sulphate and trace metals present in AMD water. Major metals of AMD water such as iron, Mn and Al were not considered. For effective treatment of AMD, the above cited metals should not be left aside.

2.9.5 Integrated approach

An integrated approach consists to remove pollutants at different stages of the system using different mechanisms. Two or more passive treatment system can also be combined to treat acid mine drainage. For instance, Champagne *et al*. (2005) assessed a combined 5 staged passive system for metals removal and sulphate reduction from acid mine drainage. The combined passive system consisted of a setup of Rock column aeration and iron removal (Stage 1), settling tank precipitation and sedimentation (stage 2), peat biofilter (stage 3), sulphate reducing bacteria and metals removal by bacterial sulphate reduction and alkalinity addition (stage 4) and anoxic limestone drain (stage 5) followed by leachate collection valve. AMD water was flowing continuously from a reservoir tank to the passive hybrid system (from stage 1 to stage 5). The novelty of the system is that there was a specific function assigned to each stage. The results revealed a net removal of metals in the following order: Al (99.7%) = Cu (99.9%) = Zn (99.9%) > Fe (99.7%) > Mn (98.6%) > Ni (98.2%) > Cd (66.5%). Sulphate concentration was reduced from 3030 mg/L to 814.9 mg/L and the pH of AMD rose to 7.2. This combined passive system is efficient in treating AMD water but it presents as drawback the generation of huge volume of sludge from anoxic limestone. Furthermore, Substrate used in stage 3 will depend of the site and AMD characteristic as well. In that regard, there will be need to investigate the site and the type of AMD before implementing the type of combined passive system.

Ordonez *et al*. (1999) investigated the efficiency of a combined three different passive technologies for AMD treatment. The integrated system was composed of successive alkalinity producing system (SAPS), an oxidation pond and a constructed wetland using *Typha sp*. The experimental procedure was designed to ensure the flow of mine water from the SAPS to the oxidation pond and to the wetland. After 30 days of experiment, the results revealed that mine water was neutralized at the SAPS level while metals were precipitated by the oxidation pond and the wetland acted as polishing stage by accumulating metals and reduce sulphate. Overall results showed that acidity was reduced at 65%, the removal efficiency of metals (Al, Mn and Fe) was 100%, 27% and 99.5% respectively whereas the removal efficiency of sulphate was 32%. From the results, it follows that the hybrid system combining different types of treatment units can be effective in mining water treatment.

In his attempt to treat AMD using integrated approach, Masindi. (2017a) investigated the integration of natural chemical (magnesite) and manufactured chemical (Barium chloride) for AMD treatment. Magnesite and AMD water were mixed in the ratio of 10g/1000ml and stirred for 60 minutes. After collection and analysis of the supernatant water, the results revealed an increase of pH from 2 to 10. The removal efficiency of metals was 99% while 40% of sulphate was removed. Barium chloride was then used to polish the resultant water and it follows that 99% of sulphate were removed. This hybrid approach allowed obtaining water that met the industrial discharge and irrigation standards as stipulated by the department of water and sanitation (DWS) water quality guidelines standard of 1996. This integrated approach however presents as drawback the production of highly toxic polluted sludge leading to another environmental problem which needs to be solved. Masindi *et al*. (2017a) evaluated the integration of basic oxygen (BOF) slag and the association of lime and reverse osmosis (RO) for the treatment of AMD. The finding revealed that at pH = 10, 75% of inorganic contaminants were removed and a huge reduction of sulphate concentration. Hardness was reduced using lime and the pre-treated water was polished using reverse osmosis to obtain product water complying with the South African National Standard (SANS) 241 drinking water specifications. This study proved the integration of BOF and RO can produce drinking water. However, his application is not cost effective at large scale thereby render its feasibility difficult.

Sulphate reducing bioreactor (SRB) and aerobic polishing cell is an integrated technology made up of an organic-rich material with limestone (SRB) and shallow pond (aerobic polishing cell). The system is designed in way that waste water flows from the reservoir tank to sulphate reducing bioreactor (SRB) to aerobic polishing cell (APC) and the metal and acidic loading rate should not exceed 0.3 moles per day. The effluent discharged into the SRB has low dissolved oxygen and excess biochemical oxygen demand (BOD). In the SRB cell, the sulphate–reducing bioreactor helps to reduce acidity by increasing the pH and reduces sulphate concentration. In this hybrid system, (SRB) species are very active in the treatment process (Gusek and Conroy, 2007; Kefeni *et al*., 2017; Zipper *et al*., 2018). Selenium is removed while Mn removal is very low and in some case nonexistent. Aluminum, copper and zinc removal is high whereas iron removal is very low. Once in the APC cell the wastewater is polished by complete removal of Fe and Mn which leads to the reduction of biochemical oxygen demand. The APC in fact acts as polishing stage and ensure sufficient retention time to allow metals oxidation and hydrolysis thereby causing precipitation and physical retention and accumulation of metals by plants. This association is efficient in improving the overall water quality of AMD water. However, it requires a large land area around 4.6 ha to be installed (Johnson and Hallberg, 2005; Gusek and Conroy, 2007; Zipper *et al.*, 2018). The loading load of wastewater in the SBR cell is very crucial since it should not exceed 0.3 moles of metal loading per day per cubic meter of organic media. Hydraulic retention time and initial sulphate concentration in AMD and the plants species are others factors contributing to the efficiency of the system (Neculita *et al.*, 2007).

2.10 Mechanisms of pollutants removal in acid mine drainage treatment

The mechanisms of pollutants removal is achieved through various processes including precipitation, absorption, bio-adsorption, ion-exchange, filtration, electrodialysis, crystallisation and phytoremediation. A brief introduction of each mechanism along with its advantages and limitation in term of AMD treatment efficiency is given below

2.10.1 Adsorption

Adsorption is a process whereby an adsorbate adheres to the surface of an adsorbent (Kurniawan, 2015). Adsorption is considered as one of the most suitable methods to remove pollutants from an aqueous solution since it allows the transfer of mass from the liquid to the surface of a solid (Kurniawan, 2015). The challenge with adsorption is that it is not selective to pollutants and sometimes it easily gets saturated. It is a good candidate for dilute solution. Masindi *et al.* (2015) evaluated the use of bentonite clay for the adsorption of heavy metals from acid mine drainage and the results revealed the feasibility of using bentonite clay mixed with AMD at different solid/liquid ratio to increase the pH and remove toxic chemical species from AMD. However, despite the efficiency of the technology, the product water must be polished to remove residual concentration of sulphate. Furthermore, this technology releases a considerable quantity of sludge if applied at large scale.

2.10.2 Precipitation

Precipitation is the most widely used technology to remove heavy metal from inorganic effluent. Zhu *et al.* (2004) describes the mechanism of heavy metal removal by precipitation as illustrated in the Equation 2.12.

$$M_2 \quad + 2OH \quad \rightarrow \quad M(OH)_2 \qquad \text{(Eqn 2.12)}$$

M and OH represent the dissolved metal ions and the precipitant respectively while $M(OH)_2$ is the insoluble metal hydroxide. pH plays a crucial role in this process because the precipitation of metals depends of the pH value. In addition, metals precipitation occurs at different pH intervals (Barakat, 2011).

Petrilakova *et al*. (2014) studied the influence of pH on selective precipitation of metals (Al, Cu, Fe, Mn and Zn) from AMD using NaOH and $Fe(OH)_3$ in two distinct experiments. The results revealed that neutralization of AMD by NaOH led to the rise of pH from 3 to 8.2 and the precipitation of 92.3% of copper, 93.3% of zinc, 96.6% of iron, 99.6% of Al and 15.9% of manganese. However, after the oxidation of Fe^{2+} to Fe^{3+} by hydrogen peroxide and neutralization by NaOH, 97.16% of iron, 95.23% of copper and 92.9% of Al were precipitated at pH range of 4.05, 4-5.5 and 4.49-6.11 respectively whereas 89.49% of Mn and 88.72% Zn were precipitated at pH range of 5.5 - 9.98 and 5.5 - 7.23 respectively.

Masindi et al. (2018b) evaluated a fractional and step-wise recovery of chemical species from AMD using calcined cryptocrystalline magnesite. Interaction BOF and AMD were mixed and the interaction led to an increase of pH and metals removal and sulphate reduction. Lime and soda ash were used to reduce residual sulphate and hardness. Minerals such as Gypsum, brucite, calcium were recovered and the reverse osmosis (RO) was used to purify the water to meet drinking water quality. The finding of the above studies clearly showed that AMD can be treated by chemicals precipitation which raises the pH to the desired values leading to the precipitation of metals. Precipitation is a simple process, low cost and convenient. However, precipitation is challenging since it requires a large amount of chemicals to reduce metals to an acceptable level and it also produces excessive amount of toxic sludge that requires further treatment thereby increasing the cost of precipitation technology (Simate and Ndlvou 2014). Furthermore, AMD treated by precipitation technology must be polished to remove residual sulphate concentration in order to obtain drinking water quality standard. As consequence, the feasibility of this technology is limited. However, it can be associated with a passive emerging technology such as phytoremediation to remove metals from toxic sludge using selected plants species.

2.10.3 Ion exchange

It is another technology widely used in the industry for the removal of heavy metals from effluent. It uses a solid as ion exchanger which is capable of exchanging either, cations or anions from the surrounding environment.

Masindi *et al.* (2015) evaluated the use of magnesite/bentonite clay nanocomposite for the removal of pollutants from acid mine drainage. The composite was mixed with AMD water at specific ratio at different time interval and its potential to neutralize AMD and reduce metals and sulphate concentration was assessed. The reaction of the composite with AMD led to an increase in pH (pH >11) and precipitation of metals thereby leading to their reduction. The removal of Al^{3+}, Fe^{3+}, Fe^{2+}, Mn^{2+} and SO_4^{2-} was optimum at 20 min of equilibration and 1g of adsorbent dosage per 100 mL of solution (1:100 S/L ratio). The composite was able to remove 99% of Al^{3+}, Fe^{3+}, Mn^{2+} and 90% of sulphate and trace elements such as Co, Cu, Zn, Ni and Pb from raw AMD water. The authors claimed that, the composite can exchange ions from its matrices for pollutants in acid mine drainage hence remediating the water. Ion-exchange is environmentally friendly, provides high flow rate of treated water and the maintenance is cost effective (Tripathi and Ranjan, 2015). It is also effective in removing dissolved inorganics. However, ion-exchange technology does not remove bacteria and deionization process of treated water can generate bacteria. It is not cost effective over long- term period. Sulphuric acid (H_2SO_4) is the most common chemical used to recharge the resin during the process of AMD treatment using ion-exchange technology (Tripathi and Rawat Ranjan, 2015). However the continuous use of sulphuric acid may increase the acidity of product water. Moreover, ion exchange technology, pollutants are removed simultaneously and his efficiency is highly sensitive to the pH of the solution (Barakat, 2011).

2.10.4 bio-adsorption

It is the use of biological components for bioremediation of AMD. This method involves the use of waste biomass to absorb heavy metals from mining water using a physicochemical process (Bishnoi *et al.*, 2004). Igwe *et al*. (2005) investigated the removal of Cr (vi) using rice husk-activated carbon from an aqueous solution and the results revealed that a maximum of metals were removed at pH ~2. This technology allowed removing various metals simultaneously but at lower removal efficiency.

Park and Lee. (2017) investigated the potential of Ca-alginate beads in treating acid mine drainage by bio-adsorption technology. The results of batch experiment revealed a removal efficiency of 95% of both Cu and Cd but under a very short period (within 3 hours) whereas the results of the column experiment showed a removal efficiency of 86% and 58% for Cu and Cd respectively. To improve the efficiency of Ca-alginate in treating AMD, others technologies such as ion-exchange and precipitation could have been incorporated since the bio-adsorption mechanism of Ca-alginate is precipitation and ion-exchange (Veglio *et al*., 2002; Davis *et al*., 2003; Ozdemir *et al*., 2005). These experiments proved that Ca-alginate beads can be successfully used as bio-adsorbent for the removal of Cu and Cd from acid mine drainage. However the operating time (3 hours) seems to be the major inconvenient of this technology. Furthermore, the temperature is not stable and this situation can compromise the efficiency of this technology. Sulphate, pH, EC, TDS and others metals (Fe, Mn, Zn, Ni) which are other important parameters to be considered when treating AMD were left aside and as consequence, this technology cannot be considered efficient for long term solution to acid mine drainage.

2.10.5 Filtration

Filtration is another method used for the treatment of AMD since it is capable of removing suspended solid, organic compounds and inorganic contaminants such as heavy metals.

It uses various types of membrane filtration such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The process of filtration allows separating different heavy metals through a semi-permeable membrane under a pressure greater than osmotic pressure caused by the dissolved solids in wastewater (Albergamo *et al.*, 2019). For instance, Juang and Shiau. (2000) studied the removal of Cu (ii) and Zn (ii) ions from synthetic wastewater using chitosan-enhanced membrane filtration. The results showed that about 100% and 95% of Cu (ii) and Zn (ii) ions were removed at pH 8.5 and 9.5 respectively. The researchers further stated that chitosan membrane significantly improve metal removal by 6-10 times compared to using membrane alone. However, Filtration technology presents some disadvantages such as replacement and disposal cost of filter when it is blocked by waste particles. Over time the replacement of filter may not be cost effective and the disposal of polluted filter may incur additional cost and new environmental problem to be solved

2.10.6 Electrodialysis

Electrodialysis (ED) water treatment technology applied to remove dissolved ions from wastewater. In this process, the inorganic contaminants are removed by separation process using semi-permeable and ion-selective membranes (Gurreri *et al.*, 2020). In this process, an application of an electric potential between the two membranes or electrodes allows the reverse movement of cations and anions towards respective electrodes. Due to the spaces alternating between cation and anion permeable membranes, cells of concentrated and dilute salts are formed. Buzzi *et al.* (2013) investigated the possibility of using ED technology to treat AMD from coal mine and recover water. The finding revealed that ED allowed separating anions and cations from AMD thereby leading to the recovery of water. Electrodialysis technology is efficient to recover clean water from AMD. However, the disadvantage of ED is the formation of metal hydroxides, which clog the membrane and thus increase the operational and maintenance costs (Mohammadi *et al.*, 2005).

2.10.7 Crystallization

Crystallization is an active treatment approach applied to reduce sulphate concentration in AMD water. For his effectiveness, AMD water must first be pre-treated by increasing the pH and reduce metals concentration by precipitation process. Raw acid mine drainage water treated used conventional active treatment still has elevated concentration of residual sulphate which is typically associated with dissolved Ca and Mg in the treated AMD water (Masindi *et al.*, 2015). The residual sulphate can then be crystallized using hydrothermal technology which is applied when the AMD was pre-treated using hydrated lime [$Ca(OH)_2$] to raise the pH and initial reduction sulphate. The pre-treated AMD then passes into a reactor where high temperature and pressure are applied to enhance the crystallization of $CaSO_4$ (anhydrite) and according to Freyer and Voigt. (2003), this process decreases sulphate levels to below 200 mg/L.

2.11 Recovery of natural available resources from acid mine drainage

Despite being globally seen as threat of great significance to the environment and associated ramification, AMD can be valorized by the recovery of chemicals of economic importance or recovery of drinking water.

MacIngova and Luptakova. (2012) developed an optimized process to recover selective metals (Al, Cu, Fe, Mn and Zn) from raw AMD. They used culture of sulphate reducing bacteria (genera *Desulfovibrio*) isolated from a mixed culture of sulphate reducing bacteria obtained from mineral water. A hybrid technology (Biological and chemical) was used during the process. Hydrogen sulphide recovered from sulphate reducing bacteria through the biological process following by the chemical stage where the hydrogen sulphide was transported by the nitrogen gas to the contactor filled with raw AMD. This chemical stage allowed the precipitation of metals sulphides which were filtrated to recover the metals. This technology produces a recovery efficiency of 99% for Al, Cu, Fe and Zn and 72% of Mn.

Demers *et al.* (2015) investigated the potential of lime neutralized sludge to reclaim sites polluted by AMD. The results revealed that sludge produced from AMD active treatment is efficient to neutralize AMD if the sludge is alkaline. However, this technology presents some inconvenient such as the stability of sludge caused by leaching pH (Zinck, 2006). In fact, at low pH, there is leaching of metals thereby increasing metals concentration which may exceed regulatory limits. Furthermore, sludge cannot be used as final reclamation process for AMD polluted site because of his short life time and it also contains toxic metals.

Masindi *et al*. (2019) developed an integrated approach to treat AMD and recover drinking water. The treatment was a 4 stages process involving the use of magnesite to neutralize AMD and recover Fe- species (stage1). The use of hydrated lime to recover gypsum from iron Free AMD water after the mixture was agitated using an agitator for 60 minutes (stage 2). Magnesite and lime treated AMD water was further treated using soda ash to recover calcium carbonate (stage 3). The alkaline water obtained in stage 3 was neutralized with hydrochloric acid (HCL) to a neutral pH followed by reverse-osmosis treatment to recover drinking water. This integrated approach allowed to recover drinking water standard from AMD and it can be very useful for countries with limited water resources which are under serious threat due to AMD pollution. It is economically feasible since valuable metals recovered can be sold to support the treatment cost. However it presents some drawbacks such as the production of toxic sludge thereby leading to another environmental problem to be solved. Furthermore, the performance of reverse osmosis technology is affected by various factors such as the membranes characteristics, the feed rate and quality, the operation pressure and temperature (Abbas, 2007). Reverse osmosis also produces a brine containing a huge quantity of salt to deal with once drinking water is recovered (Paul, 1997). The reverses osmosis is not cost effective for developing nation such as South Africa. Acid mine drainage can also be used positively by synthesizing valuable minerals of economic importance. Minerals such as sulphate, magnesium and calcium can be recovered using alkaline sulphidogenic liquor (ASL) technique (Prasad and Henry, 2009). Furthermore, there is possibility to recover valuable minerals from AMD due to the presence of elevated concentration of metals such as iron, aluminum, manganese and sulphate. Akinwekomi *et al.* (2020) developed an innovative technology to treat and recover valuable metals from AMD. The researchers recovered Fe (3) and Fe (2) goethite, hematite and magnetite from AMD using a method of selective precipitation. The resulted water was treated by filtration followed by synthesis of goethite, hematite and magnetite. The pre-treated water was used to synthesis gypsum by the addition of lime following the method described by Liu *et al.* (2016) to recover drinking water by reverse osmosis technology. This innovative technology appears to be sustainable since it allows valorizing AMD by recovering valuable metals and drinking water. The economic values of minerals can allow amortizing the treatment cost.

2.12 Future perspectives and challenges of acid mine drainage treatment

Whereas various literature sources largely focused on the combination of two different methods of treatment, there were no finding regarding the association of an active treatment such as neutralization and hybrid constructed wetland and phytoremediation. This technology will allow exploit the benefit of each types of treatment. Toxic sludge obtained after neutralization may be decontaminated using phytoremediation technology and the water obtained from a staged hybrid wetland can be further treated to recover drinking water. However, the feasibility of this novel technology will be challenging in area without available land spaces but the size of wetland can be reduced according to the available land area. This forms part of the emerging treatment technology that should be investigated by researchers and other environmental engineers.

This type of hybrid approach involves the use of natural chemical such as magnesite and staged constructed wetland to treat AMD. Magnesite in fact is used to raise pH of mine water, precipitate metals and reduce sulphate concentration while staged constructed wetland consisting of different types of CW are used to polish the pre-treated AMD. Acid mine drainage water is pre-treated using magnesite in the ration of 10:1000 (10g of magnesite for 1000 ml of AMD water). The mixture in this case is stirred to ensure homogeneity. The resultant liquid (AMD water) is then used to feed plants in a staged wetland which in fact acts as polishing stage.

At the bottom of the tank and at the bottom of each wetland, the product AMD water is collected and analyzed. At the end of the process final product water collected from the last wetland was compared with the South African water quality guidelines standard and the efficiency of this hybrid approach in treating AMD was revealed.

2.13 Legal legislation for water quality

The National Environmental Management Act (NEMA) 108 of 1998, specifies that every citizen should live in a protected environment that is suitable to his health and well-being. To have the environment preserved for present and future generation by complying with environmental law and comprehensive management of fresh water resources.

2.14 Pollution prevention and ecological degradation

Sustainable development implies that all economic activities undertaken in South Africa and precisely mining activities should be aware of the need to treat waste water before discharge to the water course (Dreyer and Mearns, 2016; Ndebele and Du Pleiss, 2018)**.**This merely means that conservation of natural resources and protection of the environment should be the focal point when undertaking economic activities in South Africa. The South African national water act, No. 36 of 1998 recognises water as a natural resource belonging to all people. It regulates the way in which water is used and provides the right for just and equitable utilisation of water resources and mitigation measures to prevent water pollution when undertaken activities (Ndebele and Du Pleiss, 2018)**.** It requires that any person or company using water resources for industrial purposes shall purify or treat such water prior to discharge into the water course. Relevant requirement for discharge of acidic and sulphate-rich water are given in **Table 2.3**.

Table 2.3: Relevant criteria for discharge of acidic and sulphate-rich water as compared to DWS water quality guidelines

Parameter	Coal AMD	Gold AMD	Neutral AMD	Irrigation water requirement (DWS)	Discharge water requirement(DWS)	Domestic water requirement (DWS)	SANS 241 standard
pH	2.3	2.5	6.5	5.0-10.0	6.5-8.4	6-8	5-9
Ec	22713	13980	500	0.0-250	> 540	70	≤ 170
Na	248.4	70.5	20.1	100	430-460	100	≤ 200
K	21.6	34.2	29.1	-----	-----	50	-----
Mg	2.3	398.7	861.8	-----	-----	30	-----
Ca	710.8	598.7	537.5	-----	-----	15	-----
Al	134.4	473.9	-----	-----	5.0-20	0.5	≤ 300
Fe	1243	8158.2	0.01	0.0-10	5.0-20	0.1	≤ 2000
Mn	91.5	88.2	0.07	0.0-10.0	0.02-10.0	0.05	≤ 400
Cu	7.8	-----	-----	-----	0.2-5.0	1	≤ 200
Zn	7.9	8.36	0.16	-----	1.0-5.0	3	≤ 5
Co	41.3	1.89	0.29	-----	0.05-5.0	20	≤ 2000
Ni	16.6	2.97	0.21	-----	-----	10	≤ 70
SO_4^{2-}	4635	42862	4603	0-500	-----	200	≤ 500

References: Gold mining AMD (Tutu *et al*., 2008),, Coal mining AMD and [†]) Neutral drainage water (Kumar Vadapalli *et al*., 2008; Masindi *et al*., 2014) irrigation water requirement, discharge water requirement and domestic water requirement (DWAF, 1996; SANS, 2011).

2.15 Summary of literature review

This literature review explored various topics in relation with this study. Acid mine drainage was defined and explained, the process of AMD formation was explained and the factors contributing to his formation were highlighted as well as the consequences of AMD on the environment and public health. Conventional AMD treatment technologies (active and passive) were explained in details but their cost and other environmental problems associated with them still preoccupying the scientific community. For instance, active technologies are effective for the treatment of persistent AMD pollution. However they are not cost effective and require constant use of chemicals and energy. In addition, active technologies produce highly polluted toxic sludge which requires additional cost for handling and disposal (Gusek and Conroy, 2007; Simate and Ndlovu, 2014; Nleya et al., 2016). Passive technologies are cost effective and environmentally friendly but the installation of passive treatment plant depends of several factors such as the characteristic of wastewater and the availability of land (wetland). Furthermore, their efficiency is influenced by the climatic and seasonal conditions which impact the availability and growth of plant in case of wetland and phytoremediation technologies. As such, an environmentally friendly approach such as a hybrid system (neutralisation and staged hybrid constructed wetland) could be ideal for the management of AMD due to the relative low cost and his imperishability.

2.16 Conclusion

The formation of AMD, metal-rich and toxic by-products from mining industry poses a major environmental problems and economic challenges for the mining industry. Treating such toxic liquid is challenging and mining companies and various governments worldwide are in quest of an appropriate technology to treat acid mine drainage. Various treatment technologies have been developed to treat acid mine drainage. However, the limitation and occurrence of secondary environmental problem associated with each technology is stressing for mining companies, governments and researchers. The long-term and environmentally friendly technologies still a matter of research being investigated by various researchers in environmental field. Following the results from single and combined hybrid system, the long-term solution of acid mine drainage may be an emerging hybrid system which combines neutralization, staged hybrid wetland and phytoremediation. Sludge released from neutralization step can further be decontaminated by phytoremediation using selective plants species.

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CHAPTER THREE

MATERIALS AMD METHODS

3.0 Introduction

Materials and methods applied to this study project were imposed by the study aim and objectives. This chapter describes succinctly how research was conducted, study design, samples collection and analysis. It also outlines ethical consideration, risks and limitations associated with the project.

3.1 Methodology

The aim of the study was to conceive an emerging hybrid approach for the treatment of AMD. In order to achieve the objectives of this study, two batches of experiment were conducted. For the first batch of experiments, three different types of wetlands equipped with *Vetiveria zizanioides* were constructed using compost soil as substrate. The three constructed wetlands were operated individually and the performance of each wetland in the treatment of AMD was assessed. For the second batch of experiment, a hybrid approach consisting of neutralization and staged constructed wetland in the treatment of AMD was assessed and the product water was compared to the Department of Water Affairs and Sanitation guidelines standard for drinking water.

3.1.1 First batch of experiment

3.1.1.1 Acquisition of plants, substrate and reagents

To construct pilot plant wetland, shoots of *Vetiveria zizanioides* were purchased from NANDADRAM ECOVILLAGE farm in Kwa Zulu Natal and transported to the University of South Africa’s sciences campus in Florida, Johannesburg. Compost soils used as wetland substrate were purchased from Garden World Johannesburg while all reagents used were of analytical grade (AG) and were purchased from Merck, South Africa

3.1.1.2 Growth of plants

Once in the campus, young plants of *Vetiveria zizanioides* were submerged under potable fresh water for two weeks in order to allow the news seeds to germinate. After that, young plants were transferred into small pots containing compost soil (soil purchased from garden world Johannesburg) as growing medium. Plants were allowed to grow naturally without the addition of nutrients. After one month, plants were transplanted to the first batch of wetlands purposively constructed for the project.

3.1.1.3 Wetland design and construction

Three different types of wetlands were constructed: Free water system constructed wetland (FWS-CW), subsurface vertical flow constructed wetland (SSVF-CW), and subsurface horizontal flow constructed wetland (SSHF-CW). Wetlands were constructed with their respective controls using the same soil previously used for the growing of seedlings as growing medium. The wetlands were constructed using a circular plastic basins measuring 62 cm of diameter (d) and 45 cm high (h) and a drum as reservoir tank to contain AMD water and fresh water for control. The wetlands were continuous flow systems where water was flowing continuously for the duration of the experiment.

3.1.1.4 Hydrology of the wetland

Once the wetlands were constructed, the following hydraulic parameters of the wetlands were determined.

1: The hydraulic loading rate (HLR)

Hydraulic loading rate (HLR) is expressed as the ration of water flow per unit area over a specific given period and was determined using the following formula (Equation 3.1).

$$HLR = \frac{Qi}{A} \qquad \text{(Eqn 3.1)}$$

Where:

Qi = water inflow (m^3/d),

A is the wetland top surface (m^2), and

HLR is the hydraulic loading rate.

2: Hydraulic Retention Time (HRT)

Hydraulic residence time or hydraulic retention time (HRT) is the average length of time that water remains in storage. In waste water treatment plant, it is the period of time holding wastewater in the treatment cell. Purposively for this project, the treatment cell is wetland. The HRT was determined using the Darcy's law.

3.1.1.5 Preliminary analyses

Prior to the experimental phase, preliminary analyses were done in order to have an insight about the quality of soil used as substrate and the quality of mine water before the treatment.

3.1.2 Characterization of wetland's substrate

3.1.2.1 Elemental and mineralogical composition of wetland substrate

Elemental and mineralogical composition of soil before and after contact with mine water was determined. Elemental composition was done using X-ray fluorescence (XRF) analysis. The analyses were performed using a Thermo Fisher ARL-9400 XP+ Sequential XRF with winXRF Software. Mineralogical composition was done using X-ray diffraction. Analyses were done using Philip PW 1710 diffractometer equipped with graphite secondary monochromatic.

3.1.2.2 Soil texture

Soil texture is the size of particles that make up the mineral fraction of the soil. Texture has influence in all aspect of soil use, both in agriculture and engineering applications. Texture is a determinant of soil characteristic because it plays an important role in water retention (Abuarab *et al.*, 2019). For the project, compost soil texture was determined according to the sense of feel method described by the United States department of agriculture.

3.1.2.3 Determination of soil pH

The pH of the soil before and after experiment was determined by adding 10 gram of air dried soil to 15 ml of distilled water. The obtained solution was stirred vigorously for 30 minutes and was then allowed to stand for 30 more minutes followed by the measurement of pH using a calibrated pH meter.

3.1.2.4 Determination of point of zero charge of soil substrate

The point of zero charge (PZC) was determined to according the mass potentiometric titration method. Two solutions (blank and sample) were prepared using 3.0 mL of 0.1 molarity of KNO_3 and 6.0 mL of deionized water. Once their respective pH were measured, 1.0 mL of 0.01 molarity of Potassium hydroxide (KOH) was added to the blank solution while 50 mg of soil sample and 1.0mL of 0.01 molarity of KOH were separately added to the sample solution. After titration of both solutions with 0.01 molarity of nitric acid (HNO_3), the point of zero charge was estimated at the cross of both titration curves.

3.1.2.5 Determination of available nitrogen and carbon

Available nitrogen and available carbon were determined using Leco CN.2000 dry combustion analyser (Leco Corporation, USA). The experiment was carried out in accordance with the recommendation by the instrument manufacturer.

3.1.2.6 Cation exchange capacity (CEC)

Cation exchange capacity of soil was determined following the method of pH =7 buffered 1 M molarity Ammonium acetate.

3.1.2.7 Determination of heavy metals concentration in wetland's substrate

Soil samples used as wetland's substrate were collected, air dried for one week, sieved and one gram (1g) was digested in a mixture of 65% concentrated HNO_3 and 35% H_2O_2 using microwave digestion. Samples were digested at 180°C for one hour. After digestion, samples were cooled to room temperature, filtered using syringe filter membrane and analysed for metals concentrations using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Shimadzu ICPE-9000, Australia).

3.1.2.8 Sampling and characterization of acid mine drainage water

Acid mine drainage was collected from a discharging point of Sibanye Gold mine in Krugersdorp, Gauteng, South Africa. During collection, pH, EC and TDS were measured in situ using a multi-parameter meter (HANNA instruments, Johannesburg, RSA). Samples were immediately cooled at 4°C and transported to the campus where metals concentrations were determined using standard methods.

3.2 Experimental procedure

3.2.1 Pre-treatment of acid mine drainage water

Prior to the treatment, the AMD water was filtered to remove total suspended solid (TSS) susceptible to clog the pipe. The flow rate of each wetland was calibrated using a drip plastic bottle and the water was fed into the system as continuous drops. The average flow per unit time was calculated using the Darcy’s Law The flow rate was maintained low and the HRT long in order to allow the water to spend more time in wetland cell and thereby improve the performance of the wetland (Conn and Fiedler, 2006; Ewemoje et al., 2015; Merino-solís *et al.*, 2015; Piñeyro *et al.*, 2016). The inlet flow rate and outlet flow rate were monitored daily to ensure a steady flow of water in and out of each wetland.

3.2.2 Product water and soil sampling

Product water samples (experiment and control) and soil samples were collected daily. Water samples (500 mL) were collected daily from the outlet of each wetland for the duration of the experiment. After collection, samples were divided into two sub-samples of 400 mL and 100 mL. Sub-samples of 400 mL were used for the analysis of pH, TDS, EC and while sub-sample of 100 mL was used for the analysis of metals (Al, Cu, Fe, Mn, Ni and Zn) and sulphate. Soil samples were also collected daily from each wetland and analysed for heavy metals content.

3.2.3 Analytical method

Samples from both wetlands (treatment and control) were analysed in triplicate following standard method. Electrical conductivity (EC), pH and TDS were determined using Hanna HI 83200 multi-parameter and the manufacturer's manual was followed with reference to standard methods for examination of water and wastewater (APHA, 2002) while analysis of water and substrate samples for metals concentration was performed using an inductively coupled plasma-optical emission spectrometry (ICP-OES) with an Agilent 5100 ICP-OES System (Agilent Technologies Inc., Santa Clara, CA, USA) and sulphate of product water samples concentration was determined using ion chromatography (IC).

3.3 Treatment efficiency of each wetland

Following the analysis of parameters of concern, the removal efficiency (RE) of each wetland was calculated gradually after every five days period for the duration of the experiment. The removal efficiency of each wetland was calculated as illustrated in Equation 3.2 (Białowiec *et al.*, 2014; Van Tran *et al.*, 2017).

$$Removal\ efficiency = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100 \qquad \text{(Eqn 3.2)}$$

3.4 Plants harvesting

Plants from each set of two wetlands (treatment and control) were harvested without damage the roots and were rinsed separately with distilled water to remove dust, soil and mineral particles. Plants were air dried for one week at 25°C weighed and followed by the determination of tolerance index as illustrated in the equation3.4.

$$Tolerance\ index\ (TI) = \frac{\text{biomass of plant growing in AMD}}{\text{Biomass of plants growing in potable fresh water}} \qquad \text{(Eqn 3.4)}$$

3.4.1 Digestion of plants and metals analysis

Plants were then separated into roots and shoots then milled to a fine powder and 01g was digested in a mixture of 5 mL of 65% nitric acid (HNO_3) and 2 mL 35% Hydrogen peroxide (H_2O_2) using microwave digestion. The digestate was cooled at room temperature and followed by filtration using a syringe filter membrane and determination of metals content in plant (roots and shoots). The concentration of metals content in roots and shoots allowed the researcher to determine the bio concentration factor (BCF) which is the quantity of metals that moved from substrate to plant and the translocation factor (TF) which is the ability of the plant to transfer metals from roots to shoots. The BCF and TF were determined as illustrated in equation 3.5 and equation 3.6.

$$BCF = \frac{Metal\ concentration\ in\ plant\ tissue}{\text{Final concentration of metals in AMD water}} \qquad \text{(Eqn 3.5)}$$

$$TF = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration in roots}} \qquad \text{(Eqn 3.6)}$$

3.4.2 Functional group and morphology of plant roots

The functional groups of grinded plant roots were determined using fourier transform infrared spectrometry (FTIR) analysis while the morphology of composite was determined using scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS).

3.5 Partitioning of metals removed between substrate, plants and external factors

Prior to the start of experiment, the initial concentration of metals in AMD water, plants and substrate was determined. At the end of the experiment (30 days), the quantity of metals retained in the wetland as well as the quantity of metals accumulated by the plants was determined. The summation of metals removed by plants and metals retained by substrate followed by comparison to the initial concentration of metals in AMD water allowed the researcher to determine the contribution of external factors in overall metals removal.

3.6 Second batch of experiment

The second batch of experiment consisted of a hybrid approach towards the treatment of acid mine drainage using the integration of neutralisation and phytoremediation. The neutralisation step consisted to use magnesite to neutralise AMD water while the bioremediation step consisted to use a staged hybrid constructed wetland operating simultaneously to polish the product water from neutralisation step. The staged hybrid constructed wetland consisted of: SSVF-CW, FWS-CW and SSHF-CW.

3.6.1 Acquisition of plants, substrate and reagents

Plant, substrate and reagents used were purchased and treated as previously described in the first section **(section 3.1.1).**

3.6.2 Acquisition and characterization of magnesite

A raw magnesite sample were collected from a magnesite mine without any form of processing and were milled to a fine powder using a Retsch RS 200 vibratory ball mill and was passed through a 32 μm particle size sieve. The sample was kept in a plastic bag until utilisation for AMD treatment. Raw magnesite was characterized for elemental and mineralogical composition, functional group and morphology. Elemental composition was done using X-ray fluorescence (XRF) analysis, mineralogical composition was done using X-ray diffraction (XRD) analysis while functional group was done using fourier transform infrared spectrometry (FTIR) and morphology of composite was performed using scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS). Characterization of magnesite was done for raw magnesite, magnesite reacted with AMD water and magnesite reacted with potable fresh water.

3.7 Experimental procedure

The experimental procedure was dictated by the aim and objectives of this section. It consisted of neutralisation and bioremediation

3.7.1 Neutralisation

Acid mine drainage was treated using magnesite. Magnesite and AMD were mixed at 1:100 (1g:100 mL) ratio. The mixture was stirred using stirrer for one hour and the product water was filtered using syringe filter membrane while the sludge was characterised for elemental, mineralogical composition, functional group and morphology of composite. The product AMD water was characterised for chemical species (pH, EC, TDS and metals) as described in **section 3.3.2.** Following the characterisation of magnesite treated water for chemicals species, the product water was then polished using staged hybrid constructed wetland.

3.7.2 Bioremediation

The AMD water previously treated with magnesite was taken to the staged constructed wetland which served as polishing stage.

3.7.3 Hydrology of the staged hybrid wetland

The flow rate of water in the staged hybrid wetland was calibrated in each wetland using a drip plastic bottle and the flow rate was the same for all three wetlands and the water was fed into the system in continuous drops. From there the hydraulic loading rate (HLR) and the hydraulic retention time (HRT) were determined.

3.7.4 Product water and soil sampling and analytical method

Like the first batch of experiment, product water was collected at the outlet of the each wetland where a collection system was installed before it flowed into the following wetland. Soil samples were also collected from each wetland. Samplings were done daily for the duration of the experiment. Immediately after collection, product water was analysed as described in the **section 3.2.2** while soil samples were analysed for metals content as described in **section 3.2.3** and characterised for elemental and mineralogical composition as described **in section 3.1.2.1.**

3.8 Treatment efficiency of the hybrid system (neutralisation and bioremediation)

The treatment efficiency of the hybrid system was determined gradually for the duration of the experiment. All parameters of concern were collected daily, analysed and the average result of five days period was considered. The treatment efficiency was determined as described in **section 3.3.**

3.9 Plant harvesting and metals content

At the end of the experiment, one plant was harvested from each wetland and treated and analysed for metals content as described in **section 3.4.1** while functional groups and morphology of roots were determined as described **in section 3.4.2**

3.10 Maintenance of wetland

Basic maintenance of wetlands is necessary since it helps to maintain the performance of the system. Maintenance of wetland was applied to keep a storm water management system at all times to ensure the steady flow water. Functional maintenance was divided into two:

3.10.1 Preventive maintenances

Preventive maintenances were conducted on regular basis to ensure the steady flow of water within the system. Preventive maintenances applied include: elimination of stagnant pool, removal of debris and trash.

3.10.2 Corrective maintenance

Corrective maintenances are applied on an emergency basis to remediate the situation and restore the steady function of the wetlands. Failure or late action to promptly take corrective measures on time may negatively affect the efficiency of the wetland. The following corrective maintenance measures were conducted when it was necessary: Removal of debris and sediment, structural repairs, dam embankment and slope repairs, fence repair, elimination of trees or woody vegetation, removal of undesirables plants (weeds), leaves removal, debris removal, cutting of old grasses. This allowed the wetland to maintain an attractive appearance and help maintain its functional integrity. The plants used for experiment were discarded by incineration according to the method described by Petridis and Dey. (2018).

3.11 Quality assurance (QA) and quality control (QC)

A quality assurance (QA) and quality control (QC) process was applied in this study to ensure the collection and production of trustworthy results. The QA/QC process embraced the collection and preservation of the samples. All experiments were conducted in three replicates and analyses done in triplicate. Data were reported as mean values and were considered acceptable when the difference within triplicate samples was less than 10%. The value below detection limit (BDL) was in line with United States Environmental Protection Agency (USEPA) for analysing of data while the accuracy of the analysis was monitored using the National Institute of Standard and Technology (NIST) water standard.

3.12 Geochemical modelling

To substantiate experimental results and determine aqueous species and mineral phases that are more likely to precipitate from activated magnesite, AMD interactions, geochemical modelling was applied. The primary aim was to determine speciation and calculate the saturation indices (SIs) of the mineral phases, based on compositions of feed AMD waters. To precisely accomplish that objective, the PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model using the WATEQ4F chemical speciation model were used (Masindi, 2016). Furthermore, the chemical species which were more likely to precipitate were determined using the modelled SI index whereby, SI values lower than unity (< -1) denote an under saturated solution, SI values equal to unity (= 1) denote a saturated solution and, lastly, SI values higher than unity (> 1) denote a supersaturated solution.

3.13 Data analysis

Acid mine water quality results from the laboratory and data gathered from the different coefficient of phytoremediation were recorded in a spread sheet on Microsoft excel and used for tables and graphical representation. The statistical package for social sciences (SPSS) was used for data analysis while analysis of variance (ANOVA) was used to determine statistically significant differences amongst the concentration of pollutants in AMD water. Significant difference was tested at 95% confidence levels.

3.14 Ethical consideration

The research proposal went through the University of South Africa ethical clearance process and the permission to start the experiment was granted for a period of one year (Appendix A). After a successful completion of the first year of laboratory work the ethical clearance was renewed for another one year (Appendix B). A formal consent was obtained from mine where real AMD was collected.

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CHAPTER FOUR

This chapter addresses the passive remediation of acid mine drainage using phytoremediation with focus on the partitioning of inorganic contaminants between the substrate, plants, and external factors.

Chapter four was prepared and submitted to **Ecological Engineering Journal**. The paper is currently under review.

CHAPTER FOUR

Passive remediation of acid mine drainage using phytoremediation: Partitioning of inorganic contaminants between the substrate, plants, and external factors

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Abstract

Acid mine drainage (AMD) and its associated toxicological effects degrade the environment and its suitability to support life. Active and passive biological technologies currently applied for AMD treatment are not environmentally friendly and unsuitable for long-term treatment thereby owing to expense. Free Water System Constructed Wetland (FWS-CW) equipped with *Vetiveria zizanioides* and compost soil as substrate for the treatment of AMD was explored. The experiments were performed in three replicates over a period of 30 days using real AMD. The water quality was monitored at 24 hours intervals and the average result of five days period was recorded. Results revealed a tolerance index of 1.02 for *Vetiveria zizanioides*. The decrease of TDS and EC from 3880 to 1400 mg/L and from 5 to 2 mS/cm respectively. The rise of pH from 2.6 to 3.1 and a net removal of pollutants in the following order: Fe (90%) > Zn (73%) > SO_4^{2-} (67%) > Mn (58%) > Cu (34%) > Al (31%) > Ni (12%). Furthermore, partitioning of contaminants revealed that metals were removed in the order: substrate ≥ plant ≥ external factors of which, the substrate contributed 77.23% (Mn), 72.01% (Al), 69.91% (Zn), 66.51% (Ni), 60% (Cu) and 56.56% (Fe). The plant contributed Fe (40.42%) > Cu (36.66%) > Ni (30.09) > Zn (27.89%) > Al (22.11%) > Mn (20.58%), and the external factor contributed 5.88 % (Al), 3.4% (Ni), 3.34% (Cu), 3.02% (Fe), 2.19% (Mn), and 2.2% (Zn). The translocation assessment revealed that Al, Fe and Ni were mainly localized in the roots whereas Cu, Mn and Zn showed greater translocation to shoot. The XRF, XRD, FTIR and SEM-EDS revealed the presence of pollutants in substrate and grass roots. The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirm that metals existed as di-and-trivalent in solution. This experiment revealed that this synergetic approach between substrate, plants and external can significantly improve the quality of AMD; however, a polishing technology is required to ameliorate the quality of product water to prescribed environmental discharge standards, specifications, and guidelines.

Keywords: Acid mine drainage; treatment; free water surface flow constructed wetland; phytoremediation; substrate; external factors; *Vetiveria zizanioides*.

4.1 Introduction

Mining exploration and minerals exploitation are amongst the most important revenue-generating industries in developed and developing countries such as South Africa, The United State of America, Australia, China, and afield (Masindi *et al*., 2018; Acharya and Kharel, 2020). Despite their immense socio-economic benefits such as job creation and boosting the GDP of any given country, mining exploitation has been associated with significant amount of environmental pollution (Simate and Ndlovu 2014; Park *et al.* 2019). This has been endorsed due to its ability to generate toxic and hazardous by-products such as acidic and metalliferous mine drainages. Specifically, acid mine drainage (AMD) is a commonly known legacy of almost all mining industries worldwide and mostly, from gold and coal mines. Acid mine drainage is produced when sulphide bearing minerals such as pyrite, arsenopyrite, and marcasite amongst others get exposed to oxidising conditions (oxygen and water) producing a highly acidic and metalliferous mine drainage (Masindi *et al.,* 2017). The product effluent commonly known as acid mine drainage

(AMD) or acid rock drainage (ARD), is chemically presented as shown in equation 4.1 (Kefeni *et al*., 2017).

$$4\mathrm{FeS}_2 + 15\mathrm{O}_2 + 14\mathrm{H}2 \rightarrow 4\mathrm{Fe(OH)}_3 3 + 8\mathrm{SO}_4^{2-} + 16\mathrm{H}^+ \qquad \text{(Eqn 4.1)}$$

Physico-chemically, AMD is characterised by low pH (pH ≤ 2 - 3.5), high electrical conductivity (EC) and total dissolved solid (TDS), notable amounts of sulphate ions and toxic heavy metals such as Al, Fe, Mn. Zn, Pb and trace level of metal such as Cu and Ni including radionuclides (Nordstrom *et al*., 2015). Due to its composition and chemistry thereof, environmental problems associated with AMD are diverse and vary in their degree of severity and toxicity (Sarmiento *et al*., 2011). Areas predominated by mining activities experience AMD contamination and this leads to the decline in myriads of ecological benefits (Jooste and Thirion, 1999; Hughes and Gray, 2013; Wang *et al*., 2018). Worldwide, there is a growing concern about the environmental legacy of mining activities and abandoned mine sites often remain the source of AMD production after mine closure and it may require decades of proper management practices to reclaim, rehabilitate, and restore the ecosystem (Naidu *et al*., 2019; Park *et al*., 2019; Spellman *et al*., 2020).

In South Africa and other regions alike, the issue of AMD is very significant and the available water resources are under serious threat of pollution from mining industries and afield (Tempelhoff *et al*., 2014). According to Masindi. (2016) around 340 ML of mine water is produced daily in the Witwatersrand basin leading to an impairment of ground and surface water due to high level of toxic pollutants that extremely exceed the set standards and targets. Ecological custodians require that this wastewater stream be treated prior releasing it to different receiving environments (Naidoo, 2017). In light of that, various technologies have been developed for the treatment of AMD (Skousen *et al*., 2017; Park *et al*., 2019; Rambabu *et al*., 2020). They include active and passive technologies. Active treatment methods rely on precipitation, adsorption, ion exchange, filtration, and freeze crystallization (Kefeni *et al*., 2017; Park *et al*., 2019). Passive treatment methods include biogeochemical processes which primarily rely on aerobic and anaerobic processes facilitated by lime drains, reactive lime barriers, biological processes, and phytoremediation (Simate and Ndlovu, 2014; Skousen *et al*., 2017;

Rambabu *et al.,* 2020). Due to the challenges of active treatment process that include cost effectiveness, generation of heterogeneous and complex secondary sludge containing toxic and hazardous chemical species, frequent energy and chemicals requirement further compound their unfriendly ecological status (Mujuru and Mutanga 2016; Masindi and Tekere, 2020). Passive AMD treatment processes have received lot of attention simply because of their zero energy requirement, and ecological footprints (Bwapwa *et al*., 2017; Rambabu *et al.*, 2020; Nguegang *et al*., 2021). Following challenges of natural wetlands and the potential of certain macrophytes to extract heavy metals from contaminated sites (Singh and Chakraborty, 2020), researchers and environmental engineers are investigating emerging passive treatment systems such as constructed wetland and phytoremediation as long-term solution towards AMD management (Nguegang *et al.*, 2021).

Employment of constructed wetlands as low cost and environmentally friendly technology for the treatment of AMD has gained lot of attention. This technology uses macrophytes which play a key role in chemical species attenuation and metals removal (Skousen *et al*., 2019; Lizama Allende *et al.,* 2020; Singh and Chakraborty, 2020; Luo *et al*., 2020). Substrate media which play a huge role in inorganic contaminants removal in constructed wetland since substrate retain metals through sedimentation process thereby enhancing their accumulation by plant (Lizama Allende *et al*., 2012). Furthermore, the study of Mustapha *et al*. (2018) has revealed that minor fraction of inorganics contaminants in constructed is removed by external factors. Recently, a wide range of literature has been focusing on the use of Vetiver grass for the treatment of wastewater and attenuation of toxic chemical species (Kiiskila *et al.*, 2017, 2019, 2020). As such, the use of Free Water Surface Constructed Wetlands (FWS-CW) with Vetiver grass can be successful in treating AMD. Vetiver grass is a perennial bunchgrass of the family of *Panacea*, it is a non-invasive perennial grass that is fast-growing with a large biomass and extensive root system (Truong and Danh, 2015; Kiiskila *et al.,* 2020). Vetiver is a hydrophyte plant (growth normally in both aquatic and non-aquatic environment), tolerant to acidic conditions and is able to accumulate various types of metals (Kiiskila *et al.*, 2019; 2020). Following the potential of natural wetland in wastewater treatment, the role of phytoremediation in removal of heavy metals and the ability of Vetiver grass to tolerate very acidic conditions and accumulate various types of metals, this study was set out to investigate an integrated approach of using a combination of free water system constructed wetland and phytoremediation with Vetiver grass for acid mine drainage treatment. Kiiskila *et al.* (2019) explored the treatment of metalliferous mine water by Vetiver grass (*Vetiveria zizanioides*) on a multiscale long-term study. The study successfully proves that Vetiver grass can be used for AMD treatment using a floating wetland. However, the use of compost soil as wetland substrate has never been examined and this will be the first study in design and execution to determine the partitioning of toxic chemicals from AMD to substrate, plants and external factors. This will enable us to understand the role and effect of each component of the system in the attenuation of toxic chemical species from AMD.

4.2 Materials and methods

4.2.1 Samples collection and characterization

Acid mine drainage water was collected at the Sibanye Gold mine in Krugersdorp, Gauteng, South Africa. *In situ* analysis was done using a multi-parameter meter (HANNA instruments, Johannesburg, RSA). These included pH, total dissolved solids (TDS) and electrical conductivity (EC). Initial concentrations of metals (Al, Cu, Fe, Mn, Ni and Zn) in AMD water were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Shimadzu ICPE-9000, Australia) and the initial concentration of sulphate was analyzed using Ion chromatography (IC) (Metrohm model 850, Switzerland). Compost soil used as wetland substrate was purchased from Garden World, Johannesburg, South Africa. Soil samples were air dried for five days, sieved and one gram (1g) was digested in a mixture of 5 mL of 55% nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide (H_2O_2) using microwave digestion. The digested soil samples were allowed to cold down at room temperature followed by filtration and analyzed for initial concentrations of metals using ICP-OES (Shimadzu ICPE-9000, Australia).

4.2.2 Acquisition of the plants and reagents

Shoot of *Vetiveria zizanioides* were purchased from NANDADRAM ECOVILLAGE farm in Kwa-Zulu Natal, South Africa and transported to the University of South Africa's sciences campus in Florida. The shoots of *Vetiveria zizanioides* were submerged under potable fresh water for two weeks in order to allow the new seeds to germinate. After two weeks of germination, the new seedlings of *Vetiveria zizanioides* were transferred into small pots containing compost soil. Reagents which were used in this study were of analytical grade and they were purchased from Merck, South Africa.

4.2.3 Characterisation of samples

The feed and product sludge were characterised using different analytical and state-of-art techniques. Elemental composition of substrate (soil) from the treatment and control wetland was done using X-ray fluorescence (XRF).The analyses were performed using a Thermo Fisher ARL-9400 XP+ Sequential XRF with winXRF Software. Mineralogical composition was done using X-ray diffraction. Analyses were done using Philip PW 1710 diffractometer equipped with graphite secondary monochromatic. Thenceforth, the shoots and roots were characterised using high-resolution scanning electron microscopy equipped with electro dispersion spectroscopy (HR-SEM-EDS) (JOEL JSM-840, Hitachi, Japon).

4.2.4 Wetlands design and optimization experiments

4.2.4.1 Wetland design

Wetlands were constructed using the same soil used as a growing medium for growing of young plants. The wetlands were constructed using a circular plastic basins of 100 litres capacity each measuring 62 cm of diameter (d) and 45 cm high (h) and a drum of 300 litres capacity as reservoir tank to contain AMD-water and fresh water for control (Figure 4.1). The wetlands were continuous flow system where water was flowing into the system in continuous drop during the experiment. The flow rate and hydraulic retention time (HRT) were the same for all the wetlands (control and experiment). Each experimental wetland was connected to a reservoir containing liquid (AMD) for treatment and tap water for control) via a conduit pipe and contained a total of 10 plants planted at equidistance each other. A control valve (drip plastic bottle) was incorporated at the baseline of each tank to regulate the flow rate of water from tank to the wetland cell. The outlet structure of each wetland cell was an orifice with valve to collect leachate for analysis (**Figure 4. 1**).

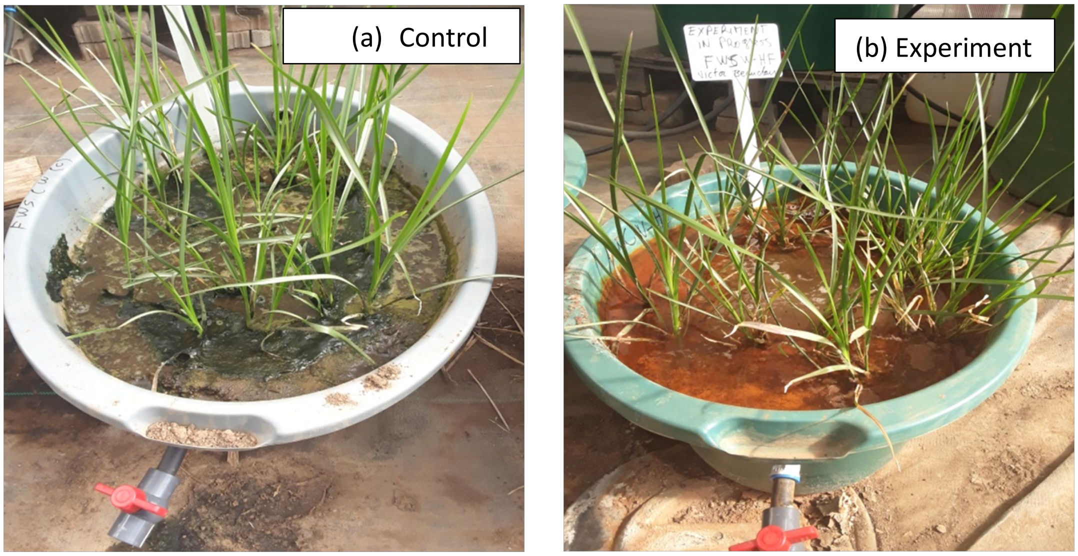


Figure 4.1 (a,b): Experimental set up of FWS-CW. (a) control (b) experiment.

4.2.4.2 Wetland experimental procedure and assays

The system was calibrated using a drip plastic bottle and fresh water was allowed to flow in continuous drop into the system for 45 days in order to ensure that the system is working properly. From there, the hydraulic loading rate (HLR) which is expressed as the average water flow per unit area over a given period was determined using equation 4.2.

$$\text{HLR or Q} = \frac{\text{Qi}}{\text{A}} \qquad \text{(Eqn4.2)}$$

Where,

Qi = water inflow (m^3/d),

A is the wetland top surface (m^2).

The calculated HLR was 1.8 cm^3 or 1.8 Litres/day. The HRL allowed the researcher to determine the HRT as illustrated in Equation 4.3 (Carleton *et al.*, 2001; Sahu, 2014).

$$\mathrm{HRT} = \frac{\mathrm{LWyn}}{\mathrm{Q}} \qquad \text{(Eqn 4.3)}$$

Where,

L is the length of wetland cell (m),

W is the width of wetland cell (m),

y is the depth of water in the wetland cell,

n is the porosity of wetland substrate (soil), and

Q is average flow through the wetland (m^3/d).

Or in the system, plastic circular basins were used as wetland cell and the surface of the top surface of each wetland was determined used Equation 4.4

$$A = \pi r^2 \qquad \text{(Eqn 4.4)}$$

Where,

π is a constant (3.14)

r is the radius of the plastic basin.

The HRT of the system was then determined using Equation 4.5

$$HRT = \frac{\pi r^2 yn}{Q} \qquad \text{(Eqn 4.5)}$$

Where,

π is a constant,

r is the radius of the plastic basin

y is the depth of water in the wetland cell,

n is the porosity of wetland substrate (soil), and

Q is average flow through the wetland (m^3/d).

$$HRT = \frac{3.14 \times (0.31)^2 \times 0.3 \times 0.6}{0.0018}$$

$$HRT = 30.17\ days$$

The determined HRT was of 30.17 days and during that period, the inlet flow rate and outlet flow rate were monitored daily to ensure a steady flow of water in and out of the wetland. The flow rate was maintained low and the HRT long to allow the water to spend more time in wetland cell thereby improving the performance of the system. Kiiskila *et al.* (2019) revealed that performance of a wetland is highly improved by high hydraulic retention time (HRT). The general health of the plants was observed daily for qualitative changes (shoot coloration, and morphology) and plants were collected at the end of the experiment for biomass assessment. The collected samples were further used for the determination of tolerance index and metals content. The soil samples were also collected for characterisation.

4.2.4.3 The performance of the system

Product water and soil substrate (experiment and control) were collected every 24 hours after the beginning of the treatment process for a period of 30 days retention time. Analysis was performed and the average results of five days period was considered. Product water samples were collected using amber glass bottles of 500 mL. After collection, samples were divided into two sub-samples of 400 mL and 100 mL. Sub-samples of 400 mL were used for in-situ analysis of pH, TDS, EC while sub-sample of 100 mL was used for the analysis of ex-situ parameters which include metals (Al, Cu, Fe, Mn, Ni, Zn) and sulphate. Prior to analysis for metal concentrations, water samples were filtered through a 0.22 um pore syringe filter membrane to remove particles and preserved by addition of two drops concentrated HNO_3 to prevent ageing and immediate precipitation of metals. After refrigeration at 4° C, filtrates were analysed using ICP-OES

(Shimadzu ICPE-9000, Australia). Sulphate was determined using IC (Metrohm model 850, Switzerland). Soils samples were air dried for five days, after that, they were sieved and one gram (1g) was microwave digested in a mixture of 5 mL of 55% nitric acid (HNO_3) and 2 mL 30% hydrogen peroxide (H_2O_2). Once digested, the samples were cooled to room temperature, the solutions were filtered through a 0.22 μm pore syringe filter membrane and analysed for metals using ICP-OES. Plants from each wetland were harvested at the end of the experiment (30 days) without damaging the roots and were rinsed separately with distilled water to remove dust, soil and mineral particles. Plants were then air dried at 25 ℃ for one week and weighed in order to determine the tolerance index of the plant using the Equation 4.6.

$$\text{Tolerance index }(\%) = \frac{\text{biomass of plant growing in AMD cell}}{\text{Biomass of plants growing in potable fresh water cell}} \qquad \text{(Eqn 4.6)}$$

Plant biomass from each wetland was further separated in two portions (shoots and roots). The Vetiver shoots and roots were air dried separately at 25°C for one week, were grinded using mortar and pestle. Thereafter, 1 g of each part was digested with a mixture of 5 mL of 55% HNO_3 and 2 mL of 30% hydrogen peroxide (AR grade; BDH). The digested sample was then filtered using 0.22 μm pore syringe filter membrane. The metal contents in the filtrate were determined using ICP-OES. After the analysis of metals concentration in plants, the bio-concentration factor (BCF) and translocation factor (TF) of the plants for each metal was calculated. Subsequently, the distributions of metals in tissues were also determined. The percentage removal of metals from the aqueous solution was determined using the following formulae.

$$\text{Removal efficiency }(\%) = \frac{\text{Initial concentration} - \text{final concentration}}{\text{Initial concentration}} \times 100 \qquad \text{(Eqn 4.7)}$$

$$\text{Metal content retained in the system} = \text{Initial metal concentration in AMD water} - \text{final metal concentration in AMD water} \qquad \text{(Eqn 4.8)}$$

$$\text{AMD contribution in substrate}' \text{ metal content} = \text{Final metal content in the substrate} - \text{Initial metals content in the substrate} \qquad \text{(Eqn4.9)}$$

$$\text{Plant metal content} = \text{Root metal content} + \text{shoot metal content} \qquad \text{(Eqn 4.10)}$$

$$\% \text{ retained by wetland substrate} = \frac{\text{metal concentration in the substrate}}{\text{Metal content from AMD}} \times 100 \qquad \text{(Eqn 4.11)}$$

$$\% \text{ removal by phytoremediation } = \frac{\text{Plant metal content}}{\text{Metal content from AMD}} \times 100 \qquad \text{(Eqn 4.12)}$$

4.2.4.4 Determination of the translocation and distribution of metals

Metals distribution throughout *Vetiveria zizanioides* roots and shoots was evaluated using ICP-OES results of each portion of plant metal content and scanning electron microscopy-energy dispersive spectrometry (SEM-EDS) analysis. Vetiver grass were harvested from AMD treatment and control wetland at the end of the experiment, treated and analyse for each portion of metal content as specified in **Section 4.2.4.3** and the morphology and elemental distribution was determined using SEM-EDS. The variation of each parameter with time was determined and the results plotted using Microsoft Excel software.

4.2.4.5 Geochemical modelling

Speciation and potential precipitating of metals during the interaction of AMD, substrate and external factors was done using the water-Q4 database. The plants-substrates were modelled using PH REdox EQuilibrium (in C language) (PHREEQC). The water potential precipitation of metals were determined using saturation indexes (SI) of which, SI ≤1 denotes under-saturation, SI ≈ 1 denotes saturation, and SI ≥ 1 denotes super-saturation.

4.3 Results and discussion

This section presents and discusses the results of the effects of FWS-CW with compost soil as substrate and equipped with *Vetiveria zizanioides.* Discuss the effect of contact time on the removal efficiency of different physicochemical properties of the feed water. The chemical and morphological properties of the feed and product minerals will also be assessed.

4.3.1 Remediation studies

This section discusses the findings from the experiments that were conducted to.

4.3.1.1 Effect of FWS-CW on pH

The final pH of 7.26 was for the control while 3.1 was for the AMD over the experimental period of 30 days. The results are shown in **Figure 4.2**.

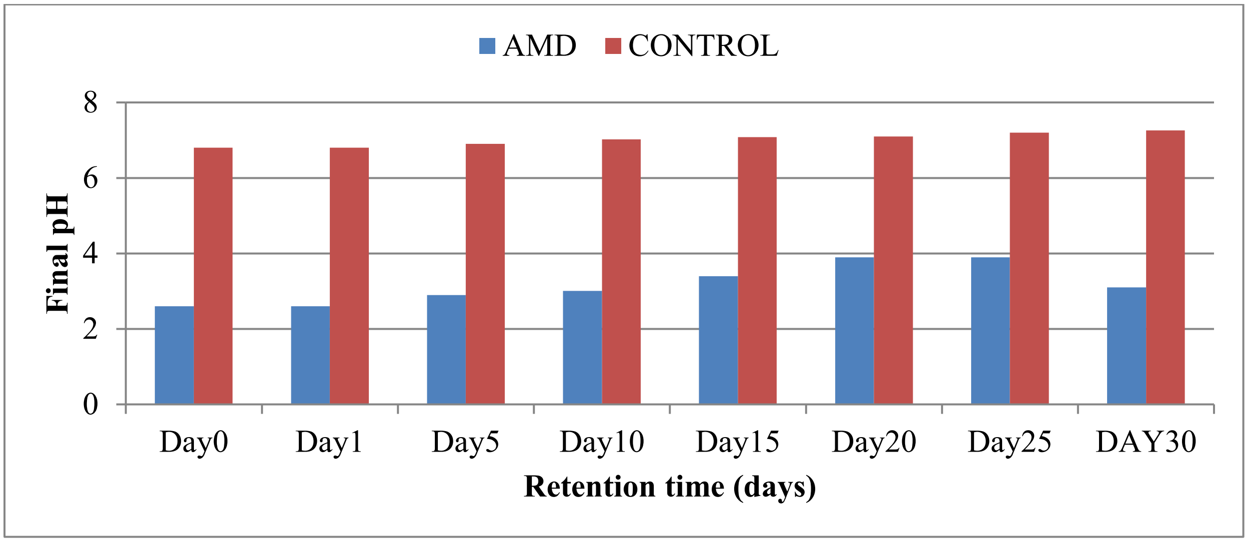


Figure 4.2: Variation in pH (treatment and control) with variation of hydraulic retention time under a hydraulic loading rate of 1.8 L/day

As shown in **Figure 4.2**, there was an increase from 2.6 to 3.1 in the final pH of AMD and from 6.8 to 7.26 in the final pH of tap water. The pH of AMD increased from day 0 to day 25 and decreased from 3.9 to 3.1; however, it was still above the initial pH of AMD. The increase in pH may be attributed to the removal of acid forming metals and mainly the removal of Al and Fe protons from the aqueous solution (Wang *et al.,* 2017). Thenceforth, the rise of pH may be the consequences of organic acids released by plants during the process of photosynthesis (Chen *et al.,* 2021). Specifically, plants growing in aquatic medium absorb free carbon dioxide (CO_2) from water and the combination of hydrogen and carbon dioxide leads to the increase of pH. The rise of pH can also be explained by the weak acid anion of metabolic substances released by plants since the literature revealed that some plants species used in bioremediation such as *Myrophyllum sp* releases acid anion which play a vital role in the pH increase of the culture medium.

4.3.1.2 Effect of FWS-CW on electrical conductivity

The effect of FWS-CW on EC was evaluated for treatment and control wetland during the 30 days hydraulic retention time and the results are shown in **Figure 4.3.**

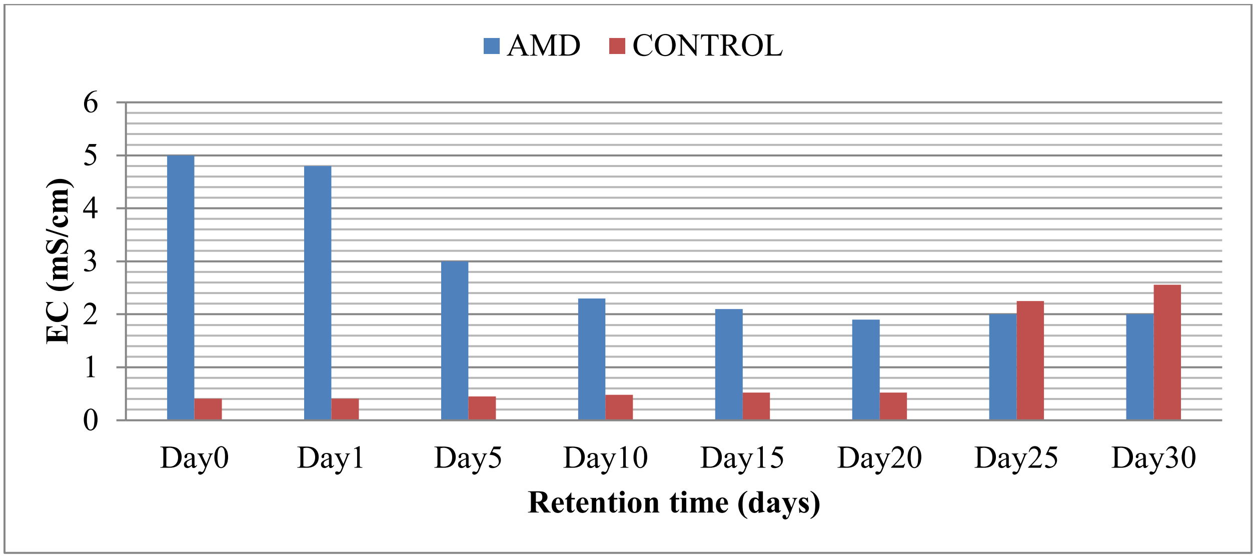


Figure 4.3: Variation in electrical conductivity (treatment and control) with variation of hydraulic retention time under a hydraulic loading rate of 1.8L/day

As shown in **Figure 4.3**, there was reduction in EC from 5 to 2 mS/cm after 30 days of retention time. Specifically, EC decreased from Day 0 to Day 20, however, a slight increase was observed from the 20th to the 25th and then remained constant until the end of experiment. This corroborated findings which were reported in other scientific studies Kiiskila *et al*. (2017) which reported a decrease of EC from 4 to 1.5 mS/cm of AMD after 30 days retention time. The reduction in EC may be attributed to the reduction in dissolved metals and other pollutants following sedimentation and accumulation by plants. This explicitly denotes that plants are absorbing metals as their nutrients hence there was a reduction in their concentration over the duration of the study. This clearly denotes that *Vetiveria zizanioides* has high affinity for metals in water hence their reduction in the aqua-sphere. Contrary, the control experiment demonstrated no reduction, instead, the EC was increasing and this could be attributed to the ion exchange process between the roots, substrate and the water bodies.

4.3.1.3 Effect of FWS-CW on sulphate concentration

The effect of FWS-CW on the removal of SO_4^{2-} from AMD is reported in **Figure 4.4.**

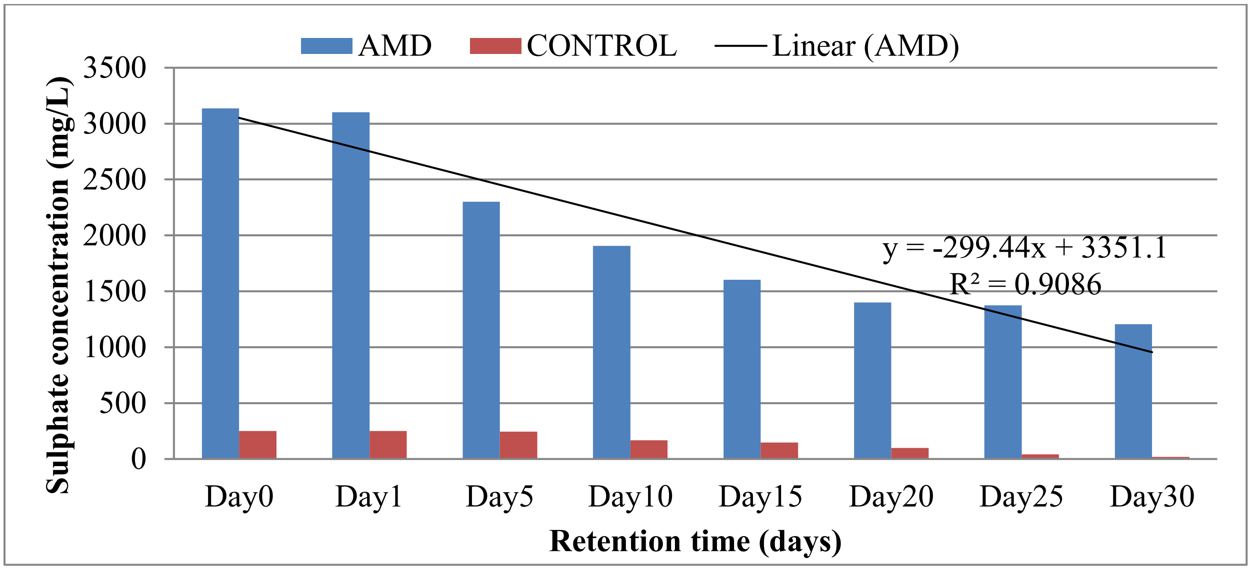


Figure 4.4: Variation of in sulphate concentration (treatment and control) with variation of hydraulic retention time under a hydraulic loading rate of 1.8L/day

As shown in **Figure 4.4**, sulphate concentrations gradually decreased with an increase in retention time (both control and experiment). Specifically, there was minute change in sulphate concentration during the first 24 hours of experiment followed by a decrease from 3102 mg/L to 1400 mg/L up to day 20. However, from day 20 to day 25, sulphate content in AMD water was almost constant followed by a slow decrease up to the 30th day. On the other hand, the concentration of sulphate in the control wetland was constant during the first five days followed by a small decrease from the 5th day up to the end of experiment (**Figure 4.4**). The regression analysis performed further confirmed the relationship between time and sulphate concentration. Longer retention time play a huge role in higher removal efficiencies of SO_4^{2-}. The decrease in SO_4^{2-} levels may be linked to the absorption by the plants since they commonly use it for amino acid synthesis particularly when they find themselves under metals stress (Sánchez-Andrea *et al.,* 2014; Buzzi *et al.,* 2013). Moreover, plants comprise small peptides such as phytochelatins or metallothioneins which comprise the thiol group of cysteine that is used to bind with metals thereby facilitating their accumulation (Sánchez-Andrea *et al.,* 2014). In order for plants to synthesize the thiol group, plants must have elevated concentration of sulphate and this can explain drastic reduction in SO_4^{2-} levels. Furthermore, SO_4^{2-} is vital part of all plants protein and certain plant molecules and is used by plants to build organic molecules (Morkunas *et al*., 2018). Lastly, the reduction in SO_4^{2-}could also be aided by thiosulphate and tetrathionate which are by-products of oxidized sulphate and may play an indispensable role in the attenuation of sulphur from acid mine drainage (Wu *et al.,* 2015).

4.4.1.4 Effect of FWS-CW on metal concentration

The effect of FWS-CW on metal concentration was evaluated over of 30 days retention time and the results are shown in **Figure 4.5 (a, b).**

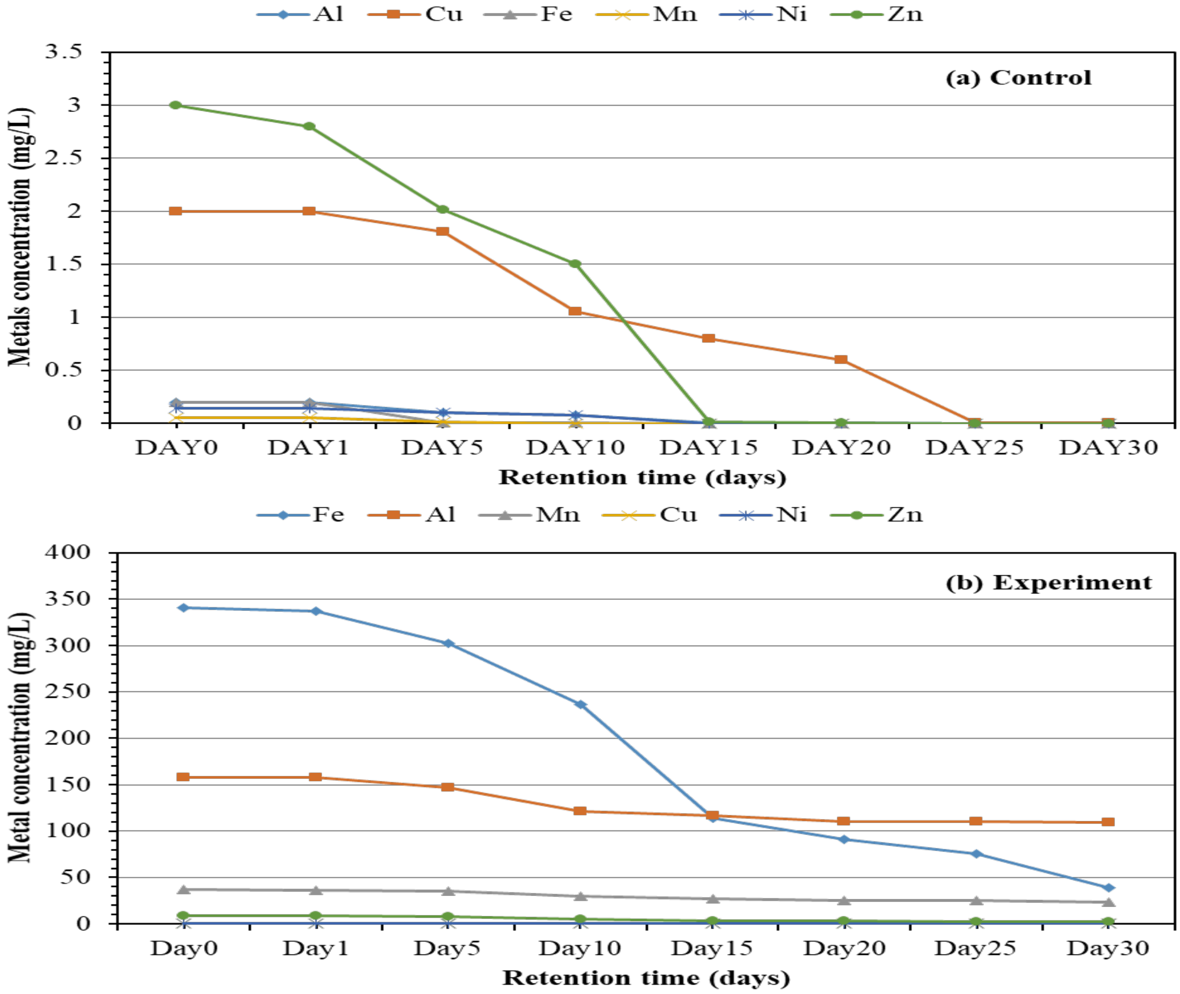


Figure 4.5 (a, b): (a) Variation of the metals concentration in the control wetland in 30 days retention time under a hydraulic loading rate of 1.8 L/day. (b) Variation of the metals concentration in the treatment wetland in 30 days retention time under a hydraulic loading rate of 1.8 L/day

As shown in **Figure 4.5 (a,b),** the levels of all metals (Al, Fe, Mn, Ni, Cu and Zn) in treatment wetland decreased with an increase in retention time. Iron decreased from Day 1 to the last day (Day 30) of retention time while other metals decreased from Day 1 to Day 20. However, the content of metals in the control wetland decreased from the first day of the experiment to day 25 and at some point was below detection limit (BDL) of 0.0001 mg/L for all metals. This finding indicated that *Vetiveria zizanioides* is great accumulator of metals from soil and water (Truong and Danh, 2015) but with different removal efficiency since the removal percentage depends of the ability of *Vetiveria zizanioides* to accumulate specific metal. Furthermore, results from this study also proved that after 30 days of retention time, *Vetiveria zizanioides* can still accumulate Fe whereas the accumulation process for other metals lasted for only 20 days **(Figure 4.5b).** Metal reduction by the system may be attributed to sedimentation in the substrate and accumulation by *Vetiveria zizanioides* for its different metabolic functions. The findings were in line with the studies of Ghadiri and Hejazi. (2018) which found that *Vetiveria zizanioides* had higher capability for remediation of heavy metals compared to *T. fescue*. In particular, iron is an essential micronutrient for plant since it plays a crucial role in metabolic process including DNA synthesis, respiration photosynthesis, and his deficiency in plant leads to common nutritional disorder. Metals such as Cu, Fe, Zn and Mn act as important cofactors for many enzymes and are essential for both mitochondrial and chloroplast functions (Morkunas *et al.*, 2018). Furthermore, zinc is amongst the eight essential micronutrients and is needed by plants in small amount but vital to plant growth since it plays an important role in a wide range of process such as growth hormone (Morkunas *et al.*, 2018) while manganese is one of the essential nutrients needed by plants for growth, it is also vital for plant's metabolism since many biochemical processes in plant such as chloroplast formation, photosynthesis, nitrogen uptake and synthesis of some enzymes are directly linked to the presence of manganese in plant (Mousavi *et al.*, 2011). These results revealed that wetland and phytoremediation operate simultaneously with wetland substrate retaining metals by co-adsorption and precipitation processes which are subsequently accumulated by wetland macrophytes. Finding from this study further revealed that *Vetiveria zizanioides* easily accumulates metals (Cu, Fe, Mn and Zn) that are essential for its metabolism and might requires more time (more than a month) to accumulate metals such as Al and Ni that are not really essential for its fundamental growth.

4.3.1.5 Role of substrate in metals accumulation

The effect of substrate on metal removal from AMD was evaluated in 30 days retention time and the results are shown in **Figure 4.6 (a, b).**

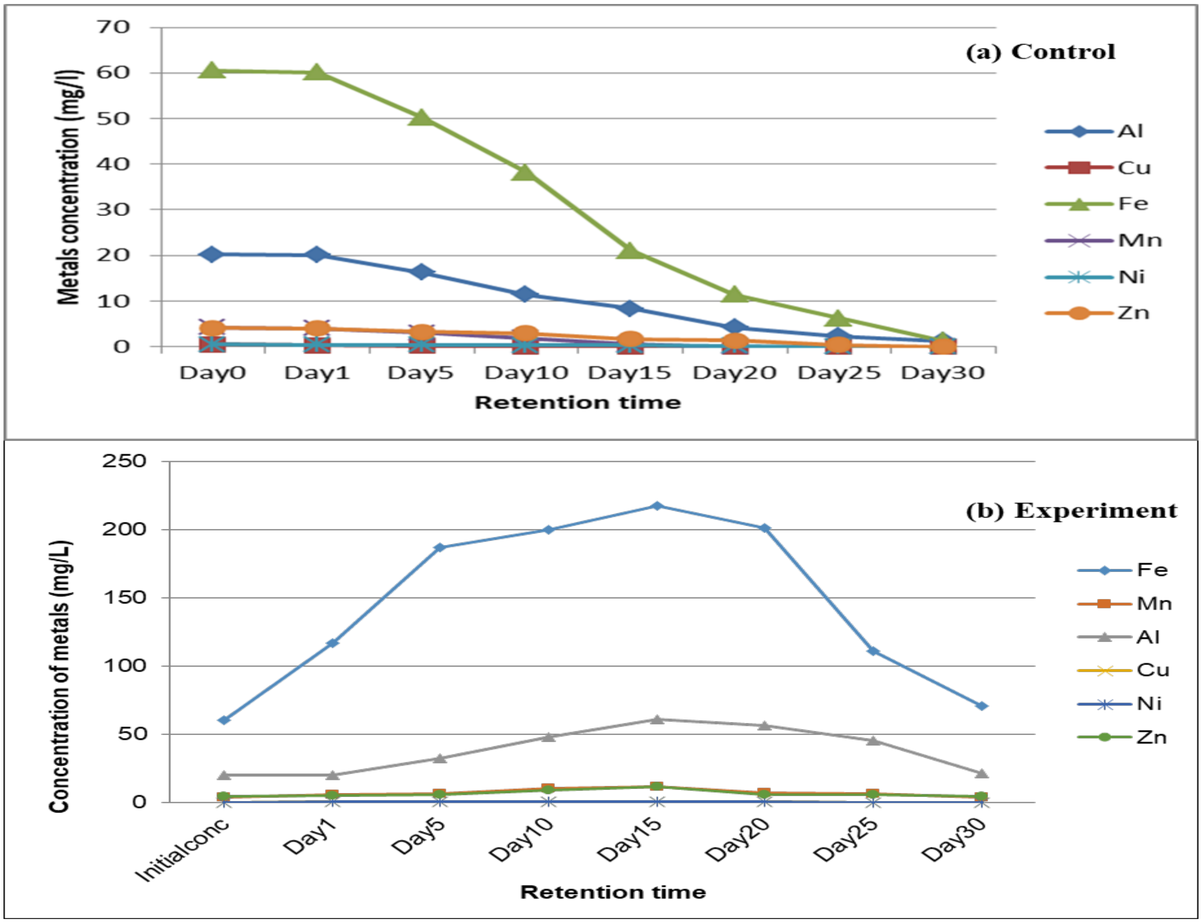


Figure 4.6 (a, b): (a) Variation of the metal concentration in substrate (control wetland) within 30 days HRT under hydraulic loading rate of 1.8 L/day. (b) Variation

of the metal concentration in substrate (experimental wetland) within 30 days HRT under hydraulic loading rate of 1.8 L/day

As shown in **Figure 4.6b**, there was an increase in the substrate metals concentrations from Day 0 to Day15 and followed by a decreasing phase until the end of experiment. On the other hand, the control experiment denoted the reduction in metals with an increase in retention time (**Figure 4.6a**). This could be attributed to the attenuation of metals by *Vetiveria zizanioides* whereas an increase in metal concentration for the experiment is the result of additional load of metals into the wetland since the water is flowing continuously. The decrease in metals concentration may be related to the presence of *Vetiveria zizanioides* which is accumulating metals from the sediment via its roots. Two different thresholds are used to determine metals concentration in wetland sediment: (i) the threshold effect level (TEL) and (ii) the probable effect level (PEL) (Kacholi and Sahu, 2018). Heavy metals concentration lower than the TEL indicates minor pollution and in this case, the ecological effects are negligible. However, if heavy metals content is higher than PEL, it may lead to serious pollution level which in turn can be associated with high toxicity condition. Reporting and comparing the above statement to this research project, it follows that after ten (10) days of the experiment, the concentration of all metals exceeded their initial concentration in the wetland sediment. However, the wetland was slightly polluted with iron, manganese, zinc, and aluminium with their concentration exceeding the TEL and the concentration of nickel and copper below the TEL. It can be assumed that in this project, soil substrate retains metals thereby allowing *Vetiveria zizanioides* to accumulate them since *Vetiveria zizanioides* has stiff stems and forms dense hedges to restrict pollutants movement and this condition greatly enhances the process of pollutants adsorption, ion exchange and precipitation as reported by (Truong and Tran, 2014). In the control wetland, metals (Al, Cu, Fe, Mn. Ni and Zn) concentration in the substrate decreased gradually to below detection limit of 0.0001mg/L at the end of the experiment (Day 30). The decreasing of metals concentration to below detection limit may be attributed to their accumulation by *Vetiveria zizanioides* (**Figure 4.6a**). The finding of this study showed that the substrate plays a vital role in metals removal by sequestering them and facilitate their accumulation by plants. Other processes such as precipitation and adsorption must precede sedimentation since plants only accumulate precipitated metals (Sheoran and Sheoran, 2006; Sheoran, 2017). The use of compost soil media in FWS-CW and phytoremediation combined system seems to be recommended due to sorption capacity and ability to form new storage sediment without clogging the wetland. As such, the results revealed that soil is suited to be used as wetland media for the removal of pollutants. The reduction of pollutants in the wetland is controlled by diverse biochemical processes and microbial interactions in wetland media (Maine *et al*., 2009). From the results above, it follows that the media is one of the most important component in FWS-CW and phytoremediation combined system since it ensures net removal of pollutant. Metal removal vary depending of the type of plant species, importance of metal in plant metabolism, the microbial diversity as well as physical and hydrodynamic characteristic such as depth and types of wetland (Vymazal, 2011) and temperature (Wang *et al*., 2017). Thenceforth, findings from this study further demonstrate that the wetland substrate aid in the removal of metals from the aqua-sphere and this will play a pivotal role in scavenging the contaminants for easy uptake by the plants as compared to the free-flow wetlands without substrate.

4.3.1.6 Removal efficiency of metals and sulphate in the experimental system

The removal efficiency of metals and sulphate by FWS-CW was determined gradually and the result is shown in **Figure 4.7.**

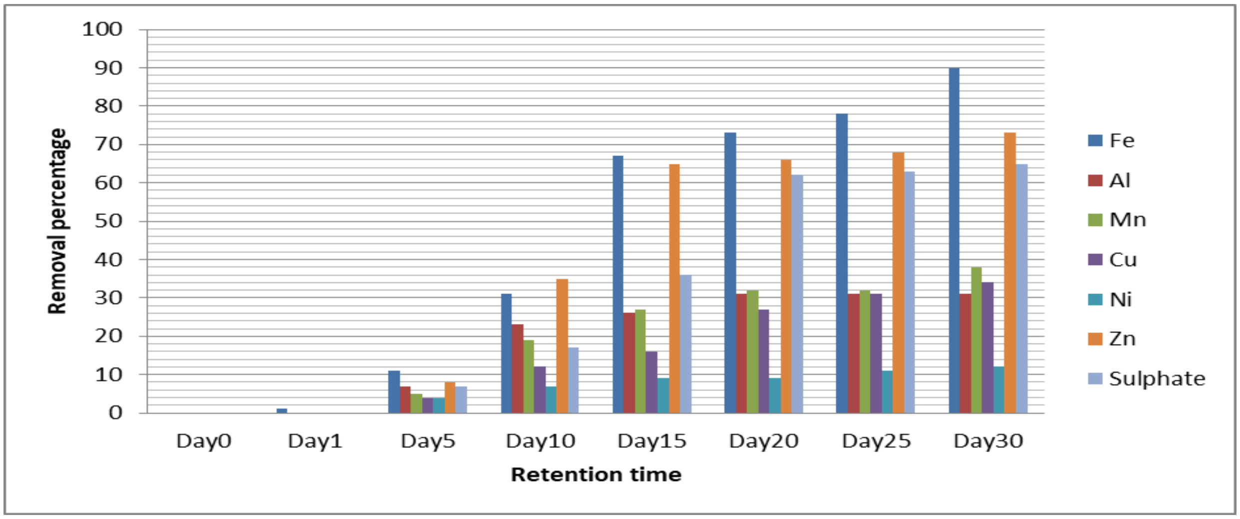


Figure 4.7: Removal efficiency of metals and sulphate by FWS-CW in 30 days retention time under a hydraulic loading rate of 1.8 Litres/day

As shown in **Figure 4.7**, the efficacy of *Vetiveria zizanioides* for the removal of metals and sulphate from AMD has been reported. The removal efficiency is basically expressed as the percentage of pollutants removed from a polluted site in terms of the ratio to the total amount of pollutant that enters the site. This study revealed that integrating FWS-CW and phytoremediation equipped with *Vetiveria zizanioides* is efficient in removing heavy metal and sulphate from AMD within a 30 days retention time. The percentage removal of metals was in the following order: Fe (90%) >Zn (73%) > SO_4^{2-} (67%) > Mn (38%) > Cu (34%) >Al (31%) > Ni (12%) (**Figure 4.7**). The metal removal started from the beginning day1 for iron and day 5 for others metals and sulphate. The removal efficiency can be attributed to the sedimentation of metals in substrate, plant accumulation and mere external factors. Specifically, iron is one of the major elements required by plants growth alongside others macronutrients which are potassium and phosphorus while zinc in plants is crucial for the development since it plays an important role in a wide range of processes such as his involvement in growth hormone production (Mudhiriza *et al.,* 2015). The finding also showed that removal efficiency of iron and zinc increased drastically during the first 15 days of experiment whereas it was not the case with other metals hence denoting that *Vetiveria zizanioides* was able to accumulate iron and zinc more than Al, Ni, Mn, and Cu. Iron and zinc are more needed by plants due to their stellar role in plant metabolism. Thenceforth, zinc is one of the eight essential micronutrients while iron is vital for the respiration and photosynthesis process in plant as well as the production of healthy green leaves (Cabot *et al.,* 2019).

4.3.2 Tolerance index, bio-accumulation, and translocation effects

This section will emphasize the tolerance index, bio-accumulation, and translocation effects of metals and oxyanions in the plants and substrate when subjected to AMD.

4.3.2.1 Tolerance index

The tolerance index (TI) in phytoremediation is an important tool to screen how plants tolerate different concentrations of pollutants. Explicitly, the TI was calculated following the method of (Diwan *et al*., 2010) as the mean weight (biomass) of a plant grown in the AMD wetland divided by the mean weight of plant grown in a control wetland and was expressed as TI.

$$\mathrm{TI} = \frac{\text{Biomass of the treated plants (g)}}{\text{Biomass of the control plant (g)}} \qquad \text{(Eqn 4.13)}$$

$\mathrm{TI} = \frac{18.69}{18.17}$, and therefore, the TI = 1.02

For this experiment, a TI value was 1.028 (> 1) which according to Kumar *et al.* (2020) reflect a net increase in biomass and suggest that *Vetiveria zizanioides* has developed tolerance. In both treatment and control wetland, all the plants were green and appeared healthy during the experimental period (30 days). The fact that *Vetiveria zizanioides* growing in AMD water remained green and healthy after 30 days was not expected by the researcher due to the toxicity and hazardous nature of AMD. However, this result was in line with the studies of Roychowdhury *et al*. (2015) and Kiiskila *et al.* (2017) thereby confirming that *Vetiveria zizanioides* has high adaptability to new environment and grow well in acidic condition. According to Audet and Charest. (2007), the TI values of less than 1 (TI < 1), is an indication of plants inability to develop tolerance to pollutants and is characterized by a net decrease in biomass. In contrary, (ii) TI greater than 1 (TI > 1) indicates that plants have developed tolerance with net increase in biomass and thereby being considered as hyper accumulator of metals. Lastly, (iii) TI values equal to (TI =1) indicates that metals pollution does not affect plants in anyway and there is not relative difference to control treatment. The concentration of each metal was determined as explained in **section 2.4.3** and the results were used to calculate the bio-concentration factor (BCF), and translocation factor (TF), including the distribution and translocation of metals through-out the plant (*Vetiveria zizanioides*).

4.3.2.2 Bio-concentration factor (BCF)

The bio-concentration factor of metals by *Vetiveria zizanioides* during the 30 days HRT is shown in Figure 4.8.

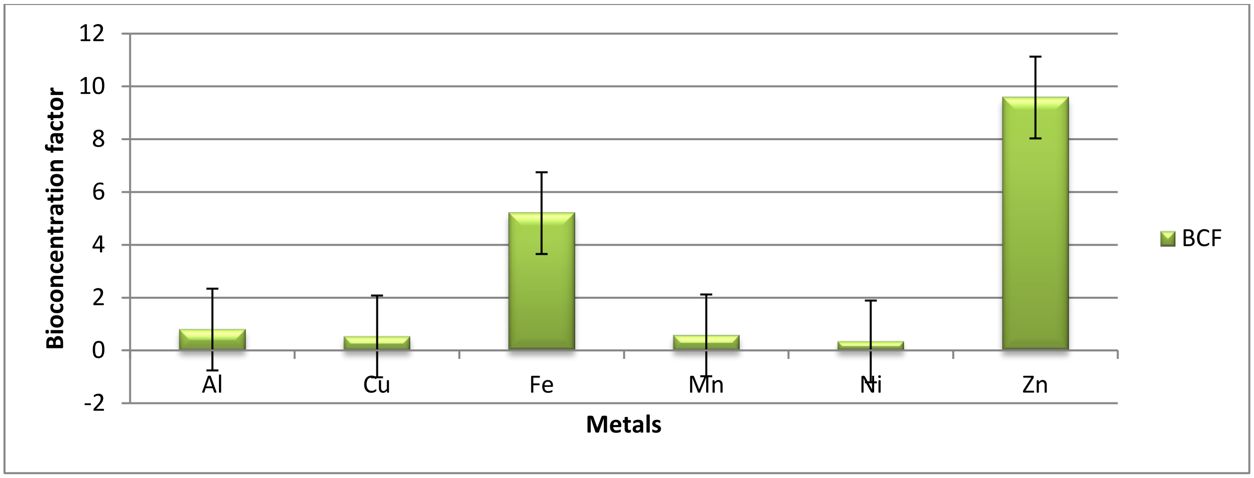


Figure 4.8: The bio-concentration factor of metals onto *Vetiveria zizanioides* for the period of 30 days HRT

As shown in Figure 4.8, the bio-concentration factor of metals onto *Vetiveria zizanioides* for a period of 30 days was reported. Specifically, the bio-concentration factor demonstrates the potential success of plant species for phytoremediation. The potentiality of plants to accumulate metals is known as BCF value (Amin *et al.,* 2018; Coakley *et al.,* 2019). This is an indication of the potential of plant to uptake a specific inorganic contaminant with respect to its concentration in the soil or water body (Sahay *et al*. 2020). Furthermore, (i) the BCF ≥ 1 is an indicator of how probable a pollutant can be accumulated by the plant species (Usman *et al*., 2019) whereas the (ii) BCF ≤ 1denotes that the plant will not accumulate the metals, and lastly (iii), the BCF = 0 denotes that the plant will accumulate minute to fair amount of pollutants. The finding of this study revealed that the BCF of Fe and Zn are 5.2 and 9.58, respectively whereas the BCF of Al, Cu, Mn and Ni were 0.79, 0.53, 0.57, and 0.34, respectively **(Figure 4.8).** According to (Suelee *et al.*, 2017), *Vetiveria zizanioides* can be considered as an accumulator of iron, and zinc but an excluder of Al, Cu, Mn, and Ni in AMD. Similarly, *Vetiveria zizanioides* accumulated iron, and zinc but was an excluder of Al, Cu, Mn, and Ni in AMD water for a 30 day retention time. However, in the control wetland, the BCF of all metals could not be determined since their final concentration in the substrate after 30 days retention time was below the detection limit. Precisely, this could be attributed to the initial concentration of metal in the medium, since bioaccumulation of metals by plants varies depending of the plant species and types of metal (Bonanno *et al*., 2018). Furthermore, minute concentration of certain metals responsible to the hyperpolarization of the plasma membrane at the root surface thereby increase the trans-membrane potential necessary for cationic uptake (Repka *et al.,* 2013). Metals in some case may promote the expression rate of certain genes responsible for cell proliferation which reduces the accumulation of metals by the roots (Khanna *et al.,* 2019). Subsequent to the BCF, the TF was also determined using the content of each metal in different portal (root and shoot) of the plants.

4.3.2.3 Translocation factor (TF)

The bio-concentration factors of metals for *Vetiveria zizanioides* over 30 days HRT is shown in **Figure 4.9.**

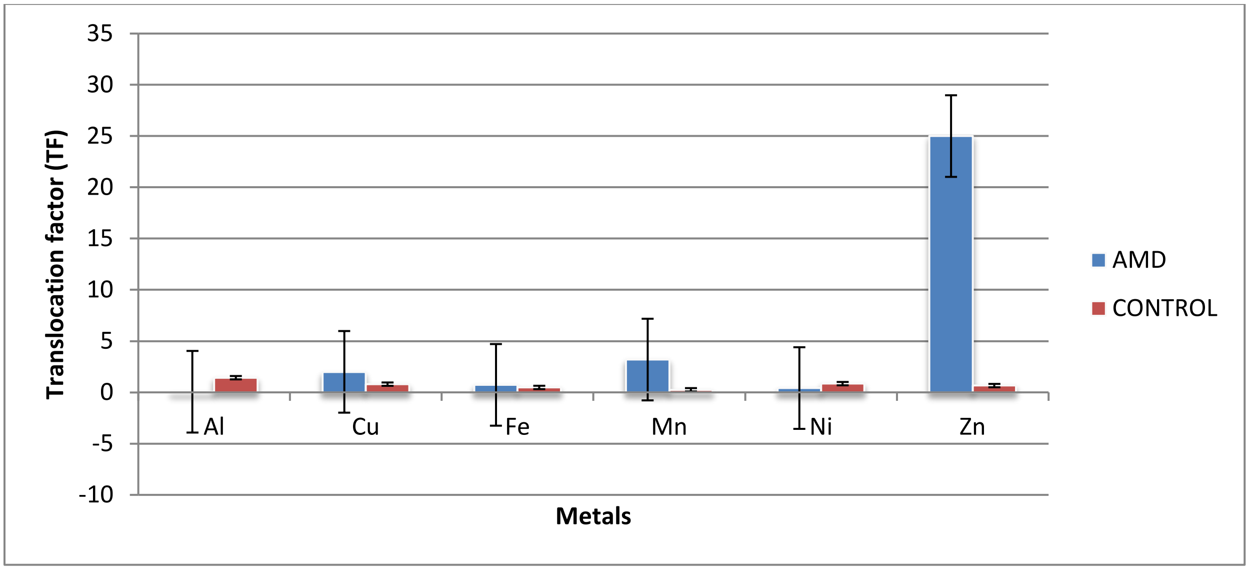


Figure 4.9: Translocation factors of metals (treatment and control wetland)

As shown in **Figure 4.9**, the translocation of metals in the roots and shoots are reported. The metal concentration in roots and shoot were determined. The obtained results revealed that the translocation factor (TF) of Cu, Mn, and Zn is greater than 1 whereas the TF of others metals is less than 1. In the control experiment, the TF of all metals was less than 1 except for Al which has a TF higher than 1 (1.43) **(Figure 4.9).** The TF ≥ 1 indicates the effectiveness of plant to transfer metals from root to shoot whereas TF ≤ 1 indicates the ineffectiveness of plants to translocate metals from roots to shoot. The low TF in control wetland may be due to low concentration of metals since it was fed by potable fresh water. Lower transfer of metals from roots to shoot may be attributed to the sequestration of metals inside the root vacuoles of plants (Vetiver) where metals are fixed as non-toxic elements (Kumar *et al.,* 2008). The low TF of Al and Ni could be a plant tolerance strategy since it is acidic condition, albeit, Al become a major constraint for the growth of plant. Specifically, at pH ≤ 5, Al^{2+} is oxidised to Al^{3+} as denoted by equation 4.12 (Bojórquez-Quintal *et al.,* 2017).

$$\mathrm{Al^{2+} + e^{+} \rightarrow Al^{3+}} \qquad \text{(Eqn 4.14)}$$

The Al^{3+} released to the medium enters into roots tip cell and delay the growth of plant (Jaskowiak *et al.,* 2018) and this can be justified by the TF of Al in control wetland which is higher than 1 (1.43). Higher concentration of Ni in the plant shoot leads to guttation by decreasing water content in plant (Hassan *et al*., 2019). This can explain the low TF of Ni (0.42) and (0.85) for both treatment and control cell respectively. Outcomes from this study revealed that there was variation of TF between the treatment and control wetland, and this may be attributed to the metals concentration within the medium of experiment. Similar results have been reported by Callahan *et al*. (2006). The TF determination in growing plant species is important in the biological monitoring of heavy metals as well as the selection of tolerant plants species.

4.3.2.4 Metal translocation and distribution

The metals translocation and distribution throughout vetiver grass portion was determined for vetiver grass from treatment wetland and the results are shown in **Figure 4.10.**
The metals translocation and distribution throughout vetiver grass portion was calculated for vetiver grass from treatment wetland and the results are shown in **Figure 4. 10** (a,b).

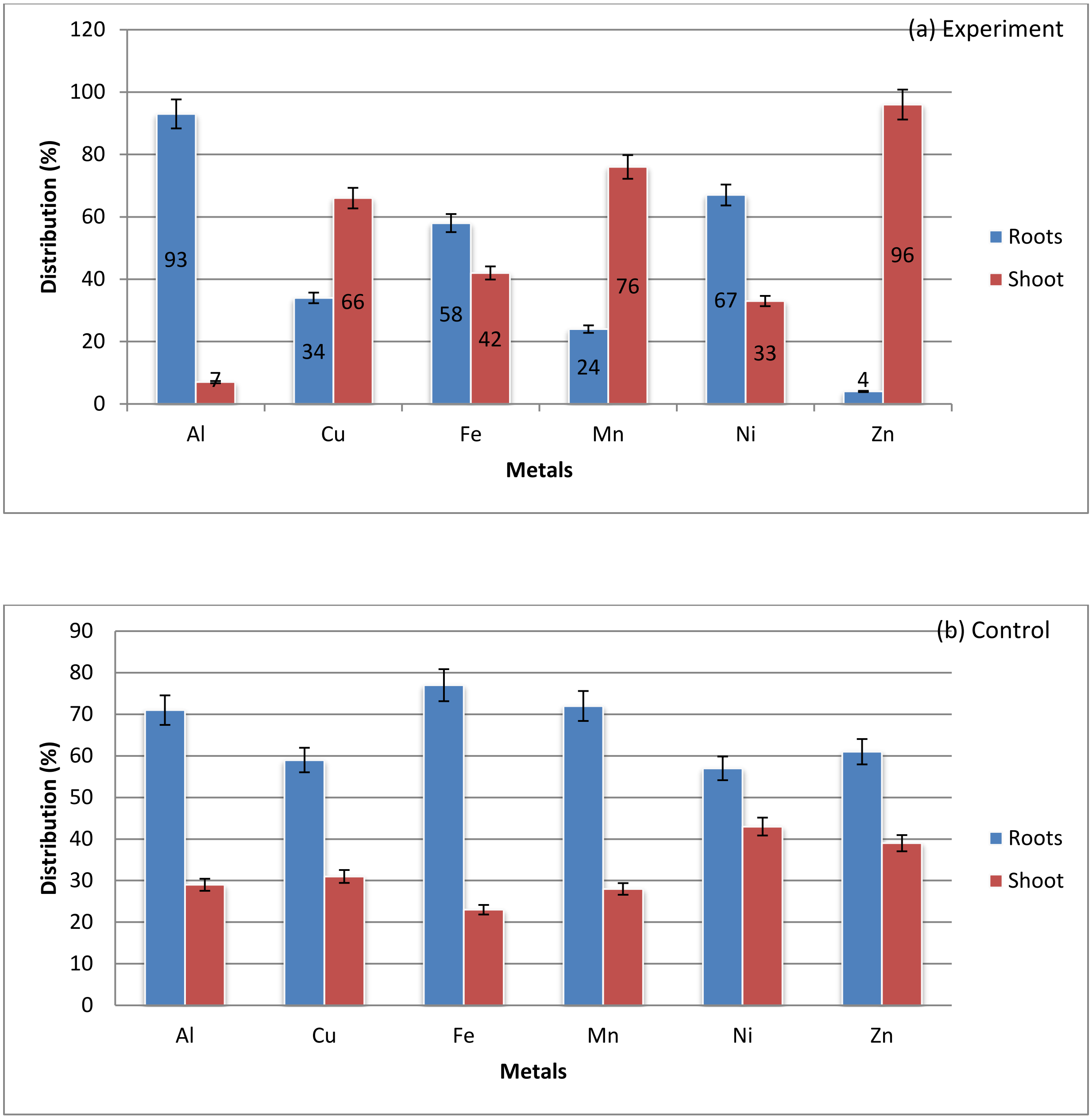


Figure 4. 10 (a,b**).** (a) Percentage of the metals distribution partitioned into roots and shoots (treatment wetland). (b) Percentages of the metals distribution partitioned into roots and shoots (Control wetland)

Figure 4.10 (a,b) represents the translocation and distribution of metals into the *Vetiveria zizanioides*. In the treatment wetland, the metals (Al, Fe and Ni) were more concentrated in the roots whereas Cu, Mn and Zn were more concentrated in the shoot (**Figure 4. 10a**). A similar trend was observed by Kiiskila *et al.* (2019) where they used floating wetland with Vetiver grass to assess the potential of the plant to clean up AMD. Vetiver grass has the potential to take up metals and distribute them to different parts of the plants according to the type of metals and the initial concentration of metals. Furthermore, previous studies conducted by Banerjee *et al.* (2016) have demonstrated moderate translocation of Zn (30 -50%) whereas the finding of this study showed higher translocation of Zn (96%). The greater translocation of nickel is in line with others studies (Banerjee *et al.*, 2016; Suelee *et al.*, 2017) while the high translocation of copper is in opposition with the study of Roongtanakiat *et al*. (2007) and Roongtanakiat and Sanoh, 2011). Low translocation of Al, Fe and Ni can be attributed to Fe plaques formed in the roots. In fact, Fe plaques act as barrier thereby delaying the accumulation and translocation of metals from roots to shoots (Singha *et al*., 2019). In phytoremediation, plaques are important features and generally described as amorphous or crystalline structures composed of metal hydroxides (Wang *et al.*, 2014; Park and Lee, 2017). In the control wetland, all metals were more concentrated in the roots (**Figure 4.10b**) and this can be attributed to the low concentration of metals in control wetland since tap water was used. The translocation of metals from roots to shoots is influenced by various factors including initial concentration of metal, plant species, and type of substrate, biochemical, physiological and anatomical. In phytoremediation process, metals are taken up by from the soil by roots and transported via the plasma membrane driven by ATP-depended proton pump that catalyse H^+ extrusion across the membrane. The different patterns of metals translocation from roots to shoots can be the attributed to cytogenetic make up and others unknown factors as reported by the study of Singh *et al*. (2010).

4.3.3 Partitioning of metals between substrate, plants, and external factors

The partitioning of metals between the substrate, plants, and external factors are reported in **Figure 4.11.**

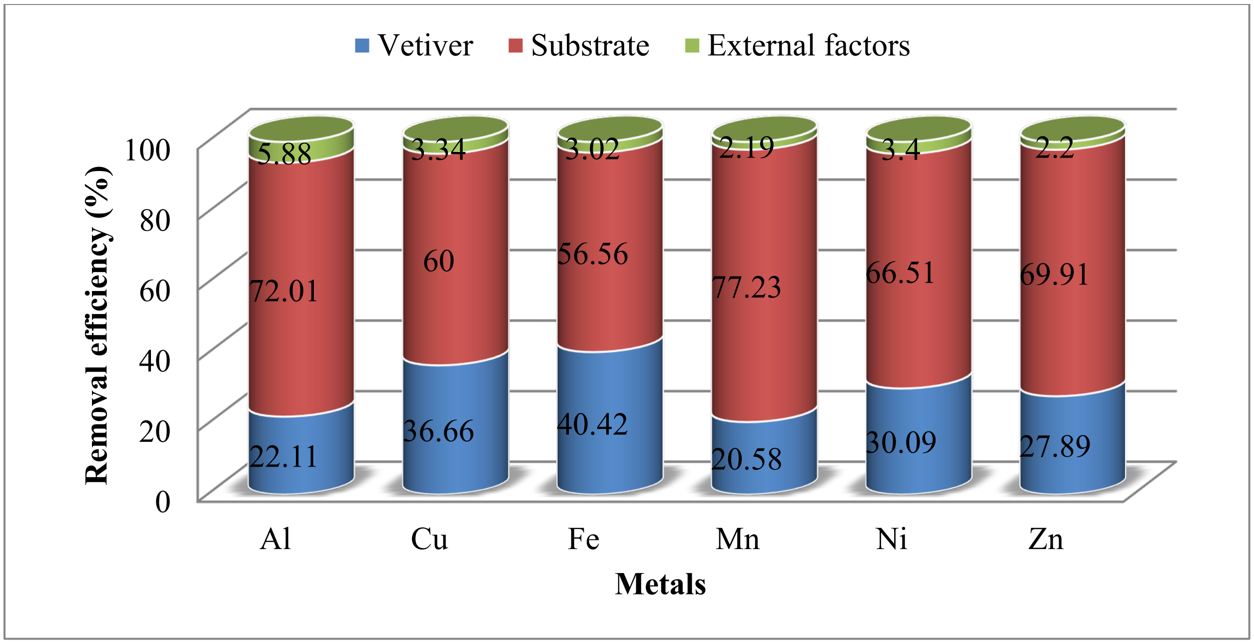


Figure 4.11: The partitioning of metals between the substrate, plants, and external factors

As shown in **Figure 4.11**, the partitioning of metals between the substrate, plants, and external factors are reported. The determination of metals concentration in product water, plant tissues and substrate allowed researcher to find out the contribution of each component in metals removal process. The results revealed that metals were more removed by wetland substrate ≥ *vetiveria zizanioides* ≥ external factors. With substrate contributing to removal of metals from AMD in the following order: Mn (77.23%) > Al (72.01%) > Zn (69.91%) > Ni (66.51%) > Cu (60%) > Fe (56.56%). The substrate plays a vital role in pollutants removal in wetland. It serves as support for plant growth, provides energy for biochemical reaction and absorbs metals via sedimentation process and enhances their accumulation by the plants roots. *Vetiveria zizanioides* contribution in overall metals removal was 40.42%, 36.66%, 30.09%, 27.89%, 22.11% and 20.58% for Fe, Cu, Ni, Zn Al and Mn respectively. *Vetiveria zizanioides* contribution in overall metals removal was small compared to substrate contribution. The small fraction of *Vetiveria zizanioides* in overall metals removal may be attributed the duration of the experiment (30 days) and it may requires more time for *Vetiveria zizanioides* to accumulate a considerable quantity of metals. Despite its smaller contribution in metals removal, *Vetiveria zizanioides* like others plants used in constructed wetland technology contribute in improving the efficiency of constructed wetland in many ways. *Vetiveria zizanioides* facilitates the settlement of total suspended solid (TSS) and provides optimum conditions for the growth of microorganisms amongst others. The external factors contributed at 5.88 % (Al), 3.4% (Ni), 3.34% (Cu), 3.02% (Fe), and 2.2% (Zn). Their contribution in overall metals removal accounted for a very small fraction including: evaporation, biological assimilation, volatilization, oxidation of metals and other minerals in the sediment may also contribute to metals removal in constructed wetland. Explicitly, it can meticulously be deduced that all metals were more retained by substrate through sedimentation process thereby facilitating their accumulation by *Vetiveria zizanioides* whereas external accounted for a very small fraction in overall metals (**Figure 4.11**). The results were in line with the study of Kiiskila et al*.* (2019) for Fe, Zn, and Cu. However, the finding cannot be conclusive since the experiment lasted for 30 days and therefore more time may be required for the plant to accumulate other metals.

4.3.4 Characterization of solid samples

4.3.4.1 Elemental composition of the substrate

Elemental composition of soil before and after interaction with AMD was characterised using XRF and the results are shown in **Table 4.1.**

Table 4.1: Elemental composition of control and experimental substrate

Elements (wt. %)	Control substrate	Experiment substrate
Fe	56.2027	84.8783
Ni	0.4405	0.2027
Cu	1.2056	10.2056
Rb	2.1014	4.4992
Ti	1.7759	2.5899
Cr	1.5332	1.0574
Sr	1.2759	3.5955
Mn	1.0113	1.9798
Zn	0.2931	0.4873
Si	0.0930	0.0472
Al	0.1393	1.0632
Na	-------	0.4580

As shown in **Table 4.1**, the levels of contaminants in the substrate for control and experiments are duly reported. Chemical species present in AMD were observed to increase after the interaction with metal-rich solution (AMD). This could be attributed to precipitation, ion exchange, and adsorption on soil matrices. As reported in Table 4.1, the Wt. percentage of Al, Cu, Fe, Mn, Rb, Sr, Ti, and Zn increased after reaction with AMD. The increasing of metals percentage after contact with AMD may be attributed to the accumulation of those metals in the soil. The percentage of Cr Ni and Si decreased after contact with AMD water indicating possible dissolution of these metals. Sodium (Na) was found to be present in AMD reacted soil and it may be originated from acid mine drainage water. Overall, findings from this technique confirmed that the soil fractions are contributing towards the removal of contaminants using the substrate. This somehow retains chemicals for the absorption process.

4.3.4.2 Mineralogical composition of the substrate

The mineralogical composition of substrates from treatment and control wetland was evaluated using X-ray diffraction analysis and the results are shown in **Figure 4.12.**

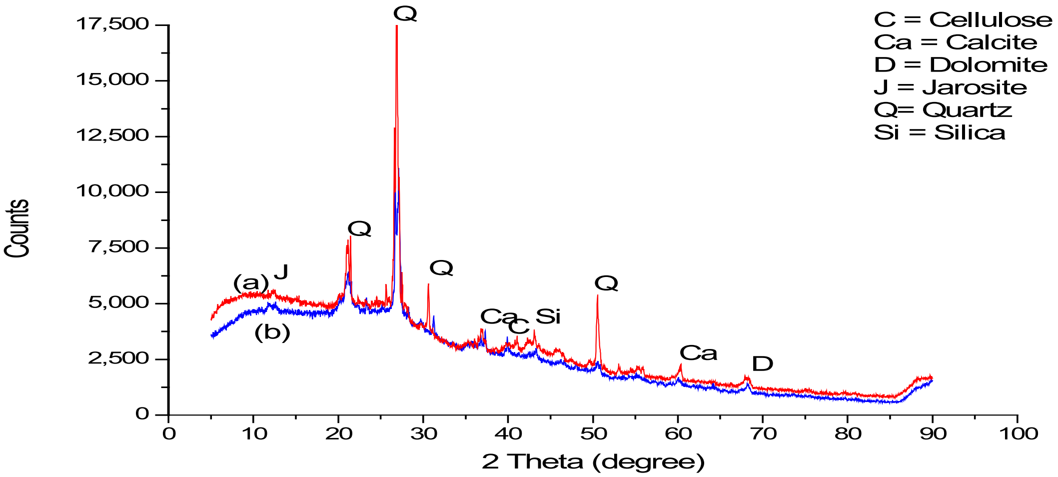


Figure 4.12: X-ray diffraction patterns of substrate: treatment (a) and control (b)

The X-ray diffraction patterns of substrate from treatment wetland and control wetland showed various peaks spread over the range 2ϴ from 12° to 68° but at different intensity. The peak at 2ϴ = 12° may correspond to Jarosite which is ferric iron-sulphate mineral (K, H_3O) Fe_3 $(SO_4)_2$ $(OH)_6$). The peaks at 2ϴ = 21.5°, 27.5°, 30° and 51° may correspond to quartz which is the major mineralogical component of compost soil used as wetland substrate (Biyada *et al.,* 2020). The peaks at 2ϴ =37.5° and 60° may correspond to calcite (Grigatti *et al.,* 2017) while the peak at 2ϴ = 41° correspond to cellulose (Grigatti *et al.,* 2017) and the peak at 2ϴ = 68° may correspond to dolomite (Yu *et al.,* 2017)

4.3.4.3 Analysis of *Vetiveria zizanioides* roots for functional group.

The Fourier transfer infrared spectroscopy (FTIR) analysis of *Vetiveria zizanioides* roots from both wetlands are shown in **Figure 4.13** while **Table 4.2** lists the identified functional group and their respective wave length.

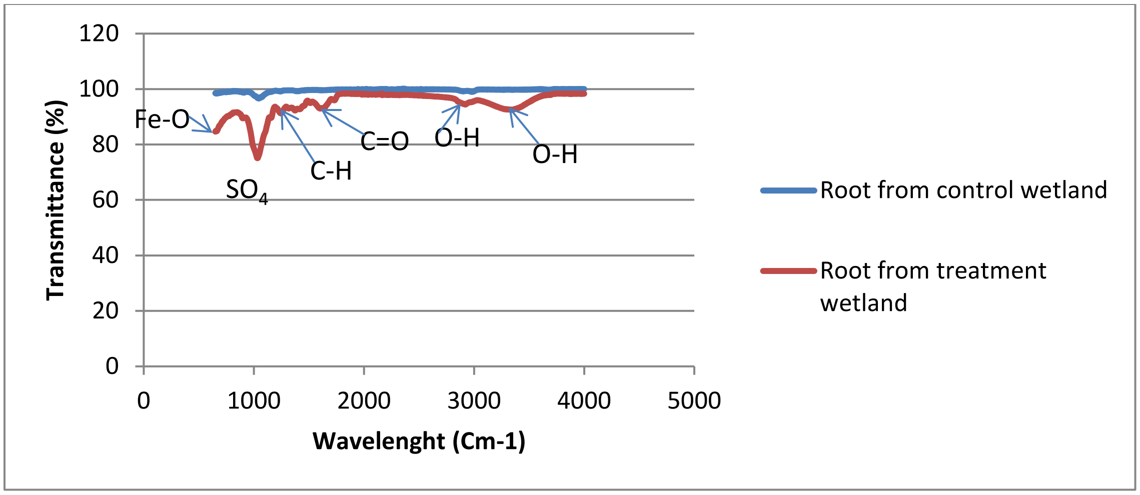


Figure 4.13: Fourier transforms infrared spectroscopy spectra of *Vetiveria zizanioides* roots grew in control and treatment wetland

Table 4.2: The metal's functional groups and their references

Wave number	Functional group	References
661	Fe-O	(Tabelin *et al.,* 2017)
1000	SO_4	(Tabelin *et al.,* 2018)
1225-1379	C-H	(McDonagh and Chinga-Carrasco, 2020).
1534-1648	C=O	(Kannappan *et al.,* 2017)
2988	O-H	(Yu *et al.,* 2017)
3316	O-H	(McDonagh and Chinga-Carrasco, 2020)

By analyzing and comparing the two spectra, it follows that the spectrum of root grown in control wetland, shows a straight band with a little stretching vibration between 991 and 1090 cm^{-1} which respectively shifted to a peak at 931 cm^{-1} and strong vibration at 1000 cm-1in root grown in treatment wetland. The spectrum of root from control wetland shows a band from 1335 cm^{-1} and signal of vibration at 2920 cm^{-1}. However, the spectrum of root from treatment wetland shows a series of vibration with band at 1263, 1364, 1378, 1522, 1576, 1767 and 1854 cm^{-1} followed by a straight band and a signal at 2993 cm-1 and a stretching vibration between 3324 and 3512 cm^{-1}. The series of vibration observed in root from treatment wetland in opposition to the spectrum of root from control wetland may be attributed to the quantity of pollutants accumulated.

4.3.4.4 Scanning Electron Microscope-Electron Dispersion Spectrometry of *Vetiveria zizanioides* roots

The morphological changes and minerals phases of *Vetiveria zizanioides* roots were assessed using SEM-EDS analysis and the results are shown in **Figure 4.14** and **Figure 4.15**

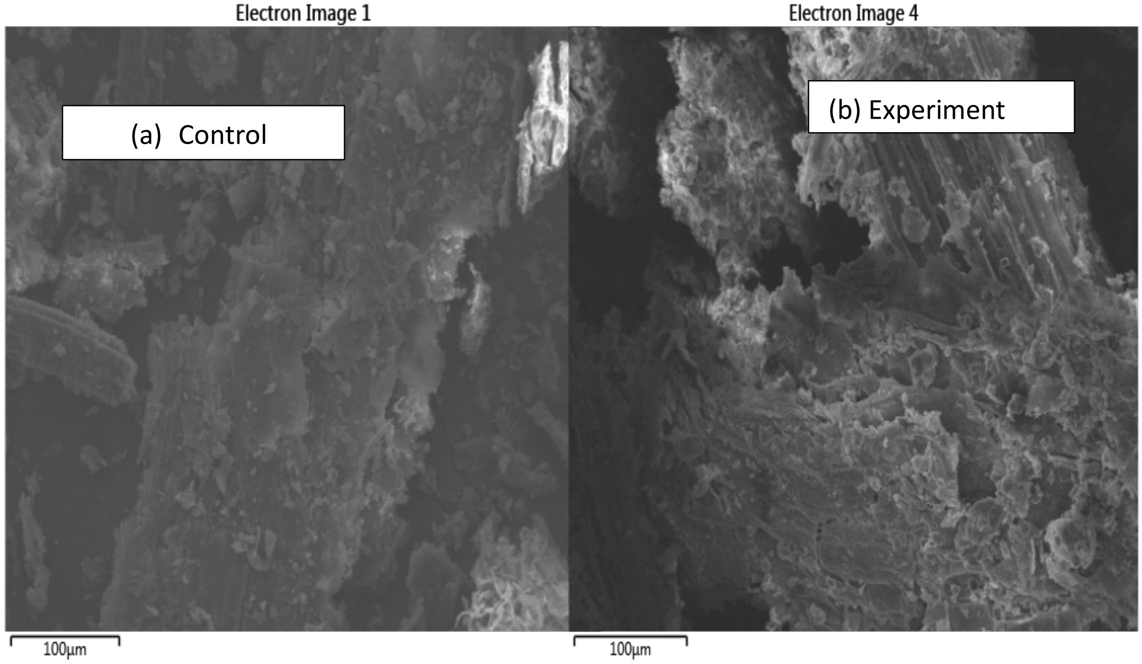
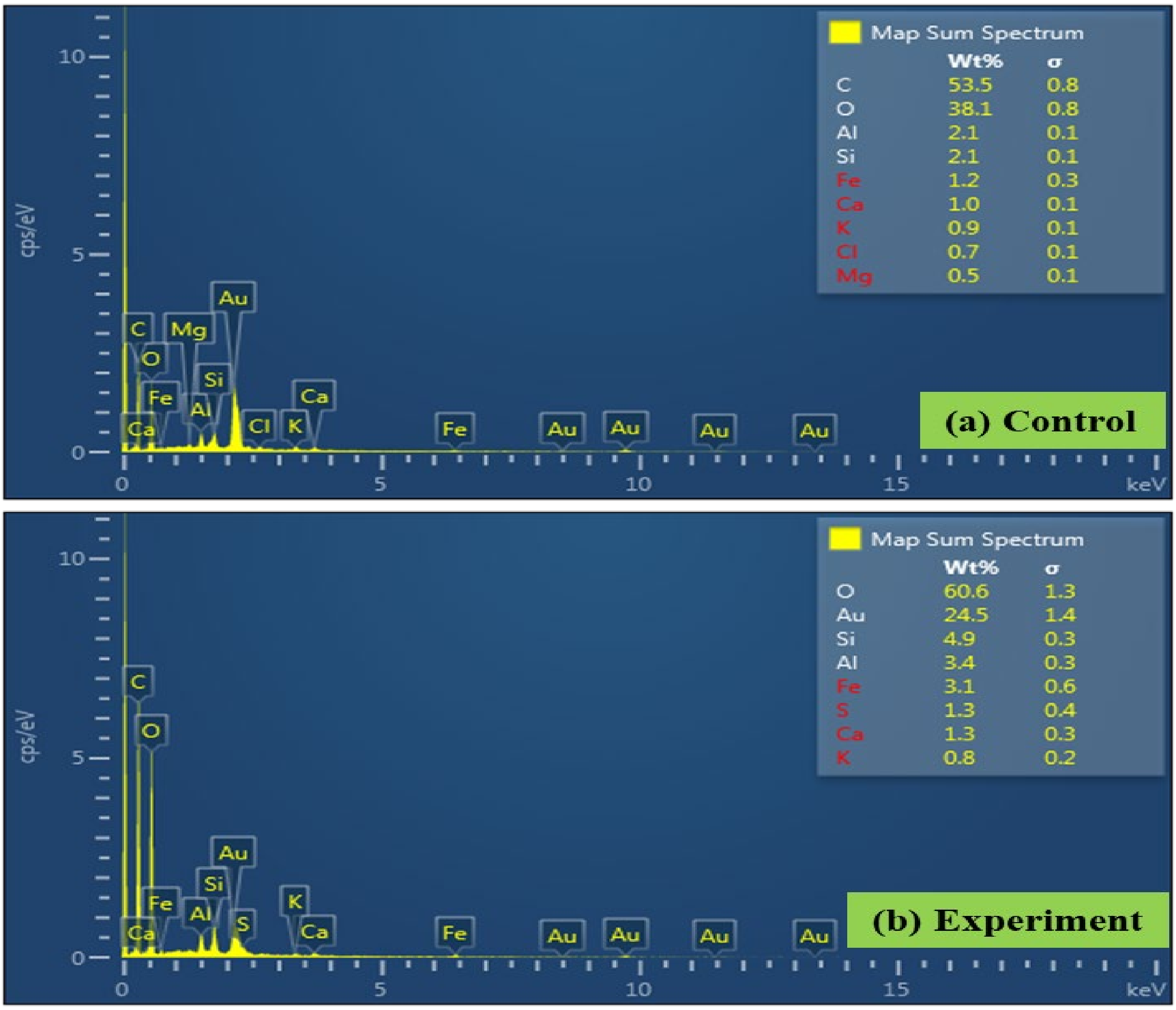


Figure 4.14: The morphological properties of *Vetiveria zizanioides*. (a) Control and (b) treatment wetland

As shown in **Figure 4.14**, the morphological properties of the roots in the control and treatment set-up were evaluated using SEM. Specifically, this was done to acquire a better understanding of the mode of interaction between AMD and *Vetiveria zizanioides.* The SEM was performed to examine structural change that may have happened in the roots of *Vetiveria zizanioides* as result of stress factors caused by the acidic media, elevated concentration of salt (SO_4^{2-}) and high level of metals in AMD. The SEM images of roots grew in AMD water (**Figure 4.14b)** and the SEM images of roots grew in potable fresh water (**Figure 4.14a**) showed a noticeable difference in morphology properties.

Roots for control experiment showed smooth scales on its morphology, however, the results for AMD-reacted-roots showed the scales on its morphology hence confirming changes in structural properties. This confirms that the plant suffered some stress. The result concurs with the finding obtained by Kiiskila *et al.* (2019) which revealed a noticeable change in the morphology of *Vetiveria zizanioides* roots grew in AMD water. In fact, plants use their cell wall as defence compartment to respond to toxic conditions and according to Rich *et al*. (2014) toxic metals are accumulated by plants using the cell wall. Plant cell wall is rich in proteins, amino acid, and phenolics which are able to bind metals in order to render them less or non-toxic (Jan *et al.,* 2015). When plants accumulate metals using the cell wall, it leads to the formation of crystal-like deposits which saturates the cell wall creating the thickening of the cell demonstrating that *Vetiveria zizanioides* has accumulated and sequestrated toxic metals away from its sensitive parts. This perhaps is a biological strategy of *Vetiveria zizanioides* to respond and adapt to very toxic environment such as AMD water.



Map Sum Spectrum

	Wt%	σ
C	53.5	0.8
O	38.1	0.8
Al	2.1	0.1
Si	2.1	0.1
Fe	1.2	0.3
Ca	1.0	0.1
K	0.9	0.1
Cl	0.7	0.1
Mg	0.5	0.1

Map Sum Spectrum

	Wt%	σ
O	60.6	1.3
Au	24.5	1.4
Si	4.9	0.3
Al	3.4	0.3
Fe	3.1	0.6
S	1.3	0.4
Ca	1.3	0.3
K	0.8	0.2

Figure 4.15: EDS of *Vetiveria zizanioides* roots from: (a) control and (b) treatment wetland

As shown in **Figure 4.15**, the elemental distribution on the roots of reacted and unreacted plants are reported. The EDS of *Vetiveria zizanioides* root from AMD treatment wetland **(Figure 4.15b)** indicated an increase in Al, Ca, Fe and Si levels in comparison to EDS of *Vetiveria zizanioides* root from control wetland **(Figure 4.15a).** Thenceforth, K and S were also observed to be present. The presence of Al is indicating availability of Al-hydroxysulphates minerals such as hydrobasaluminite and basaluminite (Nordstrom, 2020). The increase in Fe and Al can be explained by their high concentration in AMD water and their importance in plant metabolism and the presence of some kind of Al and

Fe-hydroxysulphate. The presence of Ca indicates the presence of calcite. The presence of oxygen at high level may be the results of precipitation of AMD elements to metal oxides. Fe at high level is possibly due to the phenomena of ion-exchange on soil surface (substrate) and precipitation following the slightly increase of pH. The ion-exchange process leads to the release of some metals (Zewail and Yousef, 2015) and the presence of Potassium (K) and Calcium (Ca) in roots interacted with AMD and Magnesium (Mg) in the control reacted root may be the results of ion-exchange reaction whereas the presence of sulphur compound may be attributed to the sink of heavy metals released into the environment (Zewail and Yousef, 2015; Kiiskila *et al*., 2020). Ultimately, the chemical species present in AMD were observed to accumulate in the roots of the plants hence confirming the plants are the sink of chemical species in AMD.

4.4 Chemical species for untreated and AMD-treated wetland with FWS-CW

The levels of chemical species in real AMD and product water in relation to DWS and DEA water quality guidelines (DWS, 2013) are shown in **Table 4.3.**

Table 4.3: The levels of chemical species in real AMD and product water in relation to DWS/DEA effluent quality guidelines

Parameters	Feed AMD	DWS/DEA guidelines for effluent discharge	AMD-treated water	Percentage of pollutant removed
pH	2.6	6-12	3.1	
TDS	3380	2400 mg	1450	57.1
EC	5000	150µS/cm	2000	60
Al	158	20 mg	109.04	30.98
Fe	341	50 mg	39.04	88.55
Mn	37	20 mg	23.01	37.81
Cu	4.2	20 mg	2.77	34
Zn	8.55	20 mg	2.30	73.07
Ni	3.92	10 mg	3.44	12
Sulphate	3137	2400 mg	1206.3	61.54

(**NB**: All units in mg/L except pH and EC).

As shown in **Table 4.3,** the levels of chemical species in real AMD and product water in relation to DWS water quality guidelines are reported. Real AMD was observed to comprise acidic pH (≤2.6) and elevated levels of metals and sulphate. High concentration of Fe and sulphate denotes that this AMD was formed from the oxidation of pyrite. All the parameters were observed to have elements that exceed the maximum allowed limits. High EC and TDS are attributed to elevated levels of dissolved chemical species. However, after interacting with *Vetiveria zizanioides*, the product water was observed to have improved drastically. From **Table 4.3**, the results revealed that AMD has low pH (2.6), high level of TDS and EC and this may be the consequences of elevated concentration of metals. After the treatment of AMD under free water system-constructed wetland (FWS-CW) with *Vetiveria zizanioides*, the level of metals and sulphate decreased significantly thereby leading to the decrease of TDS and EC and the raise of pH. Overall the FWS-CW using *Vetiveria zizanioides* improved the quality of AMD but without meeting the water quality standard required by department of water and sanitation (DWS). An increase in pH will be the main contributor towards the attenuation of metals (Fe and Al). These metals precipitate as hydroxides as the pH of the solution increases.

4.5 Conclusions and recommendations

This study successfully confirmed the use of a passive wetland system for the remediation of acid mine drainage with *Vetiveria zizanioides* tolerant to AMD water. The finding revealed that an integration of FWS-CW and phytoremediation using Vetiver grass is a suitable for the treatment of AMD water. Slight increases in pH rose as a result of treatment of AMD with FWS-CW equipped with *Vetiveria zizanioides,* significant decrease of EC, TDS, higher removal efficiency for Fe and Zn, moderate removal efficiency for SO_4^{2-} and Mn. A lower removal efficiency for Al, Cu and very low removal efficiency for Ni were observed. The finding further revealed that Al, Fe and Ni were more concentrated in the roots whereas Cu, Mn and Zn were more concentrated in the shoots. The efficiency of FWS-CW using *Vetiveria zizanioides* revealed the partitioning of metals removal between substrate, plant and external factors. The XRF and XRD analysis of substrate revealed the presence of element and mineral from AMD thereby confirming the role of substrate in pollutants removal while the FTIR analysis of *Vetiveria zizanioides* roots revealed the presence of various functional groups. The improvement of AMD water quality was satisfactory but did not meet the guidelines standard for effluent discharge as set by DWS, DEA and world health organization (WHO). The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirm that metals existed as divalent and trivalent in solution. Furthermore, the metals were precipitated as hydroxides and oxy-hydrosulphates. This experiment revealed that this synergetic approach can significantly improve the quality of AMD. However in order to meet the guidelines standard for effluent discharge as set by the DWS and DEA, this technology could be coupled with another treatment technology to obtain product water of acceptable standard.

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CHAPTER FIVE

This chapter assessed the fate of chemicals species in subsurface vertically flowing treating acid Mine drainage with focus on sulphate and metals.

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CHAPTER FIVE

The Treatment of acid mine drainage using Vertically Flowing Wetland: Insights into the Fate of Chemical Species

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Abstract

This study assessed the performance of subsurface vertical flow wetland equipped with *Vetiveria zizanioides* in the treatment of acid mine drainage. The focus was on pH, heavy metals and sulphate. Mine water flowed vertically to the system in continuous drop and samples were collected and analyzed daily and the average result of five days period was considered for a hydraulic retention time of 30 days. The findings showed that *Vetiveria zizanioides* was tolerant to acid mine water and a net removal of metals and sulphate was recorded with a removal efficiency in the following order: Fe (71.25%), Zn (70.40%) > Mn (62%) > Al (56.68%) > SO_4^{2-} (55.18%) > Ni (35%) > Cu (18.83%). Substrate played a huge role in the removal efficiency of metals by retaining metals in soil through various biochemical process including precipitation, adsorption, and phyto-retention. As such, it could be concluded that metals removal was partitioned between substrate, plants and external factors with substrate playing a significant role in metals removal efficiency. The finding further revealed that chemical species were more concentrated in the roots except manganese which was concentrated in the shoot (67%). The X-ray fluorescence, X-ray diffractometers, Fourier Transform Infrared, and Scanning Electron Microscopy – Energy

Dispersive Spectroscopy analysis revealed the presence of AMD chemical species in the substrate and the grass components, hence confirming that the plants are contributing in the removal of contaminants from AMD. The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirm that metals existed as di-and-trivalent complexes in AMD. Lastly, metals were precipitated in hydroxides and oxy-hydroxydes forms in the substrate, available metals were precipitated as metals hydroxides and oxy-hydrosulfates by the substrate. Subsurface vertical flow wetland could be used as a sustainable technology to remediate AMD from both active and abandoned mines. However it will be necessary to first assess the feasibility of this technology over a long period of time prior to his implementation.

Keywords: Acid mine drainage; vertically flowing wetland; treatment; phytoremediation; *Vetiveria zizanioides*

5.1 Introduction

Over many decades, the mining of gold and coal has played an indispensable role in the economy of any given country. The latter has been widely used for its high colorific value for power generation, while gold has been used for jewelry (Simate and Ndlovu, 2014). Despite its enormous socio-economic benefits, mining has been associated with numerous environmental problems, such as land destruction and the generation of acid mine drainage (AMD), among others (Kefeni *et al.*, 2017; Rambabu *et al.*, 2020**)**. Due to the nature and magnitude of its ecological impacts, AMD has been a topical issue that has been troubling the mining industry over the years (Masindi *et al.*, 2017; Masindi *et al.*, 2018). In particular, during the mining of coal and gold, the associated sulfide-bearing minerals such as pyrite and arsenopyrite which are embedded in the surrounding geology and stratas get exposed to oxidizing conditions (Simate and Ndlovu, 2014; Tabelin *et al.*, 2017a; Tabelin *et al.*, 2017). During rainfall and underground leakages, water and oxygen come into contact with pyrite and the chemical reaction that occurs lead to the formation of very acidic effluent known as acid and metalliferous drainage or acid mine drainage (Masindi *et al.*, 2019). With the use of pyrite as an example, the formation of acid mine drainage (AMD) could be explained by the following equation (Equation 5.1) (Simate and Ndlovu, 2014):

$$4FeS_2 + 15O_2 + 14H_2O \xrightarrow{\text{microorganisms}} 4Fe(OH)_3 + 8SO_4^{2-} + 16H^+ \qquad \text{(Eqn 5.1)}$$

This reaction is also mediated by microorganisms (Baker and Banfield, 2003). The acidity in AMD stimulates the drainage of heavy metals and metalloids from the surrounding geology (Sheoran *et al.*, 2010; Amos *et al.*, 2015; Tabelin *et al.*, 2018). According to the literature, AMD comprises Al, Fe, Mn, and SO_4 as major elements, and traces of Cu, Zn, Ni, Pb, Cr, As, and radionuclides, among others (Simate and Ndlovu, 2014; Masindi *et al.*, 2018; Park *et al.*, 2019; Tabelin *et al.*, 2020). These chemical species exceed the recommended limits for discharge as stipulated by the World Health Organization (WHO) (Akinwekomi *et al.*, 2017). As such, the discharge of AMD may lead to the degradation of receiving ecological systems and compartments (Masindi et al., 2018), hence affecting the integrity of the ecosystem and its ability to support life (Tabelin *et al.*, 2017; Masindi *et al.*, 2019; Rambabu *et al.*, 2020). According to Eco toxicological and epidemiological reports, constituents embodied in AMD can pose various carcinogenic, mutagenic, and teratogenic conditions to living organisms on exposure (Simate and Ndlovu, 2014; Naidu *et al.*, 2019; Rambabu *et al.*, 2020). As such, stringent regulatory frameworks require AMD effluent to be treated before it can be discharged into different receiving environments (DWAF, 1996; USEPA, 2017).

In light of the above, various technologies have been developed for the treatment of AMD, which include both active and passive treatment systems (Nleya *et al.*, 2016; Kefeni *et al.*, 2017). Specifically, active treatment technologies include ion exchange (Zewail and Yousef, 2015), adsorption (Motsi *et al.*, 2009), filtration (Aguiar *et al.*, 2018; Agboola, 2019), neutralization (Akinwekomi *et al.*, 2017; Igarashi *et al.*, 2020), and crystallization (Lewis *et al.*, 2010), whereas passive treatment methods include constructed wetlands (Sheoran, 2017), phytoremediation (Herniwanti *et al*, 2013; Herniwanti *et al.*, 2014; Kiiskila *et al.*, 2017, 2019), bio-sorption (Thongpitak *et al.*, 2019), permeable reactive barriers (PRB) (Elghali *et al.*, 2019), sulfate-reducing bioreactors (Lounate *et al.*, 2020), anoxic limestone drains (Skousen and Ziemkiewicz, 2005), and slag leach bed (Simmons *et al.*, 2002). These technologies have their advantages and disadvantages; the active treatment technologies require energy and frequent chemical inputs, whereas passive treatment systems are the opposite. Furthermore, active treatment technologies are not cost effective and require constant maintenance and skilled staff among others; furthermore, they are grossly deemed not environmentally friendly since they generate a considerable volume of sludge, which in turn leads to a new environmental problem to be solved due to secondary pollution from the highly mineralized and heterogeneous sludge (Aguiar *et al.*, 2018; Esmaeili *et al*., 2019; Lounate *et al.*, 2020). Therefore, they cannot be considered a long-term solution for AMD treatment. Following the persisting environmental impacts of AMD and the limitation of existing technologies, there is a need to investigate, develop, and implement an environmentally friendly treatment technology for the removal of contaminants from AMD. Phytoremediation has been reported as the most eco-friendly way of attenuating contaminants from different spheres of the environment, and it plays a crucial role in the reduction of heavy metals and sulfates from AMD (Herniwanti *et al.*, 2014; RoyChowdhury *et al*., 2019). Phytoremediation is an in situ intervention and there is no need for transportation and off-site processing cost except for floral plantations. In addition, the phytoremediation process prevents metals from leaching due to high metals retained intensively post extraction. The metal will then be stored in the plant biomass or released in volatile form into the atmosphere through a process known as phyto-volatilization. To this end, pollutants are removed by a wetland via (i) phyto-extraction by the plants roots (Suman *et al.*, 2018), (ii) phyto-stimulation, which is the breaking down of organics contaminants in plant root zone by microbial activity (Hawrot-Paw *et al.*, 2019), (iii) phyto-degradation, which is the metabolisation of contaminants in plant tissue to less toxic level (Zazouli *et al.*, 2014), and lastly, (iv) the phyto-stabilization process, which basically entails the de-mobilization of heavy metals in soil by plant roots (Zgorelec *et al.*, 2020).

Noting that plants play an important role in the bioremediation, the selection of plant species to be used is crucial since they will improve the overall pollutants removal process considering that metals accumulated by plants are stored in their biomass or released as volatile form into the atmosphere through the phyto-volatilization process. Various studies have revealed that *Vetiveria zizanioides* is tolerant to very harsh environments, such as the acidic and metalliferous conditions in AMD. Furthermore, it grows well in aquatic and terrestrial environments (Roongtanakiat *et al*., 2007; Kiiskila *et al.*, 2017), is tolerant to salinity conditions, grows well in all seasons, and is tolerant to heavy metals and other pollutants. However, its use with the substrate and on a vertically flowing modality has merely been considered, specifically considering AMD. The vertical flow allows for maximum reaction and interaction between the substrate and plants so as to enhance the attenuation of chemical constituents in relation to horizontal flows. To the best of our knowledge, the subsurface vertical flow constructed wetlands (SSVF-CWs) has never been explored for AMD treatment using *Vetiveria zizanioides* with the substrate. Engineering-wise, the SSVF-CW is the type of wetland where water is poured onto a surface and percolates through the soil vertically to the bottom of the cell, where it is drained horizontally to the outlet, where it is collected and characterized. Furthermore, SSVF-CWs are characterized by an abundance of oxygen and substrate pores which are intermittently filled and drained with water, thereby creating an aerobic environment for metals precipitation, facilitation of certain biochemical reactions, and aerobic decomposition (Perdana *et al*,, 2018; Marín-Rivera *et al*., 2019). In light of the above, SSVF-CWs have demonstrated their abilities to remove pollutants and other contaminants from waste water and are, therefore, considered as viable low-cost replacements of traditional methods which are expensive, require skilled personal, and generate waste that requires additional treatment (Mustapha *et al*., 2018; Marín-Rivera *et al*., 2019). The ultimate goal of this study is to highlight the accumulation and translocation of chemical species from contaminated water to the plants. The impact of the used substrate was also evaluated.

5.2. Materials and Methods

5.2.1. Acquisition of Reagents, Substrate, and Plants

All chemical compounds used in this study were of analytical grade (AG) and were obtained from Merck, South Africa. Prior to the construction of subsurface vertical flow constructed wetland (SSVF-CW), shoots of *Vetiveria zizanioides* were purchased from the NANDADRAM ECOVILLAGE farm in Kwa-Zulu Natal, South Africa and transported to the University of South Africa’s sciences campus in Florida, Johannesburg. Once on campus, the shoots of *Vetiveria zizanioides* were submerged under potable fresh water for two weeks in order to allow the new seeds to germinate. After that, seedlings of *Vetiveria zizanioides* were transferred into small pots containing organic matter used as wetland substrate (soil purchased from garden world Johannesburg, RSA) as growing medium. Plants were allowed to grow naturally without the addition of nutrients and were monitored and watered daily.

5.2.2. Sampling and Characterization of AMD Water

Acid mine drainage (AMD) water used in this study was collected at the Gold mine in Krugersdorp, Gauteng, South Africa (Latitude: 26.0963 and Longitude: 27.7752). During AMD collection, some parameters of AMD water were determined on site using a multi-parameter meter (HANNA instruments, Johannesburg, RSA). These included pH, total dissolved solids (TDS), and Electrical conductivity (EC). Initial concentration of metals (Al, Cu, Fe, Mn, Ni, and Zn) in AMD was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES), 5110 ICP-OES vertical dual view, Agilent technologies Australia, made in Malaysia. The ICP-OES was coupled with Agilent SPS 4 Auto sampler, whereas the SO_4^{2-} concentration was analyzed using an Ion chromatography (IC) (850 professional IC Metrohm, Herisau, Switzerland). All the parameters were analyzed following the ''Standard Methods of the Examination of Water and Wastewater'' (APHA, 2002).

5.2.3 Quality Assurance and Quality Control (QA/QC)

A QA/QC process was applied in this study to ensure the collection and production of trustworthy results. The QA/QC process embraced the collection and preservation of the samples. Analysis was done in triplicates, and data reported as mean values was considered acceptable when the difference within triplicate samples was less than 10%. The value below detection limit (BDL) was in line with EPA guidelines for analyzing data (Wei *et al*., 2005), while the accuracy of the analysis was monitored using the National Institute of Standards and Technology (NIST) water standards. The water-Q4 database was utilized. The plant substrate was modeled using PH REdox EQuilibrium (in C language) (PHREEQC). The water potential precipitation of metals was determined using saturation indexes (SI), in which SI ≤ 1 denotes under-saturation, SI ≈ 1 denotes saturation, and SI ≥ 1 denotes super-saturation.

5.3.3. Experimental Setup

5.3.3.1. Vertically Flowing Wetland Design and Description

The pilot plants consisted of the following: (a) one reservoir tank to contain AMD and (b) another reservoir tank to contain fresh water for control. Two circular plastic basins of 100 litres capacity each measuring 62 cm of diameter (d) and 45 cm high (h) and a drum of 300 litres capacity as reservoir tank to contain AMD-water and fresh water for treatment and control **(Figure 5.1).** The new germinated seedlings of Vetiver, described in **Section 5.1**, were transplanted into the two basins containing soil as substrate to have two wetlands (one for experiment and one for control). The wetlands were continuous flow systems, where water was flowing into the system in continuous drops for the duration of the experiment. The wetlands were connected to the tank via a perforated conduit pipe and contained a total of 20 plants planted at equidistance to each other. A control valve was incorporated at the baseline of each tank to regulate the flow rate of water from tank to the wetland cell. The outlet structure of each wetland cell was an orifice with a valve to collect leachate for the analysis. Preventive maintenance measures, such as trash and debris removal and the elimination of mosquito breeding habitats, was applied in a routine basis to maintain the operation and safe function of the wetland, while corrective maintenance measures were applied in a non-routine basis, i.e., when needed.

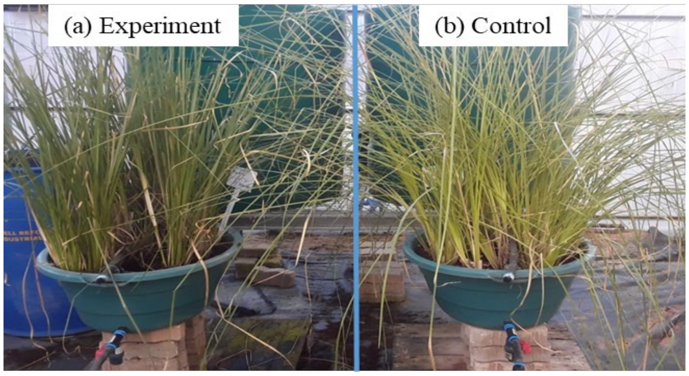


Figure 5.1 Experimental set-up of the SSVF-CW

5.3.3.2. Pre-Treatment of AMD Water and Chemical Composition of the Substrate

Prior to the treatment, AMD water was filtered to remove total suspended solid (TSS) susceptible to clog the pipe, while the chemical composition of the substrate before and after contact with water (AMD) was determined using X-ray fluorescence (XRF) and X-ray diffraction (XRD). The analyses were performed using a Thermo Fisher ARL-9400 XP+ Sequential XRF with winXRF Software. Mineralogical composition was ascertained using X-ray diffraction (XRD). The system was calibrated using a drip plastic bottle, and fed into the system as continuous drops. The average flow per unit time was calculated using the Darcy's law (Equation 5.2):

$$Q = \frac{\mathrm{Qin+Qout}}{2} = K\ A\ S \qquad \text{(Eqn 5.2)}$$

Where,

Q is the average flow per unit time,

K is the hydraulic conductivity of a unit area in the medium perpendicular to the flow direction (m^3/d),

A is the total cross-section area perpendicular to the flow (m^2), and

S is the hydraulic gradient of the water surface in the flow system (m/m).

Once the average flow rate (3 liters/day) was calculated (Q), the results were used to determine the hydraulic retention time (HRT), as illustrated in Equation (5.3) (Sandoval *et al.*, 2019), taking into account the sizes of the wetland cell and the porosity of substrate:

$$HRT = \frac{V \times d}{\mathrm{Q}} \qquad \text{(Eqn 5.3)}$$

Where,

V is the volume of the wetland cell (m^3),

d is the porosity of the wetland substrate (%), and

Q is the average flow through the wetland (m^3/d):

$$HRT = \frac{3.14 \times (0.31)^2 \times 0.43 \times 0.7}{0.003}$$

$$HRT = 30.27 \text{ days}$$

The maximum determined HRT was 30.27 days, and during that period, the inlet flow rate and outlet flow rate were monitored daily to ensure a steady flow of water in and out of the wetland. The flow rate was maintained low (3 liters/Day) and the HRT (30 days) long in order to allow the water to spend more time in the wetland cells, thereby improving the performance of the wetland, since the studies of Conn and Fiedler. (2006), Ewemoje *et al*. (2015), and (Piñeyro *et al.* (2016) revealed that the performance of the wetland is improved by high hydraulic retention time (HRT). At the end of the experiment (just after 30 days), the plants were harvested from both wetlands (treatment cell and control) for the assessment of the uptake and translocation of chemical species.

5.3.3.3. Product Water and Soil Sampling

Water samples (experiment and control) were collected daily from each wetland from the first day (Day 1) of HRT to the last day (Day 30) of HRT. An analysis was performed and the average result of five-day period was considered. Water samples were collected into amber glass bottles of 500 mL. After collection, samples were divided into two sub-samples of 400 mL and 100 mL. The sub-samples of 400 mL were used for the analysis in situ of pH, TDS, and EC, while the sub-samples of 100 mL was used for the analysis of ex situ parameters, which included metals (Al, Cu, Fe, Mn, Ni, Zn) and SO_4^{2-}. Soil samples were air dried for one week followed by an analysis for metal concentration.

5.3.3.4. Analytical Methods

Product water from both wetlands (control and treatment cell) was analysed in triplicate following standard methods. Electrical conductivity (EC), pH, and TDS were determined using Hanna HI 83200 multi-parameter and the manufacturer's manual was followed with reference to standard methods for the examination of water and wastewater (APHA, 2002). Prior to the analysis of the metal concentration, water samples were filtered through a 0.22 μm pore syringe filter membrane to remove particles and preserved by adding two drops of nitric acid (HNO_3) concentrated to prevent ageing and immediate precipitation of metals. The filtrates were refrigerated at 4 °C prior to analysis by ICP-OES. SO_4^{2-} was determined using IC. Soils samples were air dried for one week, sieved, and one gram (1 g) was digested in a mixture of 5 mL of 55% (HNO_3) and 2 mL 30% Hydrogen peroxide (H_2O_2) using microwave digestion. Once digested, the samples were cooled to room temperature and the solutions were filtered through a 0.22-μm pore syringe filter membrane and analyzed for metal concentrations by ICP-OES with a detection limit of 0.0001 mg/L and a margin of error of 10%.

5.3.4. Treatment Efficiency of Subsurface Vertical Flow Constructed Wetland (SSVF-CW)

The efficiency of SSVF-CW in AMD water treatment was determined gradually during the duration of the experiment. The parameters of concern were analyzed daily and the removal efficiency (RE) was calculated after every five-day period for the duration of the experiment (30 days). The RE was determined using Equation (5.4) **(Białowiec *et al.*, 2014; Van Tran *et al.*, 2017**):

$$RE = \frac{\mathrm{Ci}-\mathrm{Cf}}{\mathrm{Ci}} \times 100 \qquad \text{(Eqn 5.4)}$$

where Ci is the initial concentration of each parameter in the AMD water, Cf is the final concentration of each parameter after 30 days of retention time, and *RE* is the removal efficiency of each parameter after 30 days of retention time (in percentage).

5.3.5. Plant Harvesting

Plants from each wetland (treatment cell and control cell) were harvested without damaging the roots and were rinsed separately with distilled water to remove dust, soil, and mineral particles. Plants were air dried at 25 °C for one week and weighed to determine the tolerance index, as shown in Equation (5.5). The tolerance index (TI) according to Kumar *et al.* (2008) is the ratio between a variable measured in treated plants and that in control plants, and was calculated considering the dry plant weight. The tolerance index (TI) was calculated as the mean weight (biomass) of a dry plant grown in the AMD wetland divided by the mean weight of a dry plant grown in a control wetland (Ding *et al.*, 2021):

$$\text{Tolerance index (TF)} = \frac{\text{biomass of plant growing in AMD cell}}{\text{Biomass of plants growing in potable fresh water cell}} \qquad \text{(Eqn 5.5)}$$

5.3.5.1. Digestion of Plants and Metal Analysis

The plants' biomasses were separated into two samples (shoots and roots). Each part of the plant was dried and grinded using a mortar and pestle, and 1 g was digested with 10 mL of 65% HNO_3 (AR grade; BDH). The digestate samples were then filtered using a 0.22-µm pore syringe filter membrane followed by a metal content analysis using ICP-OES. The concentration of metals in each portion of the plant allowed the researchers to determine the bio-concentration factor (BCF), translocation factor (TF) and the contribution of plant, substrate, and external factors in chemical species removal. The (BCF) of each metal was calculated, as illustrated in Equation (5.6), to determine the quantity of metals that moved from the wetland to the plant **(Esmaeilzadeh *et al.*, 2016)**, while the TF indicates the ability of the plant to translocate metal from the roots to the aerial part of the plants **(Bonanno *et al.*, 2018)**. The following equations were used:

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue}}{\text{Final concentration of metals in AMD water}} \qquad \text{(Eqn 5.6)}$$

$$\text{TFx} = \frac{\text{metal concentration in shoots}}{\text{metal concentration in roots}} \qquad \text{(Eqn 5.7)}$$

5.3.5.2. Functional Group and Morphology of *Vetiveria zizanioides* Roots

Functional groups of grinded grass roots were determined using a PerkinElmer Spectrum 100 Fourier Transform Infrared Spectrometer (FTIR) equipped with a PerkinElmer Precisely Universal Attenuated Total Reflectance (ATR) sampling accessory equipped with a diamond crystal, while morphological properties and spot analysis of grass roots was determined using SEM-EDS. Grinded roots samples were mounted on carbon tape that was attached to Al ends. Samples were viewed in a JEOL 5400 LV SEM with an attached KEVEX electron detector (JEOL, Tokyo, Japan). They were viewed in low vacuum mode and the metals were detected with a Sigma EDS spectrometer.

5.3.6. Partitioning of Metals between Substrate, Plant, and External Factors

Prior to the start of the experiment, the initial concentration of metals in substrate was determined. After 30 days of the experimental period, the quantity of metal retained in the wetland (M_w) was determined using Equation (5.8):

$$M_w = X - Y \qquad \text{(Eqn 5.8)}$$

Where,

X is the initial concentration of metal in AMD water,

Y is the final metal content in AMD water, and

Mw is the metal content retained in the wetland.

The substrate's contribution (M_s) to overall metal removal was also determined; it is the difference between the final metal content in the substrate and the initial metal content in the substrate Equation (5.9):

$$M = Z - T \qquad \text{(Eqn 5.9)}$$

Where,

T is the initial metal content in the substrate and

Z is the final metal content in the substrate.

To estimate the contribution of external factors (*ExF*) in the overall metal removal, the metal concentration in the substrate (M_s) was summed with the metal concentration in plants (M_p), and the total was compared to wetland metal ((M_w) content, as per Equation (5.10):

$$ExF = Mw - (Ms + Mp) \qquad \text{(Eqn 5.10)}$$

5.4. Results and Discussion

The results for this study will be categorized into the aqueous samples and solid samples. This will emphasize the findings which were acquired from different experiments and techniques.

5.4.1. Characterization of Aqueous Samples

5.4.1.1. Effect of SSVF-CW on the Sulfate Concentration

The effect of SSVF-CW on the removal of SO_4^{2-} from AMD is reported in **Figure 5.2.**

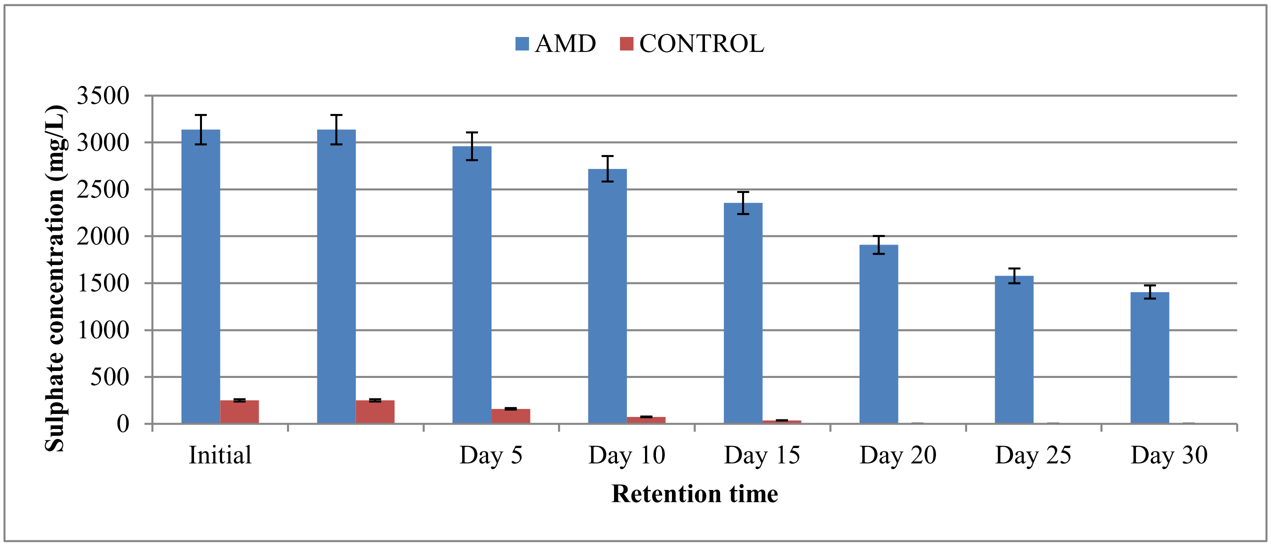


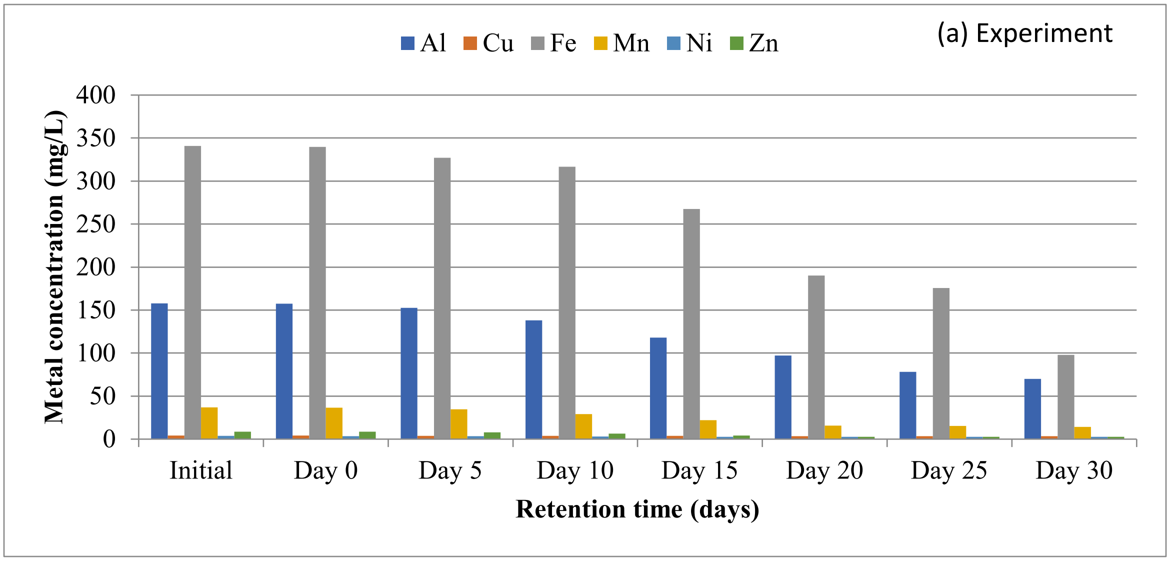
Figure 5.2: The effect of SSVF-CW on the removal of sulfate from AMD in 30 days HRT under a hydraulic loading rate of 3 liters/day

Figure 5.2 shows that the concentration of SO_4^{2-} decreased from 3137 mg/L on the 1st day of retention time to 1406 mg/L on the 30th day, resulting in a removal efficiency of 55.18% **(Figure 5.2).** The results, therefore, revealed that SSVF-CW with *Vetiveria zizanioides* can remove SO_4^{2-}, and thereby, confirms other studies (Demchak *et al*., 2001**).** However, the removal percentage depends on the retention time and the initial concentration of SO_4^{2-} in AMD. In the meantime, the concentration of sulfate in control wetland gradually decreased from day 1 to day 25 and remained constant until the end of experiment. The process of SO_4^{2-} removal in SSVF-CW can be attributed to chemicals reaction occurring within the wetland. According to Chen *et al.* (2016) and Balci *et al.*

(2017), the oxidation-reduction (redox) reaction occurring within the wetland can transform SO_4^{2-} into elemental or mixed-valence-state form of sulfur, which facilitates its absorption by plants. Others compounds, such as thiosulfate and tetrathionate, have been detected in oxidized sulfide minerals and may have a crucial role in SO_4^{2-} reduction in SSVF-CW (Balci *et al.*, 2017). The conversion of SO_4^{2-} to sulfide $(S^2)^-$ can be seen as an initial step toward SO_4^{2-} reduction in wetland due to the fact that S^{2-} can be precipitated in the presence of metals such as Mn and especially Fe from AMD water (Chen *et al.*, 2016). Dissimilatory Sulfate Reduction (DSR) and sulfide oxidation have been reported with constructed wetlands containing *Phragmites australis* and *Juncus effusus*, though DSR was the main removal mechanism (Saad *et al.*, 2016). The removal of SO_4^{2-} can also be attributed to the DBR, since SO_4^{2-} reduction generates H_2S, which can form metals S^{2-} with divalent ions, such as Fe^{2+} or Cu^{2+} (Zhang *et al.*, 2020). The reduction of SO_4^{2-} may also be attributed to the increase of the pH value following the metal solubility reduction in AMD water and especially iron due to sedimentation. Plants' uptake, metals sedimentation, and DSR may, thus, be considered as possible mechanisms of SO_4^{2-} removal.

5.4.1.2. Effect of SSVF-CW on Heavy Metal Concentration

The effect of SSVF-CW on the removal of metals from acid mine drainage (AMD) is reported in **Figure 5.3 (a,b).**



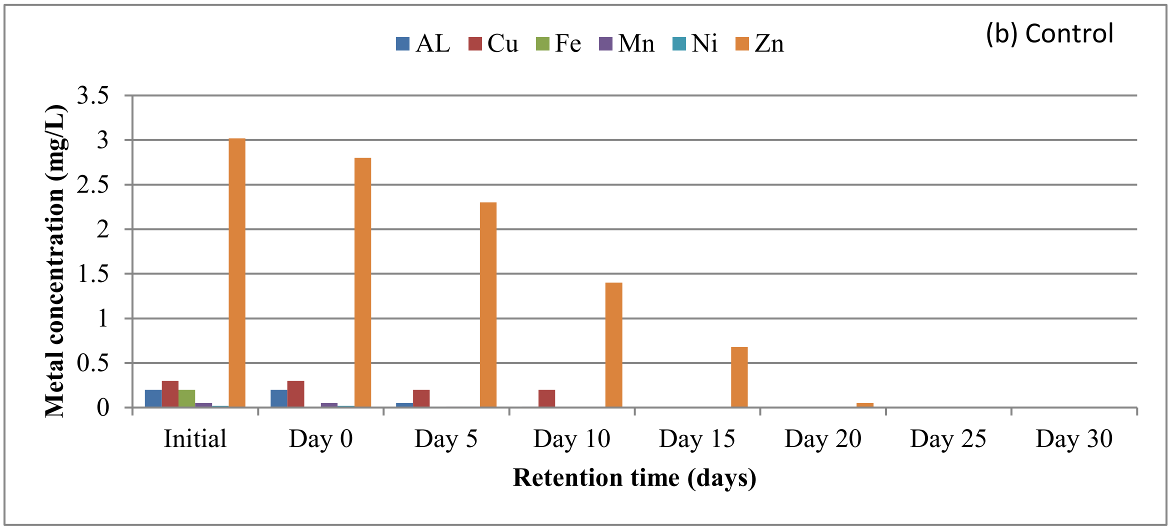


Figure 5.3 (a-b): Variation of the metal concentration in treatment wetland in 30 days of retention time under a hydraulic loading rate of 3 liters/day. (b). Variation of metal concentration in control wetland in 30 days of retention time under a hydraulic loading rate of 3 liters/day

Figure 5.3a reveals that the metal concentration significantly decreased during the 30 days of retention time but with different removal efficiency for each metal. For instance, iron decreased slowly for the first 10 days and then drastically until the end of the experiment, whereas others metals decreased slowly from the beginning until the end **(Figure 5.3a).** The metal concentration reduction can be attributed to sedimentation and accumulation by *Vetiveria zizanioides*. In the meantime, the concentrations of metals in the control wetland decreased and were below the detection limit of 0.0001 mg/L at the end of the experiment for all metals **(Figure 5.3b).** This can be explained by the sedimentation and accumulation by *Vetiveria zizanioides.* In fact, a metal such as Fe plays a crucial role in plant metabolism processes such as DNA synthesis, respiration, and photosynthesis, and its deficiency in plants leads to common nutritional disorders (Rout and Sahoo, 2015). Metals such as Cu, Fe, Zn, and Mn act as important co-factors for many enzymes and are essential for both mitochondrial and chloroplast functions (Morkunas *et al.*, 2018). Furthermore, zinc is one of the eight essential micronutrients. It is needed by plants in small amounts, and is crucial to plant development as it plays an important role in a wide range of processes, such as growth hormone synthesis (Hassan *et al.*, 2020). Many processes in plant metabolism, such as chloroplast formation, photosynthesis, nitrogen uptake, and synthesis of some enzymes, are directly linked to the presence of Mn in plant (Mousavi *et al*., 2011). In addition, the sorption of metals in oxide form is widely known, as reported by Stumm and Morgan (Stumm and Morgan, 1996). The oxide form plays an important role in metal removal in constructed wetland if Fe/Mn oxides are present.

5.4.1.3. Metal Concentration in Substrate

The effect of SSVF-CW on the removal of metals from acid mine drainage (AMD) is reported in **Figure 5.4 (a,b).**

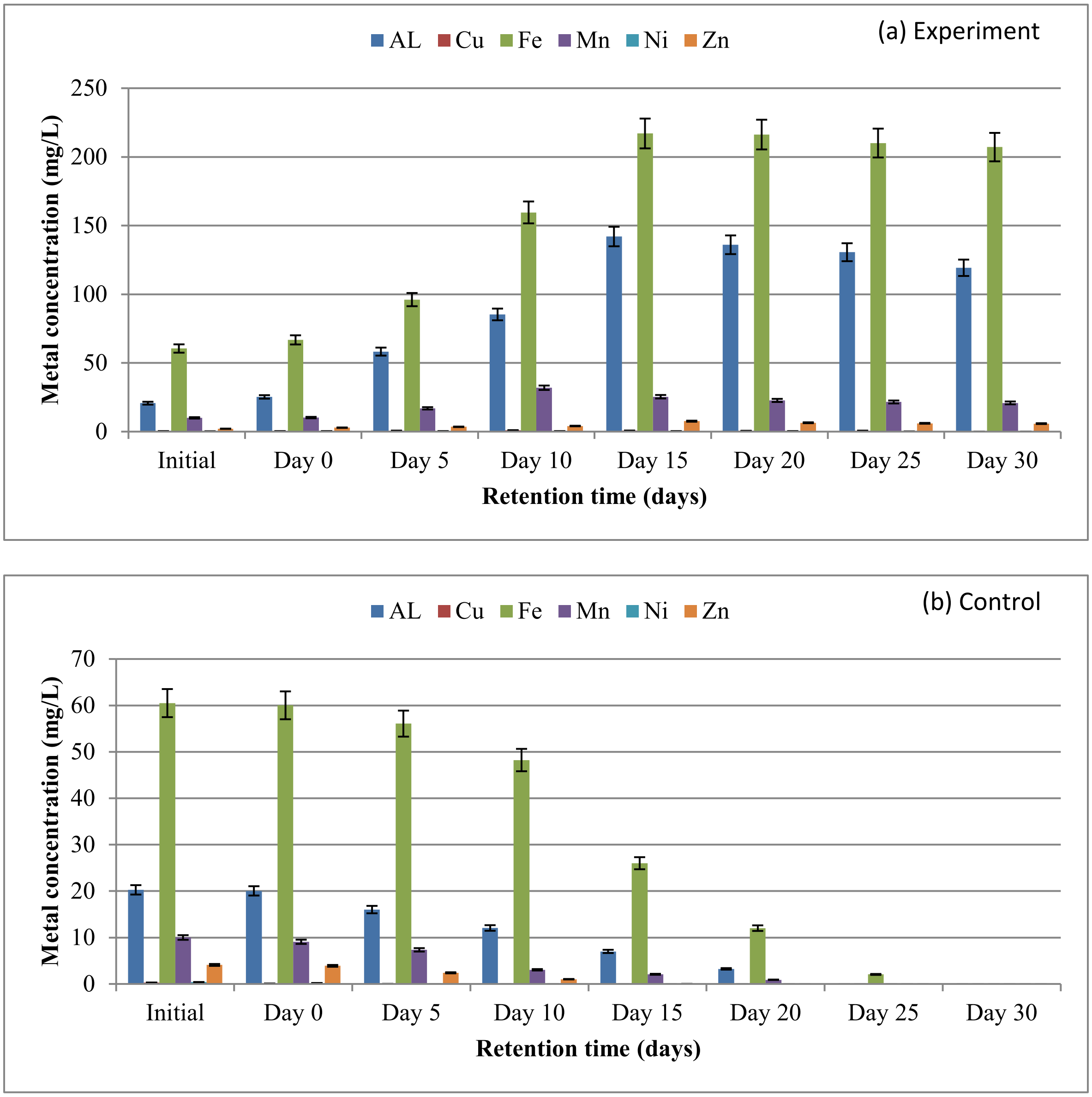


Figure 5.4 (a-b): Variation of the metal concentration in substrate (treatment wetland) within 30 days HRT and with a hydraulic loading rate of 3 liters/day. (b). Variation of the metal concentration in substrate (control wetland) within 30 days HRT and with a hydraulic loading rate of 3 liters/day

Figure 5.4a portrayed that metals content in substrate increased gradually from day 1 up to day 15. Thereafter, the removal efficacy was observed to decrease until the end of the experiment. The increase in metal content during the first 15 days of retention time may be attributed to the continuous load of metals in the wetland, since it was a continuous flow system. After 15 days, the metal content in the substrate decreased until the end of the experiment, and this may be attributed to plant accumulation of metals. In the control wetland **(Figure 5.4b),** the concentration of metals in the substrate remained almost constant for the first five days and then decreased to below the detection limit at the end of experiment; this may be the result of metals accumulation by *Vetiveria zizanioides* and external factors which also contribute to overall metal removal in constructed wetland **(Figure 5.4b).** The finding of this study showed that sedimentation plays a crucial role in metal removal by sequestering metals and facilitates their accumulation by plants. Sheoran and Sheoran. (2006) added that other processes, such as precipitation, must precede sedimentation, since plants only accumulate precipitated metals. The use of compost soil as substrate in SSVF-CW seems to be recommended due to sorption capacity and ability to form new storage sediment without clogging the wetland. The reduction of pollutants in wetland is controlled by various biochemical processes and microbial interactions in wetland media (Vymazal, 2018). From the results above, it follows that substrate in wetland is one of the most important components in constructed wetland, since it retains metals and also serves as medium for the growth of macrophytes. Metal removal in wetland varies depending on the type of plant species, the microbial diversity, and the physical and hydrodynamic characteristic, such as the depth and types of wetland (Almuktar *et al*., 2018) and temperature (Wang *et al.*, 2017). Elements have different affinities to different plant tissues once accumulated/complexed depending on their chemistry. This determines the easy or difficulty in the de-complexation from the plant tissues. Moreover, all elements are precipitated in the substrate, but their accumulation in plants depends on the pattern of metabolism of the plants.

5.4.1.4. Removal Efficiency of Metals and Sulfate

The removal efficiency of metals and sulfate by SSVF-CW is reported in **Figure 5.5**

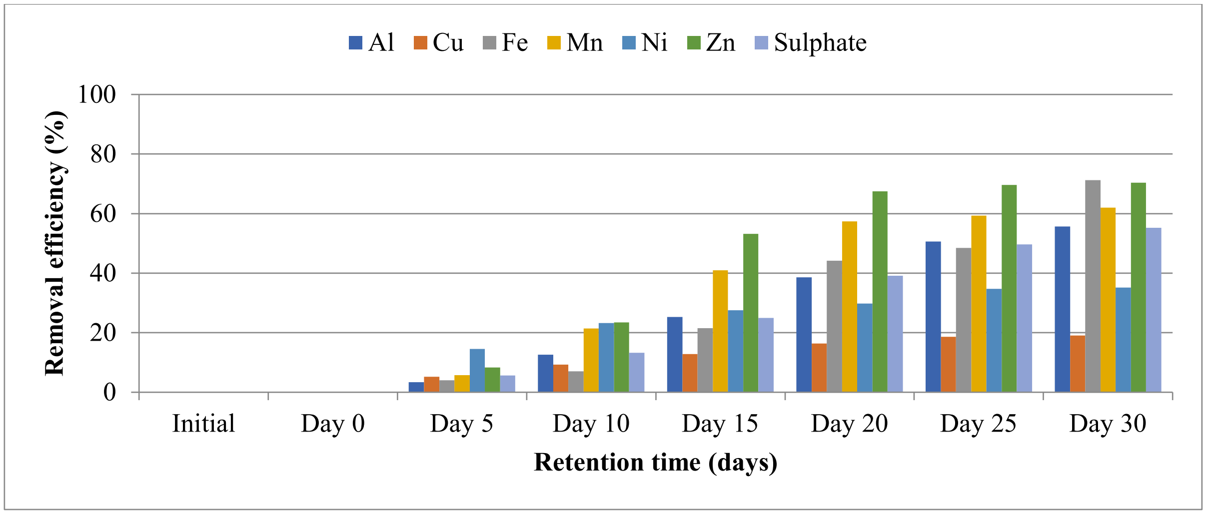


Figure 5.5: Removal efficiency of heavy metals and sulphate by SSVF in 30 days HRT under a hydraulic loading rate of 3 liters/day

The removal efficiency is the percentage of a molecule of a given compound removed or destroyed during the treatment process **(Figure 5.5).** It was calculated gradually for each metal of concern and SO_4^{2-} for the duration of experiments. The results showed that the metal removal percentage is ranked in the order: Fe (71.25%) > Zn (70.40%) > Al (68.93%) > Mn (62%) > SO_4^{2+} (55.18%) > Ni (35%) > Cu (18.83%) **(Figure 5.5).** These results were in line with the studies of Roongtanakiat *et al*., (2008) for Fe and Mn, who used vertical subsurface constructed wetland. Two processes (Sedimentation and plants uptake) contribute to heavy metal removal in SSVF-CW (Demchak *et al*., 2001). Plants accumulate metals for their metabolism, and this may be the reason why metals such as iron are easily accumulated by *Vetiveria zizanioides*. Iron is the third most important micronutrient for plant metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang, 2011). In that regards, Fe deficiency results in plant senescence. The SO_4^{2-} removal may also be attributed to the reduction of sulfur (S) in water, since S is an important nutrient for optimal plant growth. It is one of the key macro elements essential for plant growth and it is taken up from the soil/water by the plant in sulfate form (SO_4^{2-}). Sulfur is a component of methionine, cystine, and cysteine, which are three of the 21 amino acids that are the essential building block of proteins (Brosnan and Brosnan, 2006; Colovic *et al*., 2018)

5.4.1.5. Tolerance Index

For this experiment, the TI value was 1.03 (> 1), which, according to (Kumar *et al.*, 2008), reflects a net increase in plant biomass, revealing that *Vetiveria zizanioides* has developed tolerance. In both treatment and control wetland, all the plants were green and seemed healthy at the end of the experiment. The fact that *Vetiveria zizanioides* growing in AMD water remained green and seemed healthy after 30 days was not expected by the researchers, but this demonstrates that Vetiver is tolerant of a wide range of extreme conditions, such as very acidic medium (pH = 2.7) or very alkaline conditions (pH = 12) (Truong and Danh, 2015). The *Vetiveria zizanioides* is a hydrophyte (wetland plant) due to its well-developed Sclerenchyma or air cell network (Truong and Tran, 2014), hence its high tolerance degree. Vetiver is also tolerant to high concentration of a wide range of heavy metals, whether individually or a combination of several heavy metals (Truong and Danh, 2015). Following these characteristics, it can be concluded that *Vetiveria zizanioides* is an excellent candidate for a wide range of phytoremediation, as demonstrated in this study. The fact that Vetiver adapts itself to a very harsh conditions means Vetiver may have a specific cytogenetic composition allowing it to modify its metabolism according to the physico-chemical conditions of the medium. However, this was not the objective of this project and may be the subject of further research.

5.4.1.6. Bio-Concentration Factor (BCF)

The bio-concentration factor (BCF) demonstrates the potential success of the plant species for phytoremediation. A BCF greater than 1 is an indicator of how probable a metal can be bio accumulated by the plant species. It indicates the migration of heavy metals from soil to different portions of the plant including roots and shoots (Bonanno *et al.*, 2018). The BCF was calculated only for metals in the AMD-treated wetland, since the final concentration of all metals in control wetland was below the detection limit. The results are presented in **Table 5.1.**

Table 5.1: Bio-concentration factor of the metals of concern for *Vetiveria zizanioides*

Metals	**Al**	**Cu**	**Fe**	**Mn**	**Ni**	**Zn**
BCF	0.25	0.81	0.75	0.42	0.58	0.24

As shown in Table 5.1, the BCF of metals obeyed the following order: Cu (0.81) > Fe (0.75) > Ni (0.58) > Mn (0.42) > Al (0.25) > Zn (0.24). The dissimilitude of BCF results may be attributed to the variation in the heavy metal concentration in the medium and the ability of plants (*Vetiveria zizanioides*) to accumulate a specific metal. These results demonstrate that the concentration of heavy metals in plants is related to their availability in the wetland and their importance in plant metabolism. The results showed that *Vetiveria zizanioides* has not accumulated sufficient quantity of metals to be considered as hyper accumulator, since the BCF of metals was less than 1. This can be attributed to the versatile ability of *Vetiveria zizanioides* to adapt under various harsh environmental conditions, and the ability to resist a complexity of heavy metal toxicities (Roongtanakiat and Sanoh, 2011). Furthermore, low intermediate accumulation may be explained by the flow pattern of the designed system. Although the current results show that *Vetiveria zizanioides* is not a hyper accumulator, but due to its high biomass content and tolerance index (TI), which is higher than 1, and its ability to accumulate various metals simultaneously further shows its high capability for phytoremediation in relation to others plants such as *Pistia stratiotes* and *Eleocharis dulcis,* which selectively accumulate only Mn and Fe, respectively (Zuo and Zhang, 2011).

5.4.1.7. Metal Distribution and Translocation

The translocation factors of metals grown in treatment and control wetland is shown in **Table 5.2.**

Table 5.2: Translocation factor (root/shoot) of AMD and control grown vetiver

TF	Al	Cu	Fe	Mn	Ni	Zn
Treatment	0.05	0.85	0.44	2.06	0.75	0.53
Control	0.03	0.85	0.27	0.12	1.85	0.41

After 30 days of the experiment, *Vetiveria zizanioides* accumulated Al (95%), Fe (69%), and Zn (65%) in the roots **(Figure 6a**), with a TF of 0.05, 0.44, and 0.53, respectively **(Table 2).** Figure 6a shows that the majority of Cu (54%) and Ni (57%) were accumulated in the roots with a TF of 0.85 and 0.75, respectively, whereas there was great translocation of Mn (67%) with a TF of 2.06 (Table 2). However, in the control wetland, *Vetiveria zizanioides* had greater Mn (89%) and Fe contents (79%) in the roots (Figure 6b), with a TF of 0.12 and 0.27, respectively, while the majority of Al (64%), Cu (54%), and Zn (59%) were accumulated in the roots with a TF of 0.03, 0.83, and 0.41, respectively. A minority of Ni (35%) was accumulated in the roots with a TF of 1.85. The roots that grew in AMD-treated wetland showed amorphous and irregularly spaced build up along their surface from plaques, thereby reducing the translocation of metals from roots to shoots (Tripathi *et al.*, 2014) **(Figure 6a),** which is not the case for roots that grew in control wetland **(Figure 5.6b).**

The difference between the TF of Mn in AMD water and control water may be attributed to the pH of the medium. The bioavailability of Mn in soil is, therefore, influenced by both pH and redox conditions (Truong and Danh, 2015). Within a harsh environment such as AMD wastewater with various metals, including Mn and Fe, Mn oxides form co-precipitates with Fe, leading to the acidity of the medium and an enhancing of the potential redox of Mn oxides which can be easily reduced in the soil exchange sites (Adamczyk-Szabela *et al.*, 2015), thereby increasing the concentration of soluble Mn^{2+} (Watmough *et al.*, 2007), which is the predominant form of Mn in the soil solution and the most available Mn form for plants (Millaleo *et al.*, 2010). In contrast, when the pH is basic, chemical Mn^{2+} auto-oxidation is favored over MnO_2, Mn_2O_3, Mn_3O_4, and even Mn_2O_7, which are not normally available to plants (Watmough *et al.*, 2007). In addition, alkaline pH promotes the chemisorption of Mn into soil particles, decreasing their availability (Yadava, 2016). Moderate translocation of Ni in vetiver grown in both AMD and control wetlands can be attributed to the biochemical processes of sequestration and translocation, since Ni can be transported as a nickel-histidine complex (Deng *et al.*, 2018). These two biochemical processes ensure high mobility of Ni within the plant, which can justify the moderate translocation of Nickel (Soil *et al.*, 2014). The moderate translocation of Cu by vetiver grown in both wetlands may be attributed to plaque inhibition, thereby requiring more time to accumulate copper (Suelee *et al.*, 2017).

The low translocation of Al may be attributed to plant tolerance strategy, since in an acidic condition; Al becomes a major constraint for the growth of plant. At a pH value less than 6, Al^{2+} is oxidized to Al^{3+} following Equation 5.11.

$$\mathrm{Al}^{2+} + \mathrm{e}^{+} \rightarrow \mathrm{Al}^{3+} \qquad \text{(Eqn 5.11)}$$

The Al^{3+} released to the medium enters into the root tip cell and delays the growth of the plant (Roy and Bhadra, 2014). There was a greater translocation of Fe by *Vetiveria zizanioides* grown from both wetlands: 69% and 79% for AMD and control, respectively. The higher translocation of Mn and Zn in both wetlands may be attributed to the need of the aforementioned metals for plant metabolism, since Mn and Zn are essential nutrients (Suelee *et al.*, 2017). A number of factors, including types of metals, plant species, types of substrate, and the initial concentration of metals, anatomical, biochemical, and physiological factors influence the metals' translocation from roots to shoots (Wang *et al.*, 2014). In fact, metals are taken up from the soil by roots and transported via the plasma membrane driven by ATP-depended proton pumps that catalyze H^+ extrusion across the membrane. Furthermore, along with cationic nutrients, plant transporters are also involved in shuttling potentially toxic cations across plant membranes (Deng *et al.*, 2018). Cytogenetic make-up and others unknown factors can be attributed to different patterns of metal translocations from roots to shoots (Singh *et al.*, 2010).

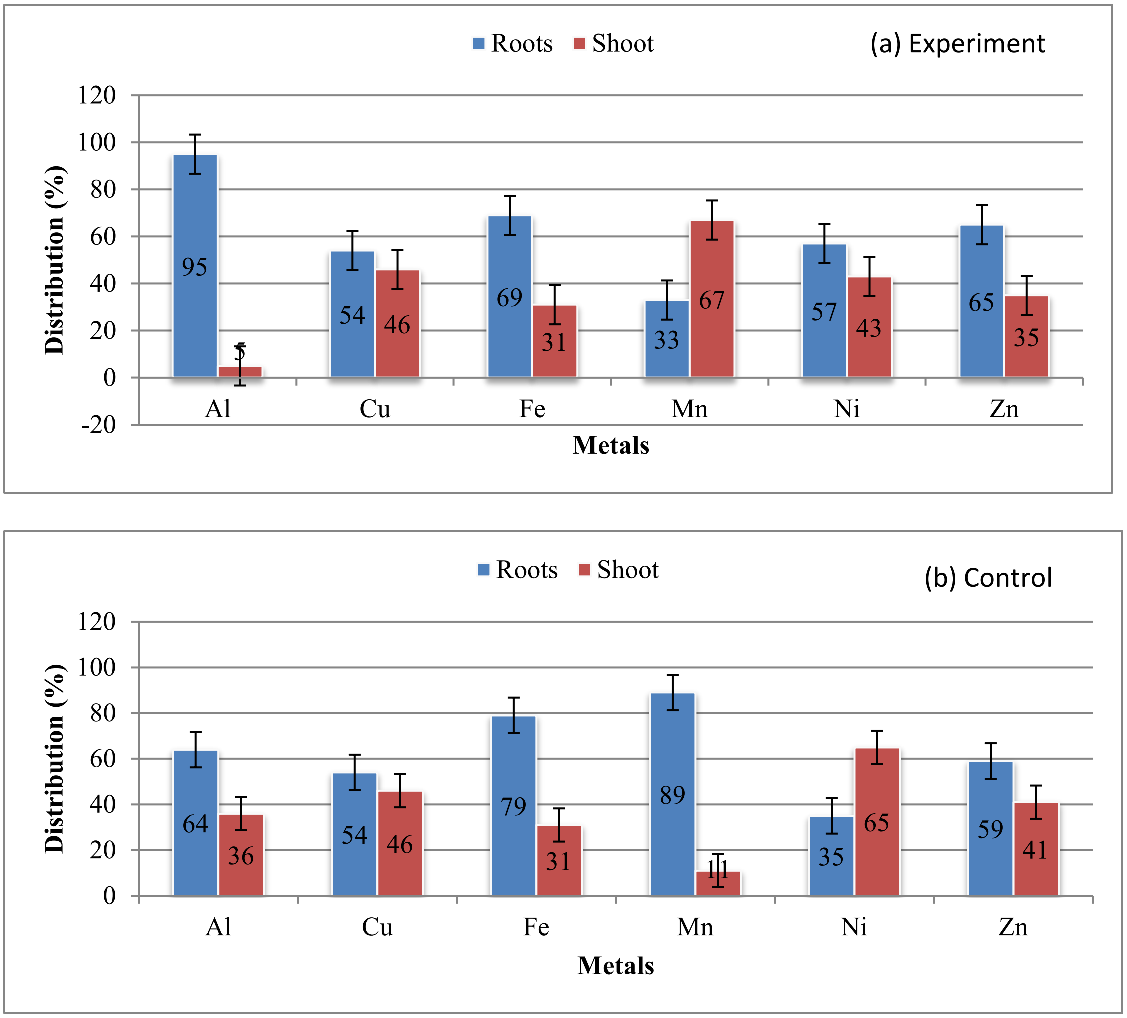


Figure 5.6 (a,b): (a), Percentages of the metal distribution partitioned into roots and shoots (treatment wetland). (b) Percentages of the metal distribution partitioned into roots and shoots (control wetland)

5.4.1.8. Metal Removal Partitioned between Substrate, Plant, and External Factors

The contribution of substrate, Vetiver, and external factors in the removal efficiency of metals was determined and the results are shown in **Figure 5.7.**

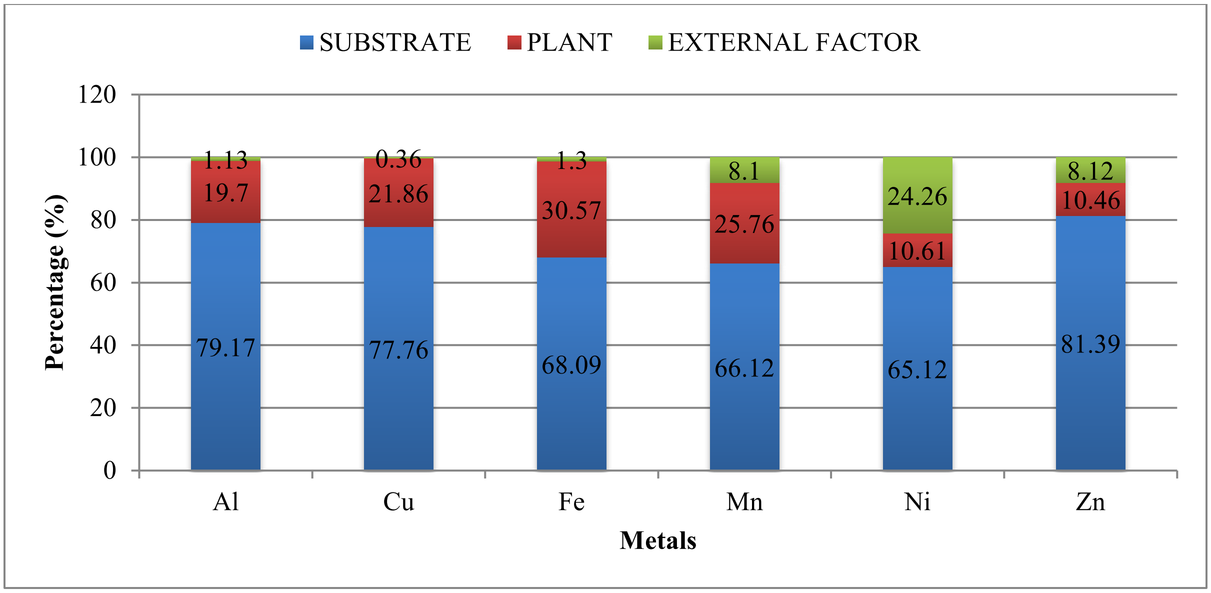


Figure 5.7: Removal efficiency of metals partitioned between *Vetiveria zizanioides*, substrate, and external factors

The determination of the final concentration of metals in AMD water, substrate, and in plant tissue allowed the researchers to calculate the contribution of each wetland component (substrate and plant) and external factor in overall metal removal process. The results revealed that metals were more removed by the wetland substrate, followed by vetiver and external factors, with substrate contributing to 81.39%, 79.17%, 77.76%, 68.09%, 66.12%, and 65.12% removal of Zn, Al, Cu, Fe, Mn, and Ni, respectively **(Figure 5.7).** The substrate provides support to plant growth, serves as an energy source for biogeochemical reactions, and also plays a major role in pollutant reduction, since it absorbs pollutants such as heavy metals, thereby facilitating their accumulation by the plants roots (Lizama Allende *et al*., 2012).

Plant contribution in the overall metal removal process was ranged in the order: Fe (30.57%) > Mn (25.76%) > Cu (21.86%) > Al (19.70%) > Ni (10.61%) > Zn (10.46%) **(Figure 5.7)** The contribution of vetiver to the metal removal was small compared to the substrate fraction in the overall metal removal, and this may be attributed to the duration of experiment (30 days) and the need of metals for plant metabolism. For instance, Cu and Zn are essential elements for plant metabolism; however, at elevated concentrations, they become toxic (Geddie and Hall, 2019) and plant (*Vetiveria zizanioides*) will not be able to accumulate considerable amounts of metals but rather significantly reduce the uptake by plant. Despite its smaller contribution in overall metal removal in SSVF-CW, *Vetiveria zizanioides*, similar to other plants used in constructed AMD-treatment wetlands, contributed to the overall efficiency of CW in many ways. It favored the settlement of total suspended solid (TSS) and provides optimum conditions for the growth of microorganisms, thereby promoting the sedimentation and accumulation of metals in CW **(**Shelef et al., 2013). The contribution of external factors was ranged in the order: Ni (24.26%) > Zn (8.12%) > Mn (8.10%) > Fe (1.3%) > Al (1.13) > Cu (0.36%) **(Figure 5.7).** They include: evaporation, adsorption, biological assimilation, decomposition, chemical transformation, and volatilization; sulfides and other minerals in the sediment may also contribute significantly to metal removal in CW (Cortes-Esquivel *et al.*, 2012). The finding was in line with the study by Mustapha *et al*. (2018), who used SSVF-CW with three different plants (*Typha. latifolia; Cyperus alternifolius; Cynodon dactylon*) for heavy metal removal from refinery wastewater and found that metal accumulation by plants and externals factors accounted only for a small fraction of the overall metal removal in subsurface vertical flow wetland.

5.4.2. Characterization of the Solid Samples

5.4.2.1. X-ray Fluorescence (XRF) Analysis

An XRF analysis was done to determine the elemental composition of initial soil, soil from control wetland, and soil from treatment wetland, and the results are shown in **Table 5.3.**

Table 5.3: The elemental composition of initial soil, soil from control wetland, and soil from treatment wetland

Samples (wt.%)	Initial Soil	Control Soil	AMD Reacted Soil
Fe	84.9504	84.9532	88.8688
Ti	4.6093	2.6774	4.3403
Rb	3.2519	3.8190	2.3403
Sr	2.7486	3.7355	2.7808
Mn	2.3030	2.6018	3.4293
Zn	0.6970	0.7001	0.4873
Si	0.1393	0.3210	0.0291
Al	----------	---------	0.0393
Na	----------	---------	0.0324
Ni	0.4505	0.0007	0.0291

From the results in **Table 5.3,** it follows that the percentage of Fe and Mn was the same for the initial and control soil. However, the percentage of Fe and Mn were found to increase after reaction of soil with AMD, and this may be attributed to the addition of metals content and formation of new phases. This corroborates with results reported in the water quality assays. The percentage of Zn, Ni, and Si decreased after contact of substrate (soil) with AMD water, indicating the possible dissolution of these metals. Al and Na were found to be present, and this may have originated from AMD water. The results also revealed that there was no noticeable difference in the elemental composition between initial soil and soil from control wetland, which may be attributed to the potable fresh water used in control wetland, which in fact does contain an insignificant quantity of chemicals.

5.4.2.2. X-ray Diffraction (XRD) Analysis

An XRD analysis was done to determine the mineralogical composition of initial soil, soil from treatment wetland, and soil from control wetland, and the results are shown in Figure 8. In addition, **Table 5.4** lists the mineral phases identified with their various peaks.

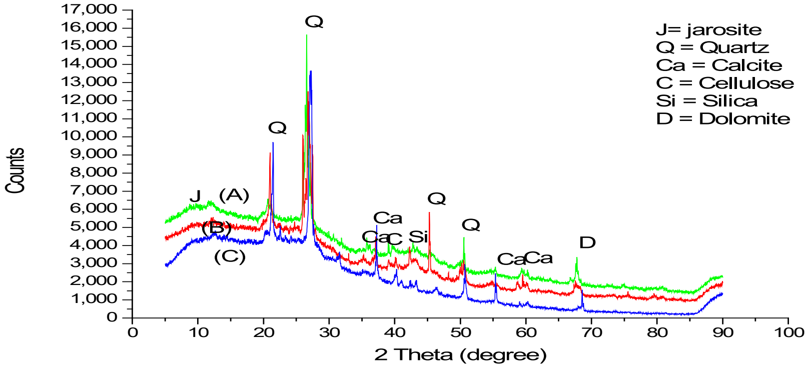


Figure 5.8 (A–C): XRD patterns of substrate: treatment (A), control (B), and initial substrate (C)

As shown in **Figure 5.8 A–C**, the X-ray diffraction patterns of substrate from experimental wetland (a) and control wetland (b) and initial substrate showed several peaks spread over the range 2Ө from 10° to 68°, although at different intensities. The peaks at 2Ө = 20.6°, 28°, 45°, and 50° may correspond to Quartz (SiO_4), which is the major elemental component of compost (Biyada *et al.*, 2020a**)**. Jarosite is present in the substrate from the experimental wetland with peak at 8, and this may be a ferric iron-sulfate mineral with the formula $(K, H_3O)\ Fe_3\ (SO_4)_2\ (OH)_6$ that originated from AMD water with acidic pH.

Table 5.4: The measured XRD and the identified mineral phase of the initial substrate, substrate from experimental wetland, and from control wetland

Substrate	2 Theta (Degree)	Mineral Phase	References
Initial soil	Quartz	21	(Biyada *et al.*, 2020b)
	Quartz	28	(Biyada *et al.*, 2020b)
	Calcite	37.5	(Sharma, Ganguly and Gupta, 2019)
	Cellulose	40.1	
	Silica	46	
	Quartz	50	(Biyada *et al.*, 2020b)
	Quartz	55	(Biyada *et al.*, 2020b)
	Dolomite	68	
Soil from control wetland	Quartz	21	(Biyada *et al.*, 2020a)
	Quartz	27	
	Calcite	37	
	Cellulose	38	(Lee, Kwag and Ra, 2010)
	Silica	44	
	Quartz	45	(Biyada *et al.*, 2020b)
	Quartz	50	(Biyada *et al.*, 2020b)
	Dolomite	67	(Grigatti *et al.*, 2017)
Soil from treatment wetland	Jarosite	8	(Lee, Kwag and Ra, 2010)
	Quartz	21	(Biyada *et al.*, 2020b)
	Quartz	26	(Biyada *et al.*, 2020b)
	Calcite	35	(Grigatti *et al.*, 2017)
	Silica	42	(Biyada *et al.*, 2020b)
	Quartz	45	(Lee, Kwag and Ra, 2010)
	Quartz	50	
	Quartz	55	(Biyada *et al.*, 2020b)
	Calcite	61	(Lee, Kwag and Ra, 2010)
	Dolomite	65	(Grigatti *et al.*, 2017)

5.4.2.3. Fourier Transform Infrared Spectroscopy Analysis

Fourier transforms infrared spectroscopy (FTIR) analysis of roots grown from both wetlands (treatment and control) was done and the results are presented in **Figure 5.9** while **Table 5.5** lists the identified metals' functional groups and their respective wavelengths.

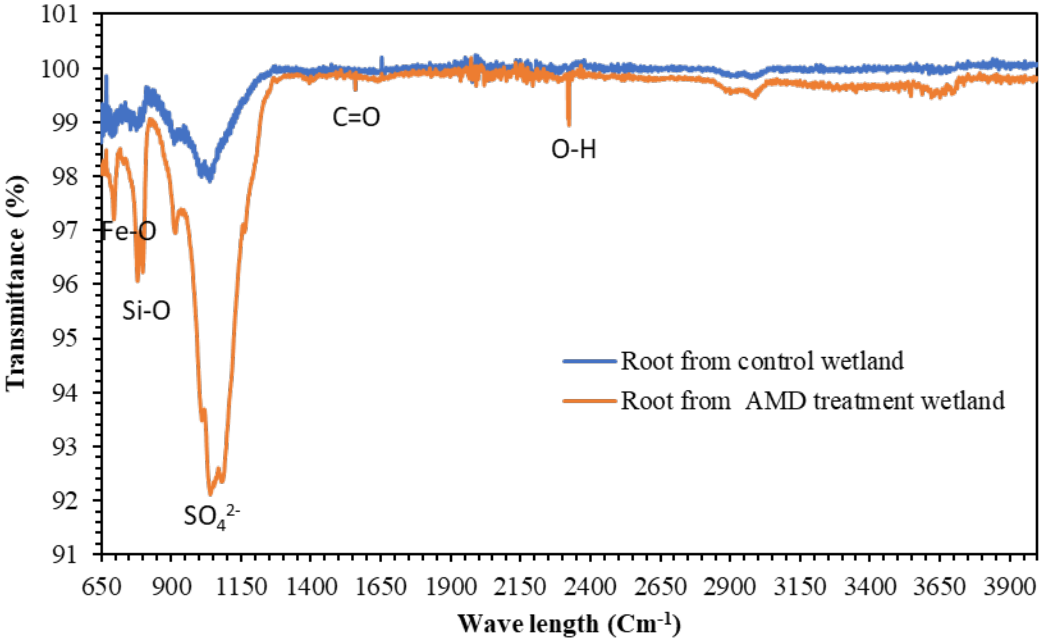


Figure 5.9: Fourier Transfer Infrared (FTIR) spectra of root from AMD treatment wetland and root from control wetland

Table 5.5: The metals' functional groups and their references

Wavenumber	Functional Group	Reference
698	Fe-O	(Tabelin *et al.*, 2017)
780	Si-O	(Yu *et al.*, 2017)
1080	SO_4	(Tabelin *et al.*, 2018)
1559	C=O	(RoyChowdhury *et al.*, 2020)
2222	O-H	(Bahri *et al.*, 2021)
2996.5	O-H	(Yu *et al.*, 2017)

FTIR spectra of *Vetiveria zizanioides* grown in AMD-treated wetland and control wetland in **Figure 5.9** indicate the complex formations between the pollutants in AMD water and different part of *Vetiveria zizanioides* cell wall. The bands in the roots grown in control wetland are characteristic of vibration, corresponding to the band at 3498 cm^{-1}. The stretching vibration of the roots from control wetland correspond to the bands at 3032, 2814.5, 1998.5, 1489.5, and 1274 cm^{-1}, while in the roots from AMD-treated wetland, the stretching vibration corresponds to the bands at 3495, 2937, 2323.5, and 1345.5 cm^{-1}. The doublet at 1026 and 1105 for roots from AMD wetland and the doublet at 857 and 966.5 for roots from control wetland may be attributed to the formation of carbonate ($CaCO_3$) (Masindi, 2016) from calcium present in roots from both wetlands, as revealed by the EDS results **(Figure 11a.b).** Furthermore, sulfates (900 –1200 cm^{-1}) (Tabelin *et al.*, 2020) and iron oxides (450–500 cm^{-1}) were also present (Tabelin *et al.*, 2017).

5.4.2.4. Scanning Electron Microscope Electron Dispersion Spectrometry Analysis

To understand the mode of interaction of AMD with *Vetiveria zizanioides* and the formation of mineral phases, a scanning electron microscope (SEM) was used to assess the change in morphology of *Vetiveria zizanioides* roots reacted with AMD water **(Figure 10a)** and *Vetiveria zizanioides* root reacted with potable fresh water **(Figure10a).** The SEM images of roots grown in AMD water **(Figure 5.10a)** and the SEM images of *Vetiveria zizanioides* roots grown in potable fresh water **(Figure 5.10b)** showed a noticeable difference in morphology. This result concurs with the findings obtained by Kiiskila *et al.* (2019). In fact, plants use their cell wall as a defense compartment to respond to toxic conditions and accumulate pollutants. Pollutants are accumulated by plants using the cell wall, which is rich in proteins, amino acids, and phenolics able to bind pollutants in order to render them less or non-toxic. When plants accumulate metals using the cell wall, it leads to the formation of crystal-like deposits, which saturates the cell wall, creating cell thickening. This demonstrates that *Vetiveria zizanioides* has accumulated and sequestrated toxic pollutants, as illustrated by **Figure 10a**. The SEM image of *Vetiveria zizanioides* roots from control wetland showed less thickening compared to the SEM image of roots from AMD wetland, thereby demonstrating that *Vetiveria zizanioides* growth in control wetland accumulated fewer pollutants.

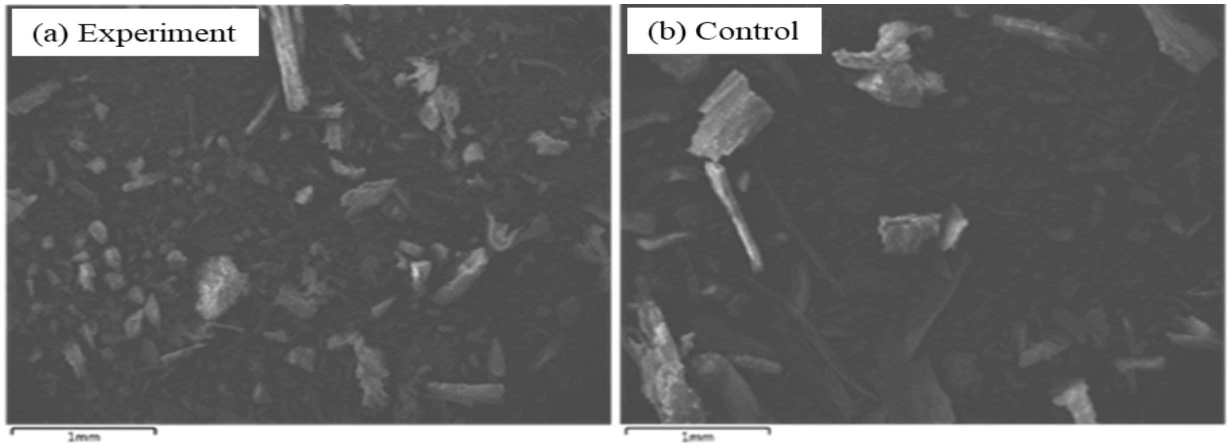


Figure 5.10 (a,b): SEM of *Vetiveria zizanioides* roots from AMD-treated wetland water (a) and control wetland (b)

The EDS results of vetiver roots grown in AMD water revealed the presence of C, O, Mg, Au, Si, and Fe. The presence of Ca indicates the presence of Calcite. The reacted *Vetiveria zizanioides* with AMD contained O, Al, K, and Si as the major components. The presence of oxygen at high levels may be the results of precipitation of AMD elements to metal oxides. High levels of Fe and Al are possibly due to the phenomena of ion exchange on the soil surface (substrate) and precipitation following the slight increase of pH. The ion exchange process leads to the release of some metals (Zewail and Yousef, 2015) and the presence of Potassium (K), Calcium (Ca), and Magnesium (Mg) in root grown in the AMD environment may be the result of an ion exchange reaction, whereas the presence of S compounds could be attributed to the sink of heavy metals released into the environment **(Figure 5.11a).** The EDS of roots from control wetland revealed the presence of the same components in addition to Na but with a very low percentage, thereby confirming the X-ray fluorescence analysis results **(Figure 5.11b)**. The components of roots from control wetland may be attributed to their presence in substrate used as wetland media.

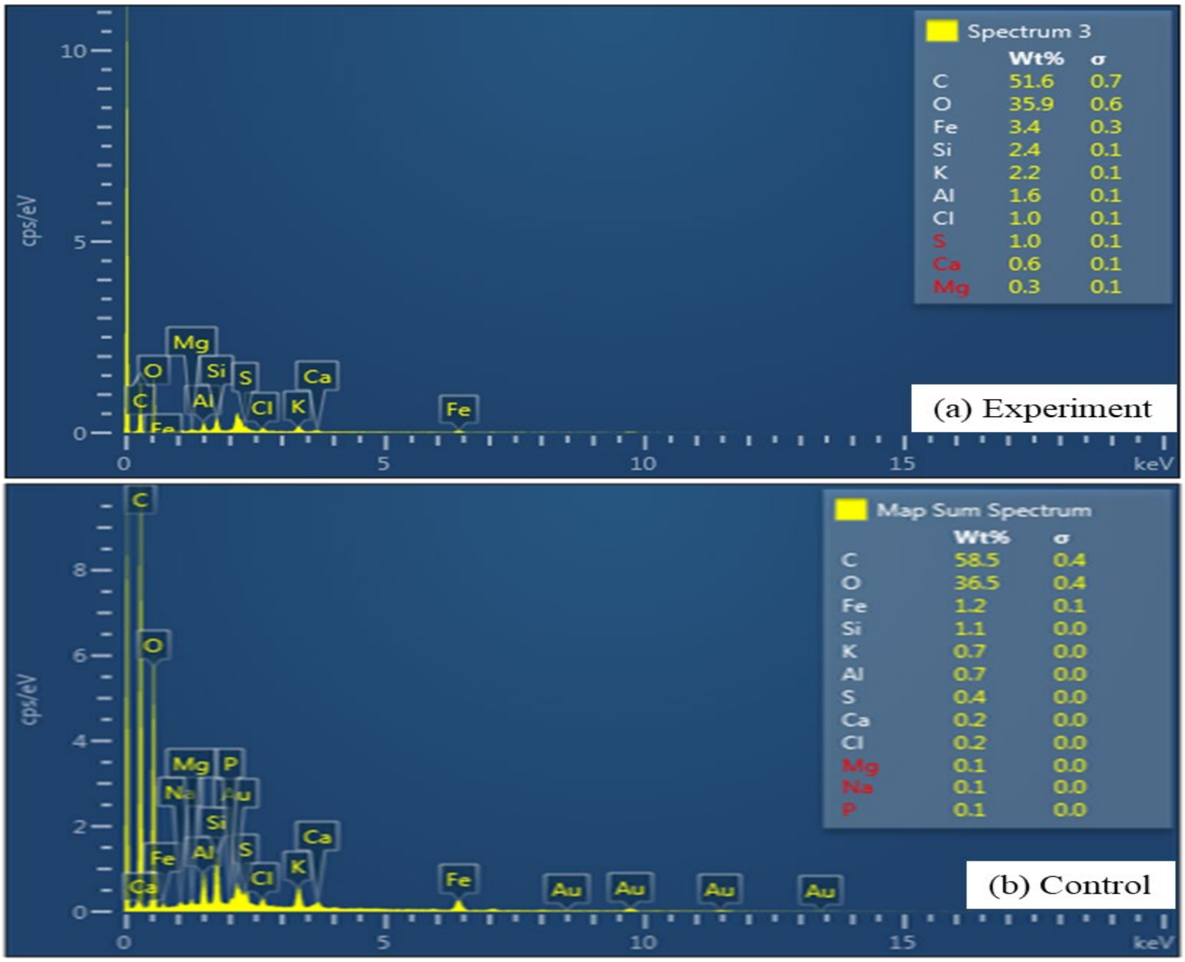


Figure 5.11(a.b): EDS of *Vetiveria zizanioides* roots from AMD-treated (a) and control wetland (b)

5.5. Chemical Species for Untreated and AMD-Treated Wetland with SSVF-CW

The results of chemical species of AMD were used to compare the guidelines standards for effluent discharge as set by department of environmental affairs (DEA) and department of water and sanitation (DWS), and the results are presented in Table 5.6.

Table 5.6: Concentrations of chemical species for untreated and AMD-treated wetland with SSVF-CW (all units in mg/L except pH and EC)

Parameters	Raw AMD	DEA/DWS guidelines for effluent discharge	AMD-treated water	Percentage of pollutant removed
pH	2.6	6-12	3.8	----
TDS (mg/L)	3380	2400	1400	58.57
EC (μS/cm	5000	150	3200	36.00
Fe (mg/L)	341	50	98.03	71.24
Mn (mg/L)	37	20	14.06	62
Cu (mg/L)	4.2	20	3.40	19.04
Zn (mg/L)	8.55	20	2.53	70.40
Ni (mg/L)	3.92	10	2.54	35
SO_4^{2-} (mg/L)	3137	2400	1406	55.18

The pH is very acidic (2.6), with high TDS and EC. Higher TDS and EC may be attributed to high levels of Al, Fe, and Mn, other macro-elements, such as Na, Ca, and Mg, and microelements, such as Cu, Zn, Pb, Co, Ni, B, Cr, Mo, Se, As, K, and Si, and a high concentration of sulfate. After treatment of AMD with SSVF-CW using *Vetiveria zizanioides*, the pH increased slightly from 2.6 to 3.01, whereas EC and TDS decreased. The concentrations of metals (Al, Cu, Fe, Mn, Ni, and Zn) and SO_4^{2-} decreased significantly after treatment with SSVF-CW, and this can be attributed to sedimentation/precipitation and accumulation by *Vetiveria zizanioides*. However, the DWAS water quality guidelines have not been met, and therefore, there is a need to associate or integrate subsurface vertical flow constructed wetland with another type of passive treatment, such as free water constructed wetland, or with active technology, such as neutralization or filtration, in order to improve the AMD water quality.

5.6 Conclusion

Subsurface vertically flowing wetland equipped with *Vetiveria zizanioides* was successfully explored for the removal of contaminants from acid mine drainage (AMD). Findings from this study further revealed that *Vetiveria zizanioides* has high tolerance to AMD, and this has been confirmed by a zero to trivial sign of toxicity response after 30 days of hydraulic retention and hydro-dynamics. Interestingly, (i) a lower removal efficiency for Ni and Cu were observed, with (ii) moderate removal of SO_4^2 reported, while (iii) higher removal efficiency was observed for Fe, Zn, Al, and Mn. The finding further revealed that the attenuation of metals could be explained by the partitioning of extracted chemical species between wetland substrate, plant, and external factors with a substrate contribution of approximately 65.12% to 81.39% with ≤31% of Al, Cu, Fe, Mn, Ni, and Zn stored in the plant. The removal efficacy of chemical species was observed to obey the following order: Fe (71.25%) > Zn (70.40%) > Mn (62%) > Al (56.68%)> SO_4^{2-} (55.18%) > Ni (35%) > Cu (18.83%). Furthermore, metals were more concentrated in the roots, except Mn, which was more concentrated in the shoot. The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirmed that metals existed as di- and-trivalent complexes in the solution. Lastly, the available metals were precipitated as metal hydroxides and oxy-hydrosulfates. A significant quantity of pollutants of concern was removed from AMD water, but we did not reach the guideline standards for effluent discharge as established by the DEA/DWS and World Health Organization (WHO). As such, we recommend the coupling with a secondary AMD treatment technology to ensure that the treated water is of an acceptable standard.

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CHAPTER SIX

This chapter assessed the performance of horizontally flowing subsurface wetland for the treatment of acid mine drainage

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CHAPTER SIX

Assessing the performance of horizontally flowing subsurface wetland for the treatment of acid mine drainage

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Abstract

Contamination of different ecological spheres with acid mine drainage (AMD) has raised numerous concerns to countries with well developed mining industry, thus calling for urgent intervention measures. Acid mine drainage constitutes toxic and hazardous chemical species that can pose severe environmental damage if not properly managed. Herein, the performance of subsurface horizontally flow constructed wetland (SSHF-CW), equiped with *Vetiveria zizanioides* for the treatment of AMD was assessed. To fulfil the goals of this phytoremediation study, the experiments were administered for a period of 30 days using authentic AMD from a gold mine in Krugersdorp, South Africa. The quality of feed and product water were monitored daily until the last day of the experiment. The results showed a slight increase in pH from 2.4 to 4.01 and a net reduction in electrical conductivity (EC), total dissolved solids (TDS), and sulphate at 47.20%, 46%, and 33.04%, respectively. Thenceforth, there was a net removal of metals in the following order: Zn (77.75%) > Fe (75.36%) > Mn (67.48%) > Al (55.05%) > Ni (44.01%) > Cu (11.36%). The results further revealed that *Vetiveria zizanioides* was tolerant to AMD with a tolerance index of 1.23 after 30 days while metals removed were partitioned between substrate, plant, and external factors. Furthermore, chemicals species accumulated by the plants were more concentrated in the roots except Mn which was more concentrated in the shoots. The XRF and XRD analyses revealed the presence of chemical species in the substrate while FTIR and SEM-EDS analysis revealed the presence of chemicals species in plant roots confirming that substrate and plant play huge role in pollutants removal from AMD. As such, it can be concluded that SSHF wetland equiped with *Vetiveria zizanioides* plays a major role in the removal of contaminants from AMD and this could be employed in derilict mines or small operation as a passive treatment technique.

Keywords: Acid mine drainage; treatment; performance; horizontal subsurface flow wetland; *Vetiveria zizanioide*.

6.1 Introduction

Acid and metalliferous drainage or acid rock drainage (ARD) originates as a by-product of minerals mining but it can also occur naturally in the environment as part of the rock weathering process (Bwapwa, 2017). When sulphide ores such as pyrite, arsenopyrite, and marcasite amongst others are in contact with oxygen and water lead to oxidation of the host rocks. The product water is rich in sulphuric acid and elevated levels of metals. High level of dissolved metals and electrical conductivity may be attributed to the acidic nature of AMD that promotes the leaching of metals (Aguila, 2018). According to literature, AMD comprises of Al, Fe, Mn, As, sulphate, oxyanions, metalloids, and radionuclides. Due to its physico-chemical properties, AMD has deteriorating consequences on the receiving environment and human health (Sangita *et al*., 2010; CSIR, 2013)**.**

Furthermore, AMD is therefore seen as the most dangerous environmental hazard derived from mining activities and it can remain active for an extended period of time after the mine closure (Moeng, 2019; Skousen *et al*., 2019). Specifically, AMD pollutes surface and ground water resources in countries with well-developed and poorly-developed mining industries (Coetzee *et al.*, 2006; Bwapwa, 2017). This has been perceived as a catastrophic threat to public health due to embodied chemical species (Masindi *et al*., 2018). For instance, the study by Coetzee *et al.* (2006) has revealed the presence of radioactive substances in AMD from the Witwatersrand basin, in South Africa. The presence of such substances increases the risk of cancer in human beings (CSIR, 2013; Moeng, 2019). Regulatory requirements, such as World Health Orgarnization (WHO), South African National Standard (SANS), Environmental Protection Agency (EPA), and departement of Water and Sanitation (DWS) standards, require AMD to be treated before it could be discharged into the environment. The treatment of AMD will play a pivotal role in curtailing the water scarcity in the country and aid in the reclamation of contaminated water resources (Masindi et al., 2019) thenceforth, another added value can be associated with the potential of the recovery of precious metals and chemicals (Akinwekomi *et al.*, 2020).

Currently, there are two types of technologies that have been widely explored for the treatment of AMD and they include (i) active and (ii) passive treatment approach (MacIngova and Luptakova, 2012a; Bortnikova *et al.*, 2020). Of which, the active method entails the injection of energy and chemicals to enhance the treatment process whereas the passive treatment method relies on natural energy. Specifically, active treatment include the neutralization and precipitation of metals using alkaline agents such as lime (Iakovleva *et al.*, 2015), magnesite (Masindi *et al*., 2015) Sodium sulfide (Bortnikova *et al.*, 2020) and soda ash (MacIngova and Luptakova, 2012b), ion-exchange (Howard *et al.*, 2009), adsorption filtration (Aguiar *et al.*, 2018) and electrodialysis (Buzzi *et al*., 2013). Active approach requires continuous input of resources such as chemicals, energy, and also skilled staff. Contrary, passive methods treat the effluent by auto adjusting to a self-operating system that does not require constant monitoring, chemicals inputs and energy inputs (Skousen *et al.*, 2017)**.** The widely employed passive treatment processes are lime drains (Maree *et al*., 2013) permeable reactive barriers (Shabalala *et al*., 2014), cascaded reactors for aeration (Oh *et al.*, 2015), biosorption (Choi, 2015), and phytoremediation (Kiiskila *et al*., 2017**)**. Furthermore, passive treatment systems also use organic matter to neutralise mine water thereby promoting the precipitation of metals and sulphate reduction (Muhammad *et al.*, 2015a). However passive treatment technologies cannot be considered as long-term solution to AMD treatment because they are not efficient in the treatment of highly concentrated raw AMD due to numerous technical problems, however, they are mainly used in abandoned mines and as a polishing step. Albeit, phytoremediation has evolved as the most promising technique for the treatment of AMD and it has been widely explored. This process is mainly governed by the affinity of plants to certain chemical species and variety of processes such as extraction (absorption) (Ashwini *et al*., 2014), translocation (Barajas-Aceves *et al*., 2015) and phyto-stabilization (Guo et al., 2014) amongst others. Furthermore, different plant species have been employed in phytoremediation techniques and they include *Vetiveria zizanioides* (Kiiskila *et al.*, 2019; Kiiskila *et al.*, 2020) *Phragmites australis* (Ding and Sun, 2021), *Eichhornia crassipes* (Palihakkara *et al.*, 2018), *Nauclea orientalis* (Tuheteru *et al.*, 2016), *Ipomea aquatica* (Biosci *et al*., 2018), and *Pistia* stratiotes (Novita *et al*., 2019). Due to unique tolerance and bioaccumulation properties, Vetiveria zizanioides has gained attention in the recent decades. Kanda *et al.* (2017) assessed the performance of sequential combination of coal ash-based adsordents and phytoremediation using Vetiver grass to remove trace metals from AMD. The authors reported that this technology increased the potentiality of *Vetiveria zizanioides* in metals removal. Kiiskila *et al.* (2017) designed a floating treatment wetland for remediating acid mine drainage-impacted water using vetiver grass (*Vetiveria zizanioides).* The authors reported increasing pH, metals accumulation and significant SO_4^{2-} removal by floating wetland using *Vetiveria zizanioides*. Furthermore, *Vetiveria zizanioides* showed tolerance with minimal change in biomass and plant growth. Kiiskila *et al.* (2020) conducted a study to investigate the metabolic response of *Vetiveria zizanioides* to AMD and the finding revealed greater changes in AMD water after 56 days exposition including the increase of pH, net reduction of metals and sulphate. The Vetiver system techniques have been widely explored in the remediation of AMD water but there is limited information on the use of combining agricultural substrate and *Vetiveria zizanioides* for the treatment of AMD. Most of them used the floating wetland with the plants suspended or a hybrid of adsorption and phytoremediation but relying on nominal irrigation techniques. To the best of our knowledge, constructed wetlands with horizontal flows equiped with *Vetiveria zizanioides* has never been evaluated and employed for the treatment of AMD. This will be the first study in design and execution to explore the use of *Vetiveria zizanioides* for AMD treatment on subsurface horizontal flow constructed wetland (SSHF-CW). This will also highlight the fate of chemical species post the treatment process and their partitioning or translocation to different parts of the system soil (substrate), and plants). The SSHF-CW is a simple, cost effective, easy to operate and environmentally friendly technology that can prove to be effective for AMD treatment (Prihatini *et al*., 2016; Andreo-Martínez *et al*., 2016; Andreo-Martínez *et al.*, 2017). The mode of operation of SSHF-CW is very simple since effluent flows horizontally under the substrate of the wetland from an inlet to an outlet valve. Depending on the researcher and climatic conditions, the nature of the substrate varies and it can either be sand, gravel, soil, root or rhizome of aquatic plants (Lizama Allende *et al*., 2012; Muhammad *et al.*, 2015a). Henceforth, the constructed wetland technology is widely used in waste water treatment (Vymazal, 2010). Like others constructed wetland, the SSHF-CW constitutes a complex system with many different components working simuoustanesly to improve the performance of the system. The ultimate goal of this study is to investigate the performance of SSHF-CW in the treatment of AMD. Insights into the contribution of *Vetiveria Zizanioides*, substrate and external factors in chemical species attenuation will also be reported.

6.2 Materials and methods

6.2.1 Acquisition of reagents, plants and substrate

All reagents used in this study were of analytical grade (AG) and were obtained from Merck South Africa. In order to construct the SSHF-CW, shoots of *Vetiveria zizanioides* were purchased from NANDADRAM ECOVILLAGE farm in Kwa-Zulu Natal and transported to the University of South Africa's sciences campus in Florida, Johannesburg while soil (Compost soil) used as wetland substrate were purchased from Garden World Johannesburg (Pty) Ltd.

6.2.2 Growth of plants

To carry out the experiments, shoots of *Vetiveria Zizanioides* were immersed under potable fresh water for aproximately three weeks to allow the germination of new seeds. Following the germination of new seeds, the seedlings were tranferred into small pots containing compost soil. No chemical fertilizer was added to the plants, they were allowed to grow naturally, monitored and watered daily.

6.2.3 Sampling and characterization of AMD water

Field AMD water used in this study was collected from a Gold mine in Krugersdorp, Gauteng, South Africa (26.0963°S and 27.8077°E). During AMD sampling, some parameters were determined in situ using a multi-parameter probe (HANNA instruments, Johannesburg, RSA). These included pH, total dissolved solids (TDS) and electrical conductivity (EC). Initial concentration of metals (Al, Cu, Fe, Mn, Ni and Zn) in AMD was analyzed using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) whereas initial sulphate (SO_4^{2-}) concentration was analyzed using Ion chromatography (IC). Parameters were analysed following the ''Standard Methods of the Examination of Water and Wastewater''(APHA, 2002).

6.2.4 Quality assurance and quality control (QA/QC)

In this study project, the QA/QC was applied to ensure the production of reliable results. All samples were analyzed in triplicate and results reported as mean values and results considered acceptable when the difference amongst the triplicate was less than 10%. The value below detection limit (BDL) was determined referring to the United States Environmental Protection Agency (USEPA) guideline for data analyses (Wei et al., 2005) while the accuracy of the analysis was monitored following the guidelines of National Institute of Standards and Technology (NIST) for water quality standards.

6.3 Experimental setup

6.3.1 Horizontally flowing wetland design and description

Two pilots experimental plants consisted of the following: two reservoirs tank of 1000 liters capacity each to contain AMD and fresh water for control. Two plastic circular basins of 100 litres capacity each measuring 62 cm of diameter (d) and 45 cm high (h) were used as wetland cell (treatment and control respectively) **(Figure 6.1).** Young *Vetiveria zizanioides* from **section 2.2** were transferred from pots into the two basins containing organic matter (substrate). Basins containing 20 plants each were connected to the reservoir tank via a pipe to have two wetlands (one for treatment and one for control). A plastic drip was incorporated at the baseline of each tank to regulate the flow rate of water from tank to the wetland cell. The wetlands were continuous flow systems, where water was flowing continuously for the duration of the experiment. The liquid water entered the plant wetland system via an inlet zone and flow horizontally under the surface of substrate until it reached the outlet zone where leachates were collected.

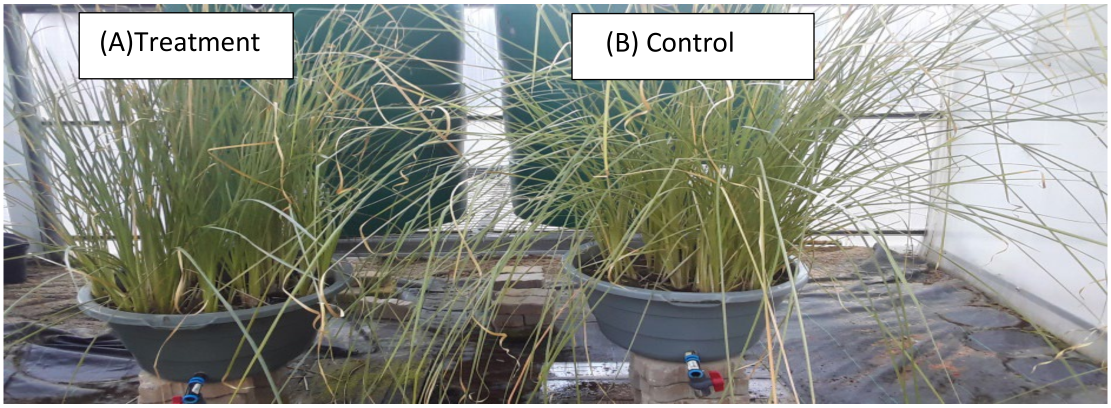


Figure 6.1: Experimental set up of the SSHF-CW system, treatment (A) and control (B)

6.3.2 Pre-treatment of AMD water and substrate characterization

Prior to experimental phase, AMD water was filtered to remove total suspended solid (TSS) susceptible to clogging the pipe. Elemental composition and mineralogical composition of substrate was determined. Elemental composition was done before and after experiment using X-ray fluorescence (XRF)**.** The analyses were performed using a Thermo Fisher ARL-9400 XP+ Sequential XRF with winXRF Software. Mineralogical composition was done using X-ray diffraction (XRD). Analyses were performed using a Philip PW 1710 diffractometer equipped with graphite secondary monochromatic.

6.3.3 Hydrology of the system

The system was calibrated using a plastic drip which allowed maintaining a very low flow rate and the water was allowed to flow continuously for four weeks. The average inflow and outflow (Qav) was determined as shown in Equation 6.1.

$$Qav = \frac{Qin\ + Qout}{2} \qquad \text{(Eqn 6.1)}$$

Where, Q_{in} is the average inflow of wastewater into the system,

Q_{out} is the average outflow of wastewater from the system,

and Q_{av} is the average flow rate of waste water through the system (m^3/d).

The calculated Q_{av} was 0.0012 m^3 or 1.2 litres. From there the hydraulic retention time (HRT) was determined as illustrated in Equation 6.2 **(IWA, 2017).**

$$HRT = \frac{As\ \times d\ \times n}{\mathrm{Qav}} \qquad \text{(Eqn 6.2)}$$

Where, As is the surface area of the wetland (m^2),

d is the water depth in the wetland (m),

n is the porosity of the wetland substrate (%)

Qav is the average flow through the wetland (m^3/d) and

HRT is the hydraulic retention time (days)

$$HRT \quad = \quad \frac{3.14 \times (0.31)^2\ 0.2\ \times 0.6}{0.0012}$$

$HRT = 30.17$ days

The determined HRT was 30.17 days and during that period, the hydraulic system of each pilot plant was monitored daily to ensure a steady flow of water within the system. At the end of the experiment (Just after the 30rd day), plants were harvested from both wetlands (Treatment cell and control) for metals analysis.

6.3.4 Product water and soil sampling

Water samples (experiment and control) and substrate (soil samples) were collected daily form each wetland from (Day 1) to the last day of experiment (Day 30). Water samples were collected into amber glass bottles of 500 mL. After collection, samples were divided into two sub-samples of 400 mL and 100 mL. Sub-samples of 400 mL were used for the in-situ analysis of pH, TDS, EC while sub-samples of 100 mL were used for the analysis of ex-situ parameters which included metals (Al, Cu, Fe, Mn, Ni, Zn) and SO_4^{2-}. Samples were analysed and the average result of five-days period was considered.

6.3.5 Analytical methods

Product water from both wetlands (control and treatment cell) were analyzed following standard methods. Electrical conductivity (EC), pH and TDS were determined in situ using Hanna HI 83200 multi-parameter and the manufacturer's manual was followed with reference to standard methods for examination of water and wastewater (APHA, 2002). Prior to analysis for metal concentrations, water samples were filtered using a 0.22 um pore syringe filter membrane to remove particles followed by preservation with two drops of HNO_3 concentrated to prevent ageing and immediate precipitation of metals. The filtrates were refrigerated at 4°C prior to analysis by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) using an Agilent 5100 ICP-OES System (Agilent Technologies Inc., Santa Clara, CA, USA). Sulphate (SO_4^{2-}) was analysed using ion chromatography (IC). Soils samples were air dried for five days, sieved and one gram (1g) was digested in a mixture of 5 mL of 55% nitric acid (HNO_3) and 2 mL 30% Hydrogen peroxide (H_2O_2) using microwave digestion. Once digested samples were cooled to room temperature, the solutions were filtered through a 0.22um pore syringe filter membrane and analyzed for metals concentrations by Agilent 5100 (ICP-OES) with detection limit of 0.0001 mg/L and margins error of 10%.

6.4 Treatment efficiency of subsurface horizontal flow-constructed wetland (SSHF-CW*)*

6.4.1 Efficiency of SSHF-CW on pH increment

The pH increment (I) was determined as illustrated in the Equation 6.3 (Prihatini *et al.*, 2016).

$$I \ = pHf - pHi \qquad \text{(Eqn 6.3)}$$

Where, *pHf* is the final pH value after 30 days retention time, *pHi* is the pH value of feed AMD water and *I* is the increment of pH after 30 days retention time.

6.4.2 Efficiency of SSHF-CW in metals and sulphate removal

The treatment efficiency of SSHF-CW in metals and sulphate removal was determined gradually during the duration of the experiment. Metals and sulphate concentration were analysed gradually and the removal efficiency (RE) of each parameters of concern was determined using Equation 6.4 (Van Tran *et al.*, 2017).

$$RE = \frac{\mathrm{Ci} - \mathrm{Cf}}{\mathrm{Ci}} \times 100 \qquad \text{(Eqn 6.4)}$$

Where,

Ci is the initial concentration of each parameter in the AMD water,

Cf is the final concentration of each parameter after 30 days retention time,

RE is the removal efficiency of each parameter after 30 days retention time (in percentage).

6.5 Plants harvesting

Plants from each wetland (treatment cell and control cell) were harvested without damage to the roots and were rinsed separately with distilled water to remove dust, soil and mineral particles. Plants were air dried at 25°C for one week and weighed to determine the tolerance index using Equation 6.5. Tolerance index (TI) is a phytoremediation factor which allows evaluating plant sensitivity to pollutants (Kumar et al., 2008). TI is the ratio between a variable measured in treated plants and that in control plants.

$$Tolerance\ index\ (TI) = \frac{\text{biomass of plant growing in AMD cell}}{\text{Biomass of plants growing in potable fresh water cell}} \qquad \text{(Eqn 6.5)}$$

6.5.1 Digestion of plants and metals content analysis

Plants were separated into roots and shoots and each portion of plant was grinded using mortar and pestle. One gram of each portion was added to a mixture solution of 10 mL of 65% HNO_3 and 4 mL of 35% H_2O_2 followed by digestion using microwave digester. The digestate samples were then filtered using 0.22 μm pore syringe filter membrane followed by metals analysis using ICP-OES. The metals content in plants allowed the researchers to determine the bio-concentration factors (BCF) and the contribution of substrate, plants and external factors in metals removal. The BCF of each metal was calculated as illustrated in Equation 6.6 to determine the quantity of metals that moved from the wetland to the plant (Ding *et al.*, 2021).

$$\mathrm{BCF} = \frac{\text{Metal concentration in plant tissue}}{\text{Final concentration of metals in AMD water}} \qquad \text{(Eqn 6.6)}$$

6.5.2 Functional group and morphological properties of *Vetiveria zizanioides* roots

Vetiveria zizanioides roots were grinded and functional groups were determined using Perkin-Elmer Spectrum 100 Fourier Transform Infrared Spectrometer (FTIR) equipped with a Perkin-Elmer Precisely Universal Attenuated Total Reflectance (ATR) sampling accessory equipped with a diamond crystal. Morphological properties and spot analysis were determined using SEM-EDS. Grinded roots samples were mounted on carbon tape that was attached to Al ends. Samples were viewed in a JEOL 5400 LV SEM with an attached KEVEX electron detector (JEOL, Tokyo, Japan). They were viewed in low vacuum mode and the metals were detected with a Sigma EDS spectrometer.

6.5.3 Partitioning of chemical species between substrate, plant and external factors

Metals content in substrate were determined before and after the experiment. The results together with results of metals content in plant and product water allowed the researchers to estimate the content of metals retained in the wetland as illustrated in Equation 6.7, the substrate's contribution in overall metal removal as illustrated in Equation 6.8 and contribution of external factors in metal removal as shown in Equation 6.9.

$$Mw = X - Y \qquad \text{(Eqn 6.7)}$$

Where,

X is the initial concentration of metal in AMD water,

Y is the final metal content in AMD water, and

Mw is the metal content retained in the wetland.

$Ms = Mz - Mt$ (Eqn 6.8)

Where,

Mt is the initial content of metal in substrate, and

Mz is the final metal content in substrate.

ExF =Mw - (Ms +Mp) (Eqn 6.9)

Where,

(ExF) is external factors in overall metals removal,

Mw is metals concentration in retained in wetland, Ms is metals content in substrate and Mp is metals content in plant.

6.6 Results and discussion

The results for this study will be categorized into the aqueous samples and solid sample studies.

6.6.1 Characterization of aqueous samples

This section will highlight aqueous parameters that were assessed to determine the fate of chemical species post the interaction of SSHF-CW with *Vetiveria zizanoides* and contaminated water.

6.6.1.1 The effect of SSHF-CW in 30 days retention time on final pH

The effect of SSHF-CW on pH in 30 days retention time under 1.2 litres/day was evaluated and the results are shown in **Figure 6.2.**

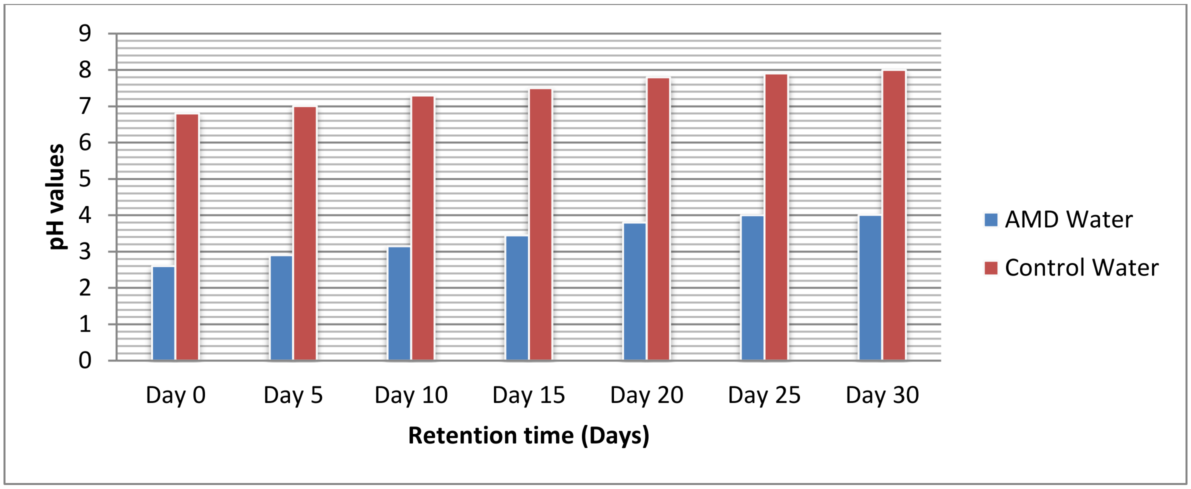


Figure 6.2: The effect of SSHF-CW on treated AMD pH in 30 days under a hydraulic loading rate of 1.2 litres/day

Figure 6.2 portrayed that the pH of the solution raised from 2.6 to 4.0 and 6.8 to 8.01 for the experiment and control, respectively. The increase in pH may be explained by the biochemical processes which occured in the substrate. In fact, for their growth, plants release acid to mineralise organic compounds and enhance nutrients absorption (Pavinato *et al*., 2008). but the acid reduces the hydrogen ion activity thereby leading to an increase in pH. In addition, some cations released from the substrate used as growing media also contribute to the reduction of ion hydrogen (H^+) activity (Fia *et al.*, 2010) and this may perhaps contribute to the increase of pH of product water after 30 days of retention time. Furthermore, the slight increase was observed for both cells (experiment and control) but at different increment values. An increase in pH may also be attributed to the SO_4^{2-} reduction in the AMD water since the contact between SO_4^{2-} and wetland substrate can promotes the growth of sulphate reducing bacteria (SRB) which led to an increase in the alkalinity of the medium thereby raising the pH of the water (Fia *et al.*, 2010). The findings are in line with previous studies conducted by (Lizama Allende *et al*., 2012) with different media and plants. In the wetland environment, plants play an important role in the fluctuation of pH. In fact during photosynthesis, aquatic plants absorb carbon dioxide (CO_2) from water and this can raise the pH of the water due to attenuation of carbonates (Long *et al.*, 2017).

6.6.1.2 The effect of SSHF-CW on Electrical conductivity (EC)

The effect of SSHF-CW on EC in 30 days retention time is reported in **Figure 6.3.**

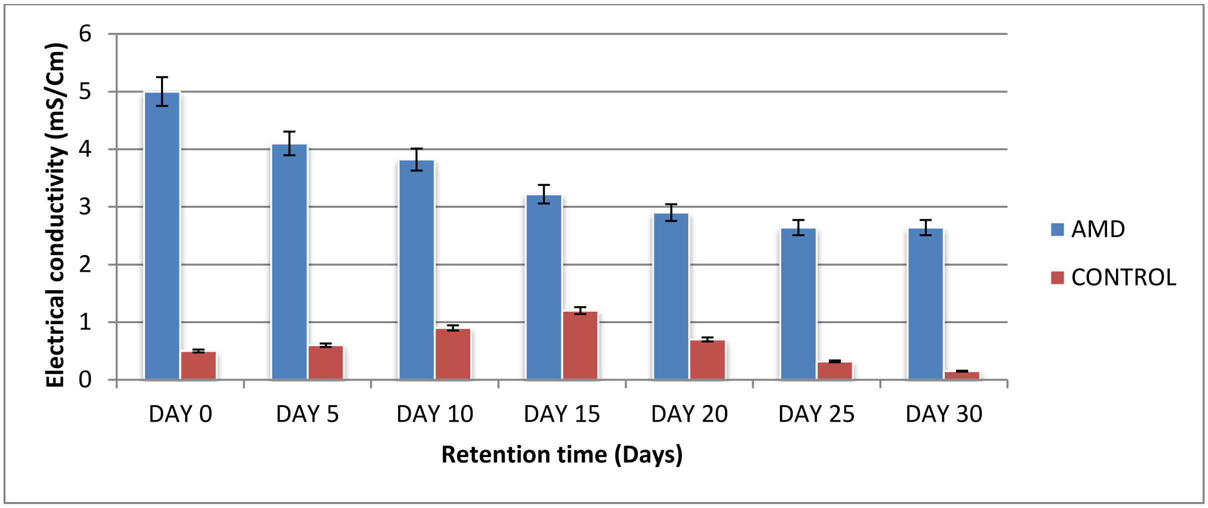


Figure 6.3: The effect of SSHF-CW on electrical conductivity in 30 days under a hydraulic loading rate of 1.2 litres/day

As shown in **Figure 6.3**, the electrical conductivity (EC) decreased from 5 mS/cm to 2.64 mS/cm within a period of 30 days whereas in the control wetland, EC remained constant (0.5 mS/cm) for the first five days, and slowly increased up to 1.5 mS/cm the 10th day and decreased from 1.5 mS/cm to 0.15 mS/cm on day 30. This reduction of EC in treatment wetland may be the consequence of chemicals species reduction from AMD leading to reduced TDS and EC due to less metals (Licciardello *et al.*, 2020). An increase in EC of the control wetland may be attributed to the presence of pollutants containing ions coming from the mixture of potable fresh water with substrate and the decrease from day 15 can be attributed to the sedimentation of pollutants in the substrate and accumulation by the plant.

6.6.1.3 The effect of SSHF-CW on Total dissolved solid (TDS)

The effect of SSHF-CW on total dissolved solid (TDS) reduction is shown in **Figure 6.4**.

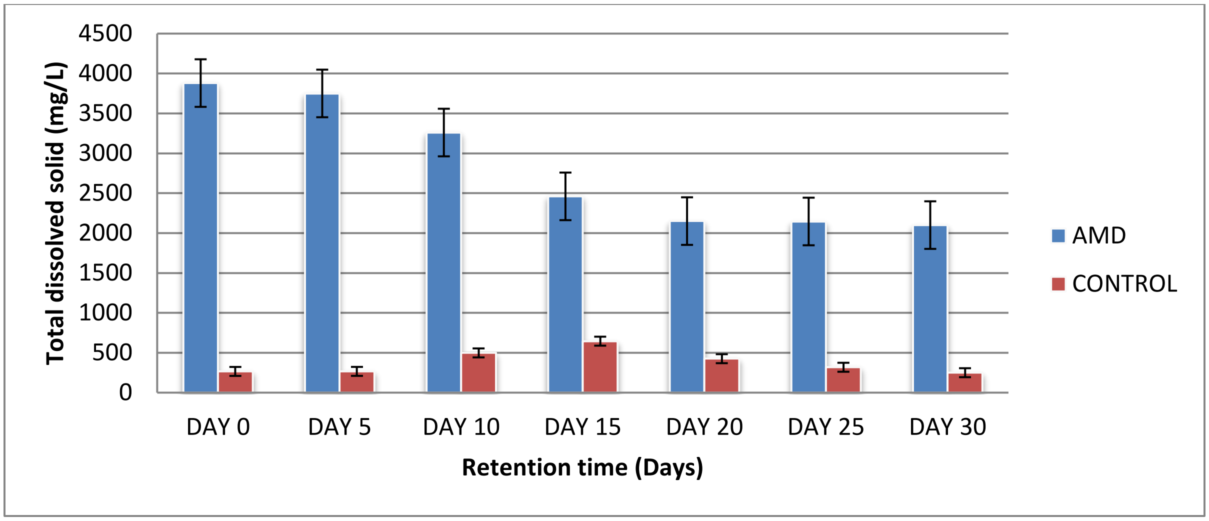


Figure 6.4: Variation in total dissolved solids with variation in the retention time (days) under hydraulic loading rate of 1.2 litres/day

The **Figure 6.4** clearly portrayed that after 30 days of the retention time, the TDS of AMD was reduced from 3880 mg/L on day 0 to 2160 mg/L on day 20 and remained almost constant. In the meantime, the TDS of fresh water (control cell) increased slowly from 266.5 mg/L to 845.08 mg/L up to day 10 and decrease to 250.02 mg/L on day 30 **(Figure 6.4).** A total dissolved solid is the concentration of all combined inorganic substance present in a liquid in dissolved form (Rusydi, 2018). TDS comprise inorganic salt such as calcium, magnesium, potassium, sodium and SO_4^{2-}. The reduction of TDS in AMD treatment cell may be the consequence of pollutants reduction in AMD water. The reduction of TDS may also be attributed to the process of sedimentation/precipitation occurring within the wetland's substrate. In fact, there is always sedimentation within the wetland substrate and this process allows some particles to settle down thereby improving the efficiency of the wetland. In some cases, pollutants adhere to the particles of organics matter in suspension and contribute to improve the efficiency of wetland (Du Toit and Campbell, 2002). The increase of TDS in the control cell may be the consequences of pollutants since the water pass through the substrate which initially contained a low concentration of salt and metals whereas the reduction may be the results of sedimentation process and pollutants uptake by plants.

6.6.1.4 Effect of SSHF-CW on sulphate concentration

The effect of SSHF-CW on the removal of SO_4^{2-} from AMD is reported in **Figure 6.5.**

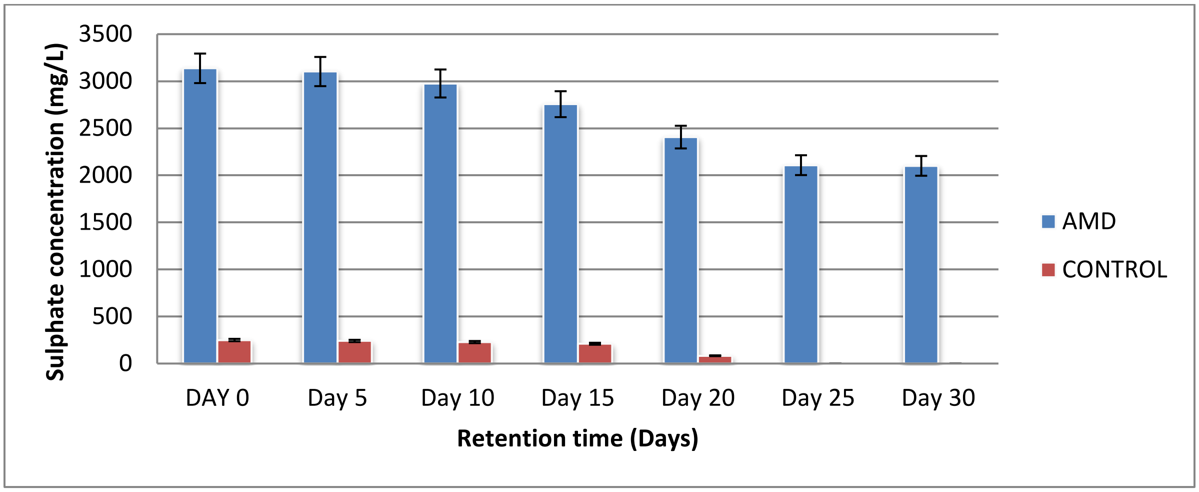


Figure 6.5: Variation in the concentration of sulphate with varying retention times (days) under a hydraulic loading rate of 1.2 litres/day

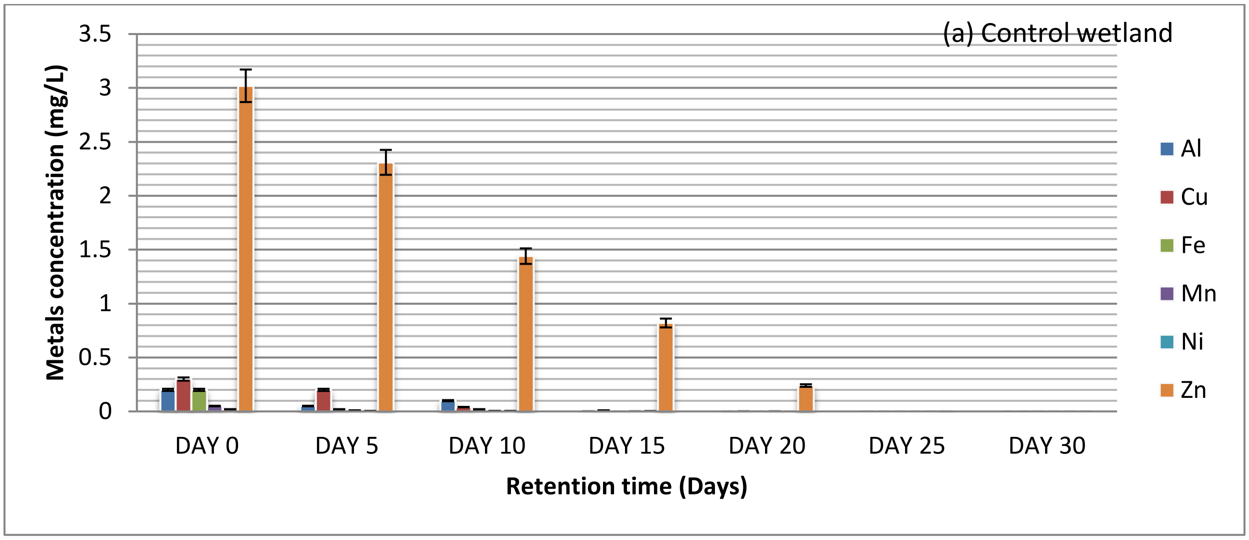
The finding revealed that SSHF-CW with Vetiver grass has the potential to reduce SO_4^{2-} concentration from 3137 mg/L to 2100.5 mg/L within 30 days HRT **(Figure 6.5).** The removal of SO_4^{2-} may be attributed to the following biochemical process. In acidic medium, SO_4^{2-} is reduced biologically as illustrated in the Equation 6.10.

$$SO_4^{2-} + CH_3COOH + 2H \rightarrow HS^- + 2HCO_3^- + 3H^+ \qquad \text{(Eqn 6.10)}$$

This equation clearly shows that electrons are transferred from the acetic acid (energy source) to the electron acceptor (SO_4^{2-}) to form bisulphide (HS^-). Depending on environmental condition; hydrogen sulphide can be released as a gas and can be ionised to HS^- and S^{2-} or precipitates as a polysulfide, elemental sulphur or metal sulphide (Bwire *et al*., 2011). This process is a key to metals removal in subsurface wetland since the study of Chen *et al.* (2016). revealed that Dissimilatory Sulfate Reduction (DSR) bacteria, which are only active in anaerobic conditions, are responsible for this process in subsurface constructed wetlands since they provide anoxic zones where DSR bacteria can be effective or in this study, SO_4^{2-} removal is correlated with the removal of metals such as zinc and this may explain why SO_4^{2-} concentration decreases simultaneously with metals concentrations.

6.6.1.5 Effect of retention time on the removal of metals from AMD

Variation in the contents of metals removed as a function of the retention time is reported in **Figure 6.6 (a, b).**



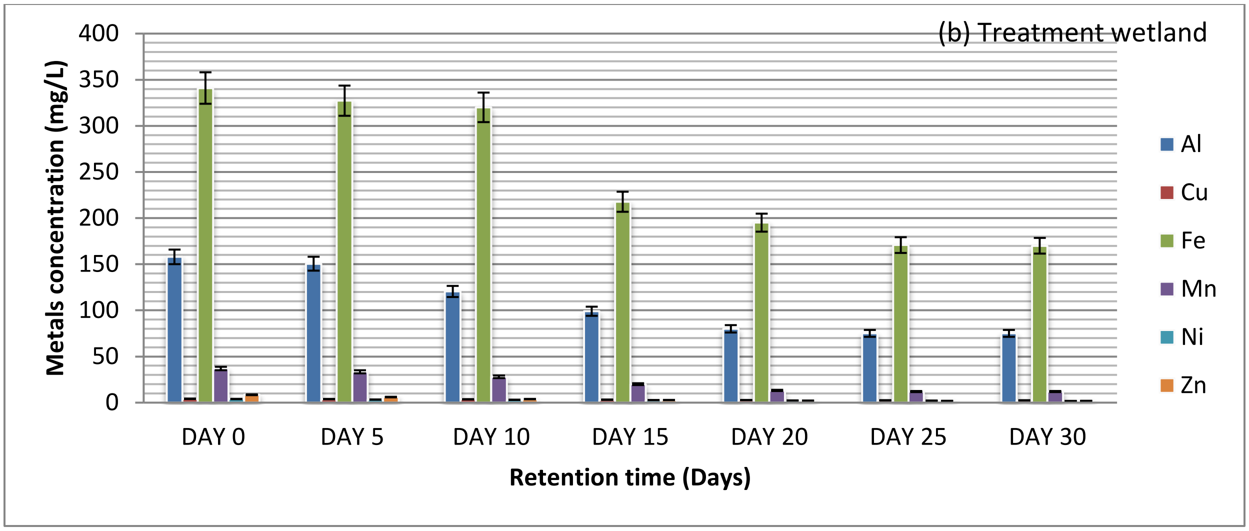
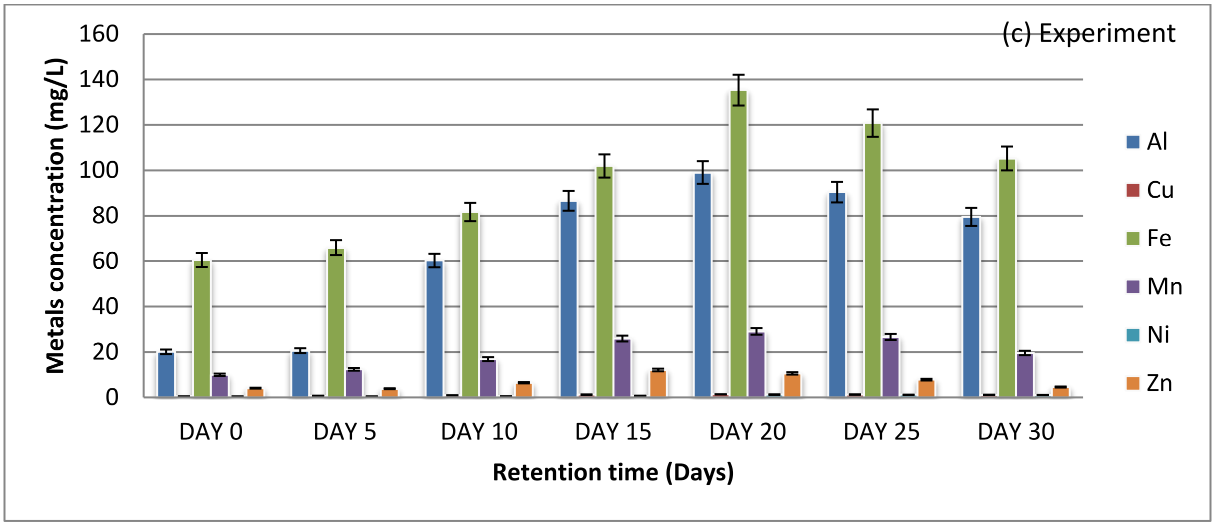


Figure 6.6 (a,b): Variation of metals concentration in control wetland (a) and treatment wetland (b) in 30 days retention time under a hydraulic loading rate of 1.2 litres/day

As shown in **Figure 6.6b**, the concentration of metal is reported in control (a) and experiment (b). For the experiment (**Figure 6.6b**), the concentration of metals was observed to decrease with an increase in retention time. Specifically, from the beginning to the end of experiment Fe decreased slowly from day 0 to day 10 and drastically from day 10 to day 25 and remained almost constant until day 30 while Al decreased slowly from day 0 to day 5 and drasticlly from day 5 to day 25 and remained constant until day 30. whereas others metals decreased slightly from day 0 to day 20 and remained constant until day 30 (**Figure 6.6b**). In the control wetland (**Fig 6.6a**), the concentration of metals decreased slowly and at the end, all metals were below detection limit The decreasing of metals in both wetlands is likely due to sedimentation and accumulation by plant. In control wetland, the decreasing of metals until below detection limit of 0.0001 mg.L can be explained by the low concentration of metal in fresh water used as control liquid, sedimentation and plant uptake. Metals removal in the SSHF-CW is influence by various biological processes such as sedimentation, filtration and plant uptake. In addition, metals are immobilized by the media and rhizomes through complexation or chelation followed by accumulation by plants (Lizama Allende *et al*., 2012; Muhammad *et al*., 2015b). Metals play a huge role in plant metabolism and homeostasis. For instance, Fe contributes to the synthesis of chlorophyll and it is essential for the maintenance of chloroplast structure (Rout and Sahoo, 2015) while Zn is one of the eight essential micronutrients and is needed by plants in small amount but crucial to plant development since it plays an important role in a wide range of processes such as growth hormone and defense mechanisms in plant (Cabot *et al*., 2019). Ni deficiency affects plants growth leading to the plants senescence, reduces nitrogen metabolism and Fe accumulation and plays a huge role in disease resistance (Bhalerao *et al*., 2015) while Cu is an essential element of many proteins and enzymes involved in photosynthesis and respiration process of plant (Printz *et al*., 2016).

6.6.1.6 Variation of metals in the substrate

Metals concentration in substrate was evaluated and the results are reported in **Figure 6.7 (c, d).**



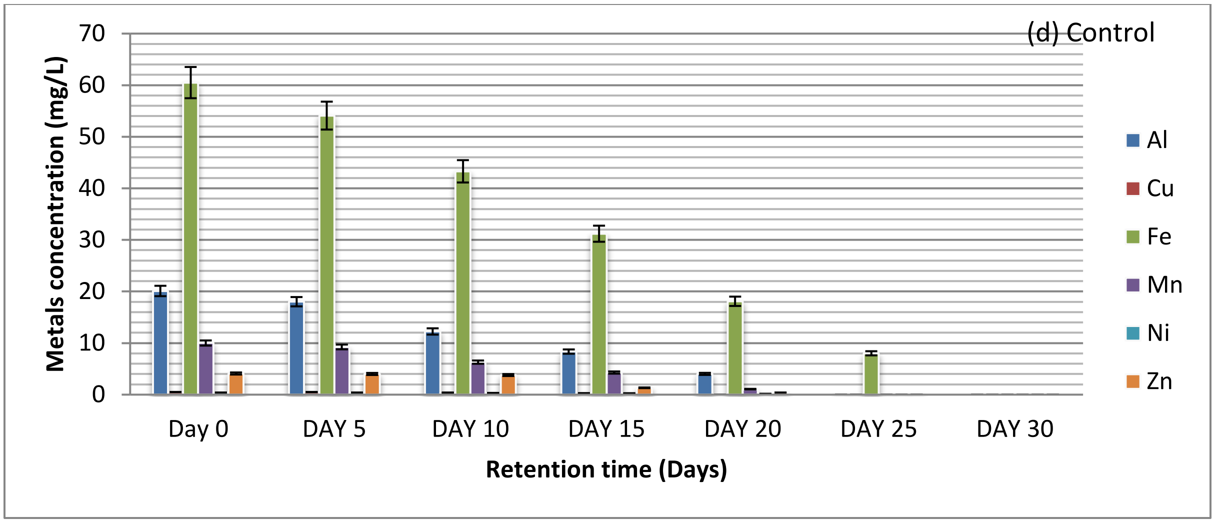


Figure 6.7 (c,d): Variation of metals concentration in substrate (c) experiment and (d) control in 30 days experiment and under a hydraulic loading rate of 1.2 litres/day

As shown in **Figure 6.7c**, the concentration of metals were observed to increase with the hydraulic retention time, from Day 0 to day 10, followed by a decreasing plateau. The increasing phase can be attributed to the continuous load of metals in the wetland since it was a continuous flow experiment. The major part of metals concentration was retained in the substrate thereby confirming results reported in the finding of Chen *et al.* (2009) and Le et al. (2020). which revealed that substrate in SSHF-CW plays an important role in metals removal by retaining them through sedimentation process thereby enhancing their accumulation by plants. The period of increasing in metal concentration was just followed by the decreasing phase likely due to metals accumulation by the plants **(Figure 6.7c).** In the meantime, metals concentration in substrate from control wetland constantly decreased from the beginning to below detection limit at the end of retention time **(Figure 6.7d)** and this may be the consequence of metals accumulation by plant. In addition, to provide the growth medium for plants and microorganisms, offers hydraulic conditions for water flow, substrate in wetland also remove pollutants directly by interception, sedimentation, adsorption, and precipitation (Saraiva *et al.*, 2018).

6.6.2 Efficiency of subsurface horizontal flow constructed wetland (SSHF-CW)

6.6.2.1 The efficiency of SSHF-CW in pH increment

The efficiency of SSHF-CW in increasing the pH of AMD water was determined and the result is reported in **Table 6.1**.

Table 6.1: Performance of the SSHF-CW in raising the pH of AMD

Wetland	pH		
	Initial pH of influent	Final pH of effluent	Increment
Treatment wetland	2.6	4.00	1.4
Control wetland	6.8	8.01	1.2

From **Table 6.1**, it follows that after 30 days HRT and under a hydraulic loading rate of 1.2 litres/day the SSHF-CW was able to increase the pH as much as 1.4 units while in the control cell, the pH was raised as much as 1.2 units. However the final pH value of AMD water was out of range of water quality guidelines standard for effluent discharge as set by the department of environmental affairs (DEA) and department of water and sanitation (DWS) for effluent discharge.

6.6.2.2 Efficiency of SSHF-CW on the removal of metals and sulphate

The removal efficiency of metals and sulphate by SSHF-CW is reported in **Figure 6.8**.

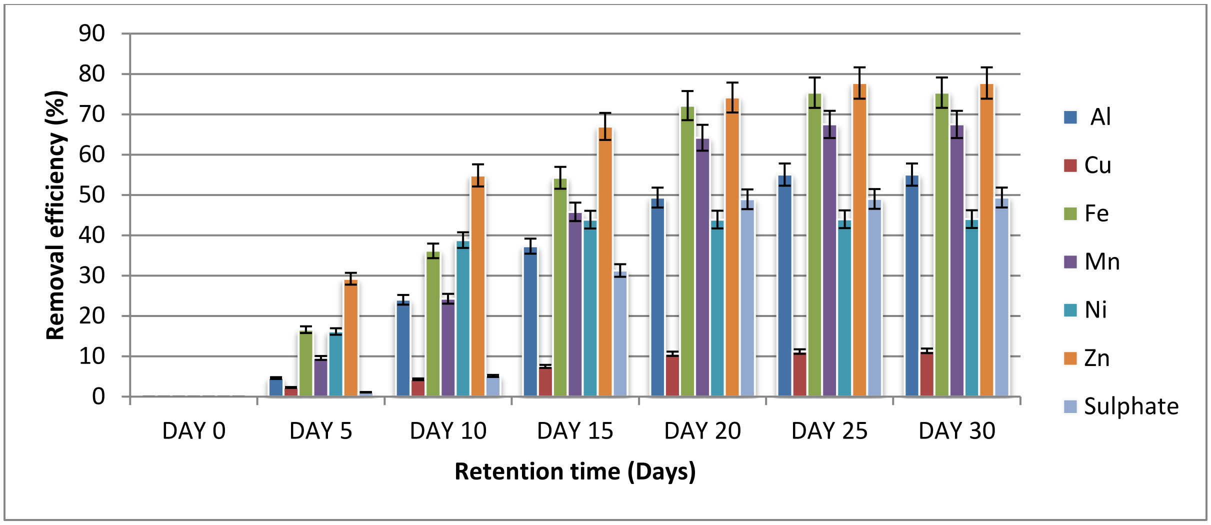


Figure 6.8: Removal efficiency of metals and sulphate by SSHF-CW in 30 days HRT under a hydraulic loading rate of 1.2 litres/day

As shown in **Figure 6.8**, the removal of chemical species follows the following order: Zn (77.75%), > Fe (75.36%), > Mn (67.48%), > Al (55.05%),> $SO4^{2-}$ (49.36%), > Ni (44.01%), > Cu (11.36%). The higher removal efficiency of Zn, Fe, Mn and Al was expected. Other studies by Vymazal. (2005) and Bakhshoodeh *et al.* (2016) have shown that the removal efficiency of Zn is usually high in SSHF-CW due to the ability of wetland substrate to retain huge quantity of Zn in response to chemical precipitate formation and cation exchange with water (Bakhshoodeh *et al.*, 2016). The removal efficiency of Fe (75.36%) in SSHF-CW is low compared to FWS-CW where it is usually very high (above 90%). The Fe removal efficiency can be attributed to the anaerobic status of the filtration substrate resulting in bacterial activities which promotes the reduction of Fe^{3+} to Fe^{2+} as illustrated in the Equation 6.11 thereby enhancing their mobility in the wetland since ferric ion (Fe^{3+}) is less mobile than ferrous ion (Fe^{2+}).

$$\mathrm{Fe^{3+}} + \mathrm{e^-} \rightarrow \mathrm{Fe^{2+}} \qquad \text{(Eqn 6.11)}$$

The 75.36% removal efficiency of Fe may be attributed to his importance in plant metabolism since Fe deficiency in plant leads to common nutritional disorder and it is an essential micronutrient for almost all living organisms and plays a crucial role in metabolic process such DNA synthesis, respiration and photosynthesis (López-Millán et al., 2013). Like Fe, the removal efficiency of Mn is low in SSHF-CW compared to free water system and this may be attributed to the anaerobic status of SSHF-CW which retain Mn in less soluble form (Mn^{4+}) thereby reducing his moblity in the system. The low Ni removal efficiency (44.01%) may be the result of Ni co-precipitation with Mn and Fe-oxyhydroxides since the co-precipitation of Ni with others Metals delays the oxydation of Ni therby reducing his mobility in the solution (Tawde and Bhalerao. 2017; Sánchez-España and Yusta, 2019). Many process such as sedimentation, complexation and plants uptake participates in metals removal in wetland. The sedimentation process allows metals to settle down however, their accumulation by plants is selective since plants uptake metals according to the need for their metabolism and this jusfifies the different paterns observed in metals removal efficiency. SO_4^{2-} removal may be attributed to plant need for his metabolism and the reduction of metals since metals reduction leads to reduction of sulphate salt such as $FeSO_4$, $ZnSO_4$, $CuSO_4$ (Larsson *et al.*, 2018).

6.6.3 Tolerance index and bio-concentration factor

6.6.3.1 Tolerance index

Tolerance index was determined as described in section 6.5 and the result was TI = 1.23. For this experiment, TI values is 1.23 (> 1) which according to Kumar *et al.* (2008) translates a net increase in biomass and revealing that *Vetiveria zizanioides* has developed tolerance and this may be justified by the fact that *Vetiveria zizanioides* grown in AMD remained green and healthy through-out the experiment. The resistivity and tolerance of *Vetiveria zizanioides* to harsh conditions may be attributed to his genetic characteristics. In fact, Vetiver has a straight and stiff stem which allows it to withstand high hydraulic loading rate of water (Suelee *et al.*, 2017). In addition, Vetiver grass up to 2m high can survive in relatively deep-water flow and as result, it can increase the retention time in a wetland thereby improving the performance of the wetland (Kafil *et al.*, 2019).

6.6.3.2 Bio-concentration factor

In wetland, the BCF is the ratio between concentrations of a chemical compound in plant to the chemical's aqueous solution concentration (Bonanno *et al*., 2018). Bio-concentration factor greater than 1 indicates the potential of the plant to uptake pollutants. In contrary, BCF lower than 1 (BCF < 1) indicates that the inability of plants to accumulate pollutants. The BCF was calculated for all metals in treatment wetland and only for Cu in control wetland since the final concentration of other metals were below detection limit and the results are shown in **Figure 6.9**.

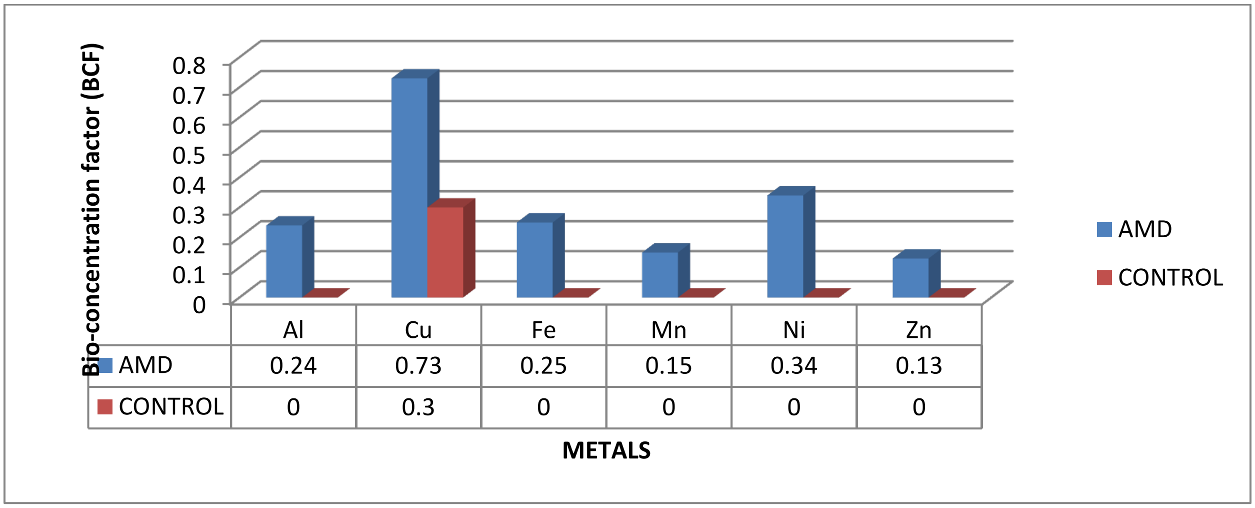


Figure 6.9: Bio-concentration factor of metals

As shown in **Figure 6.9,** the bio-concentration factor for different elements (metals) varied significantly. Figure 6.9 depicted that in the treatment wetland, the BCF of all metals were lower than 1 (BCF < 1) and ranged in the following order: Cu (0.73) >Ni (0.34) >Fe (0.25) > Al (0.24) > Mn(0.15) > Zn (0.13). In this experiment, none of the metals was sufficiently accumulated by *Vetiveria zizanioides* during the 30 days experiment and this merely mean that after 30 days, metals were more retained by the wetland substrate and it may require more time for *Vetiveria zizanioides* to accumulate sufficient quantity of metals to be qualified as hyper-accumulator. However, in the control wetland, the BCF of Cu was 0.3 whereas the final concentrations of most metals (Al, Cu, Fe, Mn, and Zn) were below the detection limit hence the BCF of the aforementioned metals could not be determined. The finding clearly revealed that *Vetiveria zizanioides* used in SSHF-CW may require more time (more than 30 days) to accumulate sufficient quantity of metals. The variation in BCF values of metals may be explained by the importance of the given metal in plant metabolism. For instance, metals such as Cu, Fe, Zn and Mn act as important co-factors for many enzymes and are all-important for both mitochondrial and chloroplast functions (Millaleo *et al.*, 2010; Zawierucha *et al.*, 2020). The BCF values may also be the results to the concentration of metals in the substrate since the BCF of Cu is higher in the treatment than in the control cell. The determination of metal content in plant and substrate allowed the researcher to calculate the quantity of metals retained by the wetland substrate.

6.6.4 Contribution of substrate, plant and others factors in overall metals removal

The contribution of substrate, Vetiver and other factors contribution in metals removal were determine and the results are presented in the Table 6.2.

Table 6.2: Heavy metals removal by the wetland based on substrate, plant and external factors contributions to removal process

Treatment(%)	Al removal	Cu removal	Fe removal	Mn removal	Ni removal	Zn removal
Substrate	93.2	70.7	60	73.31	53.08	69.77
Plant	4.1	20.46	35.17	18.22	29.85	20.75
External factor	2.7	8.77	5.02	8.45	17.06	9.48

As shown in **Table 6.2**, the contribution of *Vetiveria Zizanioides* in metals (Al, Cu Fe, Mn, Ni and Zn) removal is a minor fraction to overall removal with *Vetiveria Zizanioides* contributing to 4.1%, 20.46%, 35.17%, 18.22%, 29.85% and 20.75% removal of Al, Cu, Fe, Mn, Ni, and Zn, respectively while the wetland substrate contributed to 93.2%, 70.7%, 60%, 73.31%, 53.08 and 69.77% for Al, Cu, Fe, Mn, Ni, and Zn, respectively to the overall metal removal. In the wetland system, metals are firstly retained in the sediment (Liang *et al.*, 2017). However, biochemical process occuring in the wetland system, sulfides and others minerals in the sediment may also contribute to the removal of metals in constructed wetland (Bakhshoodeh *et al.*, 2016). Despite the minor contribution of plant (*Vetiveria Zizanioides*) to the overall metals removal, it plays a crucial role in the treatment of wastewater using constructed wetland. For instance, it promotes the settling of suspended solids (Shelef *et al.*, 2013). Like others plant used in constructed wetland, the Vetiver's rhizosphere provides the substrate and supporting media the suitable condition for in the growth of microorganisms, which play an important role in overall metals removal in constructed wetland (Stottmeister *et al.*, 2003; Kumar and Dutta, 2019; Shahid *et al.*, 2020). In addition, oxygen transported by vetiver from the aerial parts to the roots creates a suitable condition in the rhizosphere for the proliferation of bacteria and for the promotion of various chemicals and biochemical reactions which accelerate metals sedimentation in the substrate and further accumulation by plant (Tawde and Bhalerao., 2017; Kafil *et al.*, 2019).

6.7 Characterization of the solids samples

6.7.1 X-ray fluorescence analysis

The elemental composition of raw and reacted soil samples are shown in **Table 6.3.**

Table 6.3: Elemental composition of raw and reacted soil samples

Sample (wt%)	Initial soil	Control soil	AMD reacted soil
Fe	83.6917	82.9824	89.0974
Rb	5.3379	5.0927	5.0196
Sr	3.3187	4.2368	3.0746
Ti	4.3548	3.6349	3.4322
Mn	1.9175	2.6888	2.6185
Zn	0.9745	0.9276	0.6774
Zr	-----------	0.2212	1.1057
Cl	-----------	0.2156	---------
I	-----------	----------	2.3647
Cr	0.3846	----------	0.3182
Ca	-----------	----------	0.4676

A shown in **Table 6.3**, after the reaction of the substrate with AMD, the following elements, Zr, I, Cr and Ca were found to be present. Thenceforth, the levels of Fe and Mn were observed to increase indicating the formation of new mineral phase whereas Ti, Zn, Rb and Cr decreased indicating possible dissolution into aqueous solution.

6.7.2 X-ray diffraction analysis

The X-ray diffraction analysis was done to determine the mineralogical composition of soil from experimental and control wetland and the results are reported in **Figure 6. 10**.

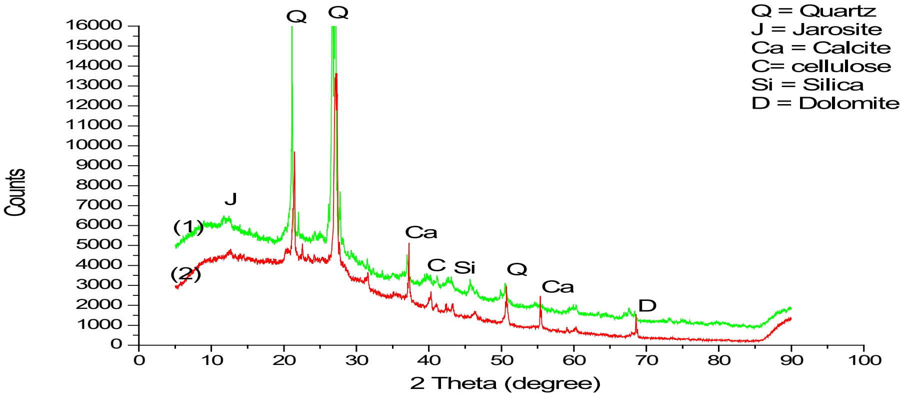


Figure 6.10 (1-2): XRD patterns of substrate: experiment (1) and control (2)

Figure 6. 10 depicted that X-ray diffraction patterns of substrate from experimental wetland (1) and control wetland (2) showed various peaks spread over 2Ө between 12° and 68° but at different intensity. The peak at 2Ө = 21°, 27.5° and 51° correspond to quartz (SiO_2) which is the major mineral composition of compost soil (Biyada *et al.*, 2020). The peak at 2Ө = 37°, 51° correspond to calcite which supply calcium for plant nutrition (Sharma *et al.*, 2019). Peak at 2Ө = 40° corresponds to cellulose which can be originated from plant cell wall (Biyada *et al.*, 2020). The peak at 2Ө = 46° may correspond to silica present in compost soil. Silica affects the binding of nutrients element to soil particle (Grigatti *et al.*, 2017). The peak at 20 = 68° may correspond to Dolomite which is one of fertilizer found in compost soil (Shaaban *et al.*, 2015).

6.7.3 Fourier transforms infrared spectroscopy analysis

The metals functional groups of raw and reacted roots are presented in the **Figure 6. 11.**

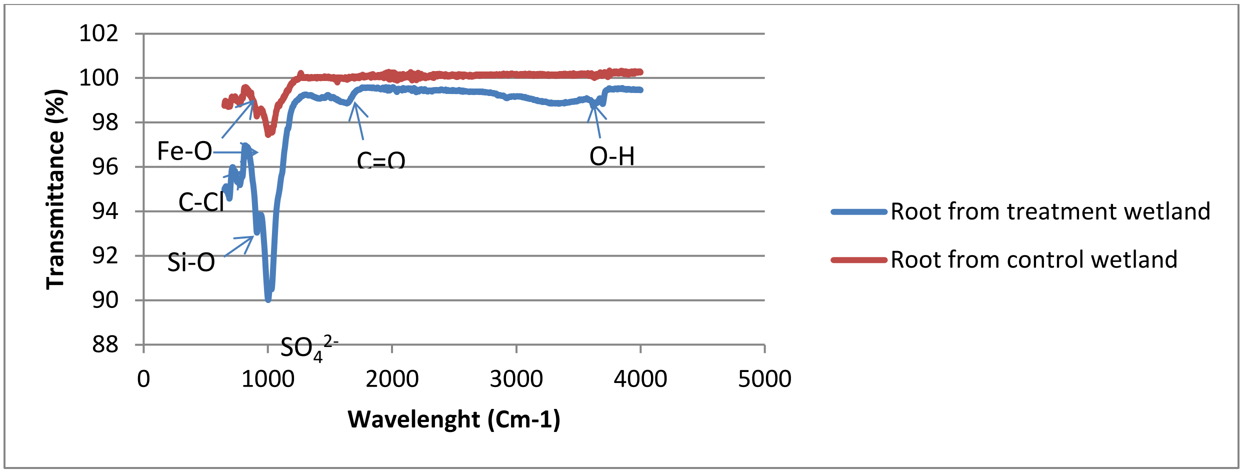


Figure 6.11: The metals functional groups of raw and reacted roots

Table 6.4: Metals functional group and their references

Wave length	Functional group	References
805.5	C-Cl	(Nayak *et al.*, 2018)
883.5	Fe-O	(Tabelin *et al.*, 2017)
966.5	Si-O	(Yu *et al.*, 2017)
1034.5	SO_4	(Tabelin *et al.*, 2018)
1711	C=O	(Kannappan *et al.*, 2017)
3707	O-H	(Yu *et al.*, 2017)

As shown in **Figure 6.11,** the spectrum of both roots begins by a serie of stretching vibration with a peak at 805.5 cm^{-1} for both roots followed by a strong vibration between 920 and 958.5 cm-1 for control root and between 966.5 and 1034.5 cm^{-1} for root from treatment. After the vibration, the spectrum of root from control wetland shows a straight band from 1264.5 to 3974 cm^{-1} while the spectrum of root from treatment wetland shows a stretching vibration with band at 1648 cm^{-1} and a doublet with band at 3617.5 and 3707 cm^{-1}. The vibration observed from both root may be attributed to stress factor while the stretching vibration observed in root from treatment wetland may be the results of pollutants accumulation.

6.7.4 Scanning electron microscope-electron dispersion spectrometry analysis

To understand the mode of accumulation of metals by vetiver roots *Vetiveria zizanioides* and the formation of mineral phases, scanning electron microscope (SEM) and Energy dispersive X-ray spectroscopy (EDS) were performed for roots from both wetland (treatment and control wetland) and the results are shown in the (**Figure 6. 13 and Figure 6.14).**

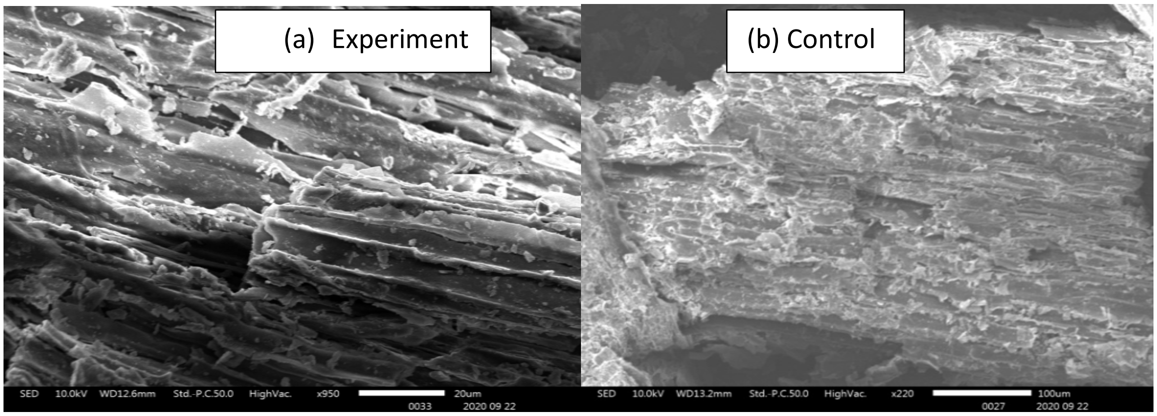
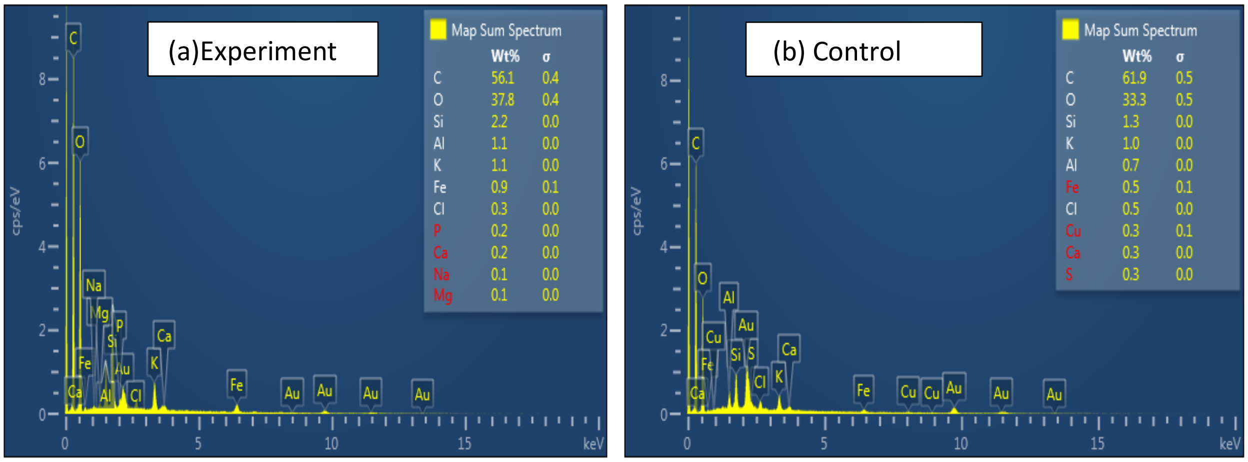


Figure 6.13 (a,b): SEM image of root from treatment wetland (a) and from control wetland (b)

As shown in **Figure 6.13**, the results reveal noticeable difference in image with root from treatment wetland showing an aggregate of pallettes while SEM image of root from control wetland shows a sort of large cubic mass. The aggregate of pallettes observed in the SEM image of root from treatment wetland may be attributed to metals and others pollutants accumulated by plant thereby leading to elevated percentage of pollutants such as Al, Fe, K and Si as shown in the electron dispersive spectroscopy (EDS) analysis of roots from treatment wetland **(Figure 6.14b).** The presence of Ca, C, Mg and O in both EDS may be attributed to metals such as manganese, iron, and carbonate whereas the presence of silicon in roots is from both wetland **Figure 6.14 (a and b**) may be attributed to silicon in biological plant material thereby confirming the finding of (Nylese *et al*., 2015) which revealed high percentage of silicon in plant material after EDS analysis.



Map Sum Spectrum

	Wt%	σ
C	56.1	0.4
O	37.8	0.4
Si	2.2	0.0
Al	1.1	0.0
K	1.1	0.0
Fe	0.9	0.1
Cl	0.3	0.0
P	0.2	0.0
Ca	0.2	0.0
Na	0.1	0.0
Mg	0.1	0.0

Map Sum Spectrum

	Wt%	σ
C	61.9	0.5
O	33.3	0.5
Si	1.3	0.0
K	1.0	0.0
Al	0.7	0.0
Fe	0.5	0.1
Cl	0.5	0.0
Cu	0.3	0.1
Ca	0.3	0.0
S	0.3	0.0

Figure 6.14 (a,b): Elemental composition of roots from experimental wetland (a) and control wetland (b)

6.8 Chemical species of untreated and AMD- treated with SSHF-CW

The results of chemical species of treated AMD were used to compare Department of Water Affairs and Sanitation (DWS) and Department of Environmental Affairs (DEA) guidelines standards for effluent discharge and the results presented in **Table 6.5.**

Table 6.5: Concentrations of chemical species for untreated and AMD treated with SSHF-CW (all units in mg/L except pH and EC)

Parameters	Feed AMD	DWS/DEA guidelines for effluent discharge	AMD treated water	Percentage of pollutants removed
pH	2.6	6-10	4.01	1.41 (I)
TDS (mg/l)	3380	2400	2100	37.86
EC (μS/cm)	5000	1500	2640	47.2
Al (mg/L)	158	20	71.02	55.05
Fe (mg/L)	341	50	84.02	75.36
Mn (mg/L)	37	20	12.03	67.48
Cu (mg/L)	4.2	20	3.72	11.36
Zn (mg/L)	8.55	20	1.90	77.75
Ni (mg/L)	3.92	10	2.19	44.01
Sulphate (mg/l)	3137	2400	1588.57	49.36

As shown in **Table 6.5**, the pH is very acidic (2.6) with notable levels of TDS and EC. This may be the results of high level of broken down of chemical species. After the treatment of AMD, the pH increases slightly from 2.6 to 4.01 whereas EC and TDS notably decreased. The concentration of metals (Al, Cu, Fe, Mn, Ni and Zn) and sulphates were observed to have decreased significantly after the interaction with SSHF-CW and this is attributed to the settling of metals or deposited as sediment followed by accumulation by *Vetiveria Zizanioides*. However, the DWS water quality guidelines has not been met except for Ni and therefore, there is need to associate or integrate subsurface horizontal flow constructed wetland with another type of water treatment technique to enhance the performance of the system.

6.9 Conclusion

This eco-friendly and passive study successfully demonstrated that SSHF-CW improved the quality of AMD water with *vetiveria zizanioides* tolerating acidic environment showing no sign of de-generating with tolerance index above 1. The results showed a slight increase in pH from 2.4 to 3.8 and a net reduction in electrical conductivity (EC), total dissolved solids (TDS), and sulphate at 47.20%, 46%, and 33.04%, respectively. Thenceforth, there was a net removal of metals with Zn registering the highest removal percentage of 77.75%, followed by Fe (75.36%), Mn (67.48%), Al (55.05%), Ni (44.01%), and Cu (11.36%). Selected pollutants were significantly romoved from AMD water after 30 days retention time however they failed to meet the DEA and DWS limits for effluent discharge. Furthermore, the metals removal was partitioned between substrate, plants accumulation and external factors with substrate contributing more for metals removal except with Cu and Ni where the contribution percentage of substrate was 10.05 and 30.59 respectively. Metals accumulation by plant accounted only for a small percentage of overall metal removal. In light of the highlighted findings, this study would need an integration with another effective technology to enhance its performance and ensure that the product water comply with the stipulated specifications, guidelines and standards. This will play an enormous role in curtailing the impacts of AMD on the environment and other ecological compartments.

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CHAPTER SEVEN

This chapter investigated the potential of a hybrid approach towards the treatment of acid mine drainage using the integration of neutralisation and phytoremediation

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CHAPTER SEVEN

The treatment of acid mine drainage using a combination of MgO-NPs and a series of constructed wetland planted with *Vetiveria zizanioides:* A hybrid and stepwise approach

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Abstract

In this study, a cost-effective, efficient, and low-maintenance hybrid system for the treatment of acid mine drainage (AMD) was duly developed. The system comprised a nano-and-biotic system integrated together in a step-wise modular. Specifically, the treatment chains were made up of different stages of which, stage 1 focused on activated magnesite for the neutralisation of AMD and stage 2 focused on the polishing of product water using staged hybrid constructed wetland equipped with *Veteveria zizanioides* grass as phytoremediation plant. In stage 1, the feed AMD was treated with magnesite at a ratio of 1:100 (1g/100 ml), at 500 rpm, and 1 hr of hydraulic retention time (HRT) whilst in stage 2, the final water was explicitly fed into the phytoremediation system for polishing using constructed wetlands (CWs) equipped with *Vetiveria zizanioides* in subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow

(SSHF-CW) mode. In this stage, the product samples were characterised every 24 h per 5 d interval for a period of 30 days. The feed and product sludge and plants were characterised using the state-of-art analytical techniques. Furthermore, the PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model was used to determine the speciation and fate of inorganic contaminants. Overall, the removal efficiency of metals registered the following sequence, Fe (99.8%) ≥ Al (99.5%) ≥ Mn (99.24%) ≥ Zn (98.36%) ≥ Cu (97.38%) ≥ Ni (97.7%). The electrical conductivity (EC) recorded ≥ 86%. The pH was observed to increase from 2.9 to 8.8 and 10.2 for neutralisation and phytoremediation, respectively. A system denoted significant removal of metals during the neutralisation stage while the bioremediation step served as the polishing stage, specifically for residual sulphate. Thenceforth, the substrate, grass and external factors played a huge role in residual metals removal. The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirm that metals existed as di-and-trivalent complexes in solution. Lastly, available metals were precipitated as metals hydroxides and oxy-hydrosulphates. The product water conformed to prescribed standards, specifications, and guidelines. In light of the findings, this study proved that a synergy of neutralisation and bioremediation could potentially yield the desired results in mine water management and this could be deployed to mines in a quest to curtail ecological impacts of AMD.

Keywords: Acid mine drainage; treatment; hybrid technology; activated magnesite; staged constructed wetland; *Vetiveria zizanioides*.

7.1 Introduction

Water is natural resource that plays a fundamental role in the development of earthly organisms (Masindi *et al*., 2018a). However, the inter-play between clean water and anthropogenic activities has raised numerous concerns due to the alarming rate of pollution introduced to clean water bodies thus rendering them unusable (Mavhungu *et* *al.*, 2019). Amongst the leading cause of water pollution, acid mine drainage (AMD) which originates from the mining of coal and gold has been a pollutant of grave concern (Sebogodi *et al.*, 2020). As such, national, regional, and international research communities have taken a firm stance in finding short-and-long term solutions to address the threat posed by gigantic volumes of AMD from active and derelict mines since AMD can compromised the water sustainability in countries with well-developed mining industry (Naidu *et al.*, 2019; Rambabu *et al.*, 2020). Thenceforth, the perilous and cumulative effect of AMD ultimately diminishes or destroys the ability of the ecosystem to foster life (DeNicola and Stapleton, 2002; He *et al.*, 2015). Specifically, AMD is generated from the oxidation of sulphide bearing minerals in different lithology and geological strata (Akinwekomi *et al.*, 2017; Park *et al.*, 2019). According to literature, AMD comprises acidic pH (≤ 2 5), SO_4^{2-}, and metals such as Al, Fe, Mn. Zn, Ni, Cu, As, Cr, and Pb, including radionuclides and rare earth metals (Nordstrom *et al.*, 2015). In light of the physicochemical properties, chemicals therein can pose severe toxicological effects to living organisms on exposure (Sarmiento *et al.*, 2011). Stringent regulatory frameworks require AMD to be treated prior to release into the receiving environment although numerous companies fail to comply with such regulation possibly due to various reasons (USEPA, 2017). As such, companies require proper management practices to reclaim, rehabilitate, and restore the ecosystem hence minimizing the effects of AMD on the environment (Naidu *et al.*, 2019; Park *et al.*, 2019; Spellman *et al.*, 2020). Acid mine drainage is mostly treated using active or passive method despite the high cost of active treatment systems and labour intensive compared to passive treatment approaches that largely rely on natural ecological process to treat AMD (Skousen *et al.*, 2017). In-turn, the challenges of passive treatment systems have been observed for heavily polluted streams hence calling for advanced techniques and approaches to acquire synergistic effect towards AMD treatment (Simate and Ndlovu, 2014; Masindi and Tekere, 2020), and these will include hybrid or integrated approaches. The cost to rehabilitate the impacts of AMD due to active and abandoned mines is posing huge economic challenges (Kefeni e*t al.*, 2017; Naidu *et al.*, 2019). These exorbitant costs could hamper the much needed treatment of AMD where it is required (Liu and Lal, 2012). The development and implementation of low-cost and low-maintenance treatment approaches are therefore vital for states and government where AMD pollution is prevalent (Acharya and Kharel, 2020; Vaziri *et al*., 2020). It is for this reason why various active and passive treatment technologies are used for the management of AMD. Specifically, active treatment processes require the use of neutralisation agents (Akinwekomi *et al*., 2017), adsorbents (Mohan and Chander, 2006), ion exchange resins (Nleya *et al*., 2016; Felipe *et al*., 2020), membrane technologies (Agboola, 2019; Naidu *et al.*, 2019) and desalination (Demetriou *et al*., 2010)**.** The challenge with active technologies is the use of virgin chemicals, generation of heterogeneous and highly mineralised sludge, production of brine and regenerates wastewater and high energy inputs (Masindi and Tekere, 2020). On the other hand, passive treatment technologies include constructed wetlands (Wieder, 1993; Allende *et al*., 2014; Luo *et al.*, 2020), biological barriers (Moodley *et al*., 2018; Park *et al*., 2019), permeable reactive barriers (Shabalala *et al*., 2014; Naidu *et al*., 2019), aerobic wetlands (Johnson and Hallberg, 2005), anaerobic wetlands (Skousen *et al*., 2019), and lime drains (Skousen *et al.,* 2017)**.**

Nonetheless, biological approaches such as constructed wetlands (CWs), have gained significant momentum and interest to cost-effectively treat medium to low flow AMD effluents (Rambabu *et al*., 2020). Passive treatment systems have challenges of poor performance in concentrated solutions, clogging of the system and the need for maintenance (Simate and Ndlovu, 2014). Even though the use of active and passive technology have gained much attraction (Masindi and Tekere, 2020), their use as hybrid system is limited in literature. However, neutralisation and phytoremediation had been used for the treatment of AMD. For instance, Gwenzi *et al*. (2017) studied the use of NaOH-activated coal ash and phytoremediation with *Vetiveria zizanioides* for the treatment of AMD while RoyChowdhury *et al*. (2019) assessed a combined chemical (Aluminium based and calcium based WTR) and phytoremediation with *Vetiveria zizanioides* for the treatment of AMD. To the best of the authors' knowledge, the combination of activated magnesite and a series of CWs planted with *Vetiveria zizanioides* has never been used for the treatment of AMD. This will then be the first study in design and execution to explore the use of neutralisation with activated magnesite and phytoremediation using a series of CWs planted with *Vetiveria zizanioides* for the treatment of AMD. Other researchers have explored the use of integrated systems for the treatment of AMD under the circular economy concept whereby AMD is valorised and beneficiated for the synthesis of valuable minerals and the reclamation of drinking water (Masindi *et al*., 2019; Masindi and Tekere, 2020). The intervention proposed is a cost-effective, efficient, and low-maintenance AMD remediation technology which will make use of a hybrid passive approach consisting of nano-biotic system to treat AMD.

The proposed approaches, namely magnesite (Masindi *et al*., 2018b) and the *Vetiveria zizanioides* (Kiiskila *et al*., 2019; Nguegang *et al*., 2021) have been tested separately using AMD effluents with promising results. *Vetiveria zizanioides* has the ability to adapt to a wide range of ecological conditions, they are often observed to thrive in AMD environments and have been shown to effectively remove metals under different pH conditions (Kiiskila *et al.*, 2019, 2020)**.** Findings from our previous studies have demonstrated that *Vetiveria zizanioides* can however be susceptible and not fulfil this function optimally when exposed to high concentrations of toxic metals in acidic waters (Kiiskila *et al*., 2019, 2020; Nguegang *et al*., 2021). A published and patented study by Masindi *et al.* (2015) successfully proved that activated magnesite can remove close to 90% of metal species and 60% of SO_4^{2-} from AMD. The optimum conditions were observed to be 60 min of equilibration, 1 g dosage and 1:100 (solid/liquid) S/L ratios. Owing to their drawbacks which include elevated levels of residual SO_4^{2-} in the magnesite process and poor performance of the *Vetiveria zizanioides* for concentrated AMD solutions. It is for this reason why a hybrid treatment approach is proposed for the amelioration of AMD. This will then leverage on the weaknesses of each other and ensure that product water that complies with specified limits, and standards are produced from the AMD treatment process. In this study, the principal attempt will be to increase the pH of AMD water to basic level and precipitate metals in the first phase and attenuate elevated levels of toxic chemical species and partially SO_4^{2-} at pre-treatment. In the next phase (second phase), the series of CWs planted with *Vetiveria zizanioides* will be added to the treatment system with the view of further polishing the water by removing the residual SO_4^{2-} and chemical species to required standard for effluent discharge. The quality of product water will be tested and assessed to establish if it is complying with the prescribed water quality standards before being discharged into a receiving water body. This study was set up to find out if the hybrid system consisting of activated magnesite and a series of CWs planted with *Vetiveria zizanioides* can treat AMD to required standard for effluent discharge as set by the department of environmental affairs (DEA) and the possibility of reclaiming drinking water standard as set by the department of water and sanitation (DWS) in the republic of South Africa. According to the authors' knowledge, this proposed technology is novel and it has never been tried anywhere else. This will be used in a hybrid system to enhance the functionality of the individual components compared to when used in stand-alone system.

7.2 Materials and methods

7.2.1 Samples collection and characterization

Real acid mine drainage (AMD) was collected at the Sibanye Gold mine in Krugersdorp, Gauteng, South Africa. Onsite characterizations were ascertained by multi-parameter meter (HANNA instruments, Johannesburg, RSA). These included pH, total dissolved solids (TDS) and Electrical conductivity (EC). Initial concentrations of metals (Al, Cu, Fe, Mn, Ni and Zn) in AMD water were determined using ICP-OES and the initial concentration of sulphate was analysed using Ion chromatography (IC). The soil which was used as wetland substrate was purchased from garden world, Johannesburg and once in the campus, soil samples were air dried for five days, sieved and one gram (1g) was digested in a mixture of 5 mL of 55% nitric acid (HNO_3) and 2 ml of 30% Hydrogen peroxide (H_2O_2) using microwave digestion. Once the digested soil samples were cooled to room temperature, the solutions was filtered and analysed for initial concentrations of metals using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

7.2.2 Acquisition of the samples, plants and reagents

Cryptocrystalline magnesite with ≤ 32 µm particles size was procured from Sterkfontein carbonates (Pty) Ltd. Shoot of *Vetiveria zizanioides* were purchased from NANDADRAM ECOVILLAGE farm in Kwa-Zulu Natal, South Africa and transported to the University of South Africa's sciences campus in Florida. Once in campus, the shoots of *Vetiveria zizanioides* were submerged under potable fresh water for two weeks in order to allow the new seeds to germinate. After two weeks of germination, the seedlings of *Vetiveria* *zizanioides* were transferred into small pots containing soil. Reagents used in this study were of analytical grade and they were purchased from Merck, South Africa.

7.2.3 Characterisation of the solid samples

Raw magnesite and AMD reacted magnesite were characterised using different analytical and state-of-art techniques. Elemental composition of substrate (soil) before and after the contact of the substrate with AMD was done using X-ray fluorescence (XRF). Thenceforth, the shoots and roots were characterised using high-resolution scanning electron microscopy equipped with electro dispersion spectroscopy (HR-SEM-EDS). Metals-Functional groups of magnesite and grass roots were determined using Perkin-Elmer Spectrum 100 Fourier Transform Infrared Spectrometer (FTIR) equipped with a Perkin-Elmer Precisely Universal Attenuated Total Reflectance (ATR) sampling accessory equipped with a diamond crystal.

7.2.4 Treatment of real mine water

This section is divided into two categories of which phase 1 focuses on the neutralisation of AMD using cryptocrystalline magnesite and phase 2 focuses on the use of staged constructed wetland in the bioremediation step. The optimised conditions are discussed herewith.

7.2.4.1 Phase 1: Neutralisation - cryptocrystalline magnesite

This section is based on the findings which were reported in the previous study of our group whereby cryptocrystalline magnesite was used for the neutralisation and attenuation of inorganic contaminants from AMD (Masindi *et al*., 2018b). The experiments were optimised using the one-factor-at-a-time (OFAAT) approach whereby the effect of contact time, dosage (w/v-relationship), and temperature were duly explored. Findings were observed to be 1:100 (w/v or 1 g/100 ml), 500 rpm of mixing, and 1 hr of hydraulic retention time (HRT) (Masindi *et al*., 2015; Masindi *et al*., 2019). Similarly, the neutralisation process obeyed the same conditions. Quality control procedures were explored and the results reported as mean values.

7.2.4.2 Phase 2: Bioremediation - Wetland design

Two series of multi-stage wetland (treatment and control) were constructed and disposed as follow: subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW). The wetlands types were selected taking into account their efficiency and specificity in metals removal. For instance, better removal efficiency had been achieved for Al, Mn, Ni and Zn in subsurface constructed wetland (Sheoran and Sheoran, 2006; Sheoran, 2017) while As, Cu and Fe can be efficiently removed by both wetland with removal efficiency close to 90% (Knox, 2017; Sheoran, 2017; Pat-Espadas *et al*., 2018). The use of different flow system allowed the researchers to find out the impact of each flow system in pollutant removal. The SSVF wetland was connected to the reservoir tank via 25mm PVC pipe and others wetlands were connected each other following the aforementioned order. The outlet structure of each wetland cell was an orifice with valve to collect leachate for analysis. The wetlands were constructed using circular basins of 100 litres capacity each measuring 62 cm of diameter (d) and 45 cm high (h) and a drum of 1000 litres capacity as reservoir tank to contain AMD water and fresh water for control. The control wetland was fed by tap water mixed with activated magnesite at the same ratio of 1: 100 (1 g/100 ml). Each experimental wetland contained a total of 20 plants planted at equidistance from each other (**Figure 7.1**). The wetlands were continuous flow system where water was flowing in continuous drop into the system for the duration of the experiment.

Figure 7.1: The fabricated hybrid system for the treatment of AMD using the integration of magnesite and staged constructed wetland equipped with *Vetiveria zizanioides*.

7.2.5 Hydrology of the system

Hydrological factors in constructed wetland include hydraulic loading rate (HLR), hydraulic retention time (HRT), evapotranspiration (ET) and water balance within the system.

7.2.5.1 Hydraulic loading rate (HLR) of the system

The system was calibrated using a medical drip plastic bottle and the water was fed into the system in continuous drop. The inflow loading rate (ILR) and outflow loading rate (OLR) were determined as expressed in the Equation 7.1 and 7.2

$$\mathrm{ILR} = \frac{\mathrm{Qin}}{\mathrm{A}} \qquad \text{(Eqn 7.1)}$$

Where;

Q_{in} is the inflow of water to the system (m^3/d),

A is the total surface area of the three wetlands (m^2), and

ILR is the inflow loading rate to the system (m^3/d)

$$\mathrm{OLR} = \frac{\mathrm{Qout}}{\mathrm{A}} \qquad \text{(Eqn 7.2)}$$

Where:

Q_{out} is the outlow of water from the system (m^3/d),

A is the total surface area of the three wetlands (m^2), and

OLR is the outflow loading rate from the system (m^3/d).

The average flow rate of the water within the system was determined as illustrated in the Equation 7.3

$$\mathrm{Q_{av}} = \frac{\mathrm{Q_{in} + Q_{out}}}{2} \qquad \text{(Eqn 7.3)}$$

Where:

Q_{in} is the water inflow into the system (m^3/d)

Q_{out} is the outflow of water from the system (m^3/d)

The determined average flow rate within the system was 0.0035 m^3 or 3.5litres/day. The flow rate was used to determine the hydraulic retention time (HRT) of the system

7.2.5.2 Hydraulic retention time (HRT) of the system

The hydraulic retention time (HRT) of the system was determined using the Darcy's law formula as illustrated in Equation 7.4 taking into account the number of the wetland in the system, and the fact that the sizes of the three wetlands are the same as well as the porosity of substrate.

$$\mathrm{HRT} = \frac{3\pi r^2 \mathrm{yd}}{\mathrm{Qav}} \qquad \text{(Eqn 7.4)}$$

Where:

π is the constant (3.14),

r is the radius of each wetland cell (m),

y is the depth of water in the wetland cell (m),

d is the porosity of the wetland substrate (%), and

Q_{av} is the average flow of water within the system (m^3/d).

$$\mathrm{HRT} = \frac{3\left(3.14\times(0.31)^2 \times 0.2\right)\times 0.59}{0.0035} = 30.52 \text{ days}$$

$$HRT = 30.52 \text{ days}$$

The flow rate was maintained low (3.5 litres/day) and the HRT long (30.52 days) in order to allow the water to spend more time in wetland cell and thereby improve the performance of the wetland since various studies have revealed that the performance of the wetland is highly improved by low hydraulic rate and long hydraulic retention time (Demetriou *et al*., 2010; Agboola, 2019). In this study, the experimental phase lasted for 30 days and three replicates were done using different substrate and different plants to ensure the reproducibility of the results.

7.2.6 Leachates and substrate sampling and plants harvest

Leachate (500 ml) from the outlet of each wetland and substrate (soil samples) from the bottom of each wetland were daily collected for a period of 30 days retention time. Leachates were divided into two sub-samples of 400 mL and 100 mL. Sub-samples of 400 mL were using for the measurement of pH, TDS, EC while sub-sample of 100 mL was further divided into two sub-samples for analysis of metals (Al, Cu, Fe, Ni, Mn and Zn) and SO_4^{2-}. Plants were harvested from each wetland, air dried and analysed for metals contents.

7.2.7 Analytical methods

Leachates, from both wetlands (control and treatment cell) were analysed following standard methods. Electrical conductivity (EC), pH and TDS were determined using Hanna HI 83200 multi-parameter and the manufacturer's manual was followed with reference to standard methods for examination of water and wastewater (APHA, 2002). Prior to analysis for metals concentration, water samples were filtered through a 0.22 um pore syringe filter membrane to remove particles and preserved by adding two drops of nitric acid (HNO_3) concentrated to prevent ageing and immediate precipitation of metals. The filtrates were refrigerated at 4°C prior to analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES). SO_4^{2-} was determined using ion chromatography (IC). Soils samples were air dried for five days, sieved and one gram (1g) was digested in a mixture of 5 mL of 55% (HNO_3) and 2 mL 30% Hydrogen peroxide (H_2O_2) using microwave digestion. Once digested samples were cooled to room temperature, the solutions were filtered through a 0.22 μm pore syringe filter membrane and analysed for metals concentrations by Agilent 5100 Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Plants biomasses were separated into two samples (shoots and roots). Each part of plant was dried and grinded using mortar and pestle and 1g was digested with 10 mL of 65% HNO_3 (AR grade; BDH). The digestate samples were then filtered using 0.22 μm pore syringe filter membrane following by analysis using (ICP-OES).

7.2.8 Contaminants removal efficacy

The treatment efficiency of hybrid system (neutralisation and bioremediation) in AMD water treatment was determined gradually during the duration of the experiment. All parameters of concern were analysed daily and the removal efficiency (RE) was calculated gradually every 5 days interval for the duration of the experiment (30 days). RE was calculated for all pollutants of concern using Equation (7.5) (Demetriou *et al.*, 2010) and the increment was calculated for pH (Prihatini et al., 2016) as illustrated in Equation 7.6.

$$\mathrm{RE} = \frac{\mathrm{Ci-Cf}}{\mathrm{Ci}} \times 100 \qquad \text{(Eqn 7.5)}$$

Where:

C_i is the initial concentration of each parameter in the AMD water,

C_f is the final concentration of each parameter after hybrid treatment, and

RE (in percentage) is the removal efficiency of each parameter after hybrid treatment

$$\mathrm{I} = \mathrm{pHf} - \mathrm{pHi} \qquad \text{(Eqn 7.6)}$$

Where: pH_f is the final value of pH of the treated AMD, pHi is the initial pH value of AMD water, and I is the increment of pH.

7.2.9 Geochemical modelling

Speciation and potential precipitation of metals during the interaction of AMD, substrate and external factors (Parkhurst, 1995; Hart, 2002). The water-Q4 database was utilised. The plants-substrate was modelled using PH REdox EQuilibrium (in C language) (PHREEQC). The water potential precipitation of metals were determined using saturation indexes (SI) of which, SI ≤1 denotes under-saturation, SI ≈ 1 denotes saturation, and SI ≥ 1 denotes super-saturation.

7.3 Results and discussion

This section will discuss the removal efficacy of different physicochemical properties of the feed water. The chemical and morphological properties of the feed and product minerals will also be assessed.

7.3.1 Remediation studies

This section will discuss the findings which were observed from the experiments that were conducted to fulfil the goals of this study.

7.3.1.1 Effect of a hybrid system on pH

The effect of neutralisation and bio-remediation on pH was evaluated over a period of 30 days and the obtained results are reported in **Figure 7.2**.

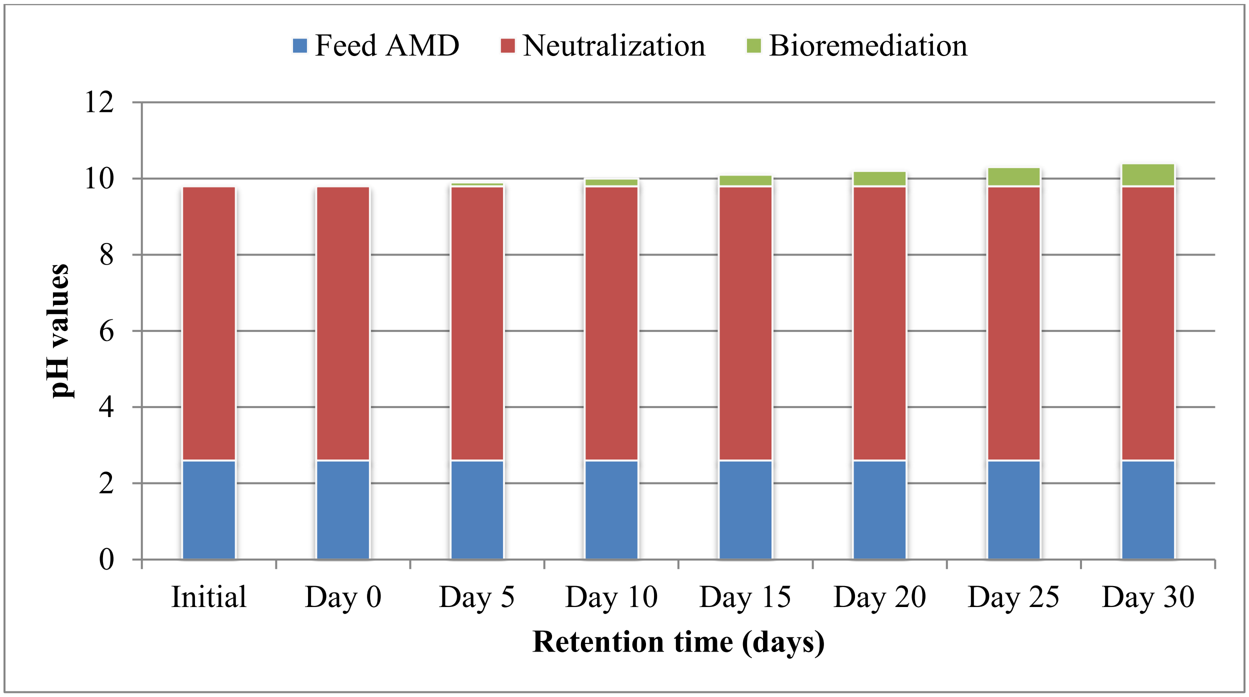

Figure 7.2: Variation in pH as a function of neutralisation with activated magnesite and bio-remediation using a series of constructed wetland planted with *Vetiveria zizanioides*

As shown in Figure 7.2, the effect of neutralisation and bioremediation on pH was evaluated over a period of 30 days. Explicitly, the results revealed that the hybrid system was able to increase the pH of AMD from 2.6 to 10.4 corresponding to a total increment of 7.8. The neutralisation step of the hybrid system raised the pH from 2.6 to 9.8 while the bioremediation step raised the pH from 9.8 to 10.4. In light of the findings, it can be noted that the neutralisation treatment steps contributed to 92.30% of total pH increment while the bioremediation step contributed to 07.70% (**Figure 7.2**). The rose of pH in the neutralisation treatment step is the result of AMD treatment by activated magnesite. In fact, the contact of AMD with activated magnesite at 1:100 ratio (1g:100 ml) for one hour duration, led to the consumption of hydrogen ions (H^+) from sulphuric acid by magnesite resulting to the attenuation of acidity and increase of pH (Masindi *et al.*, 2015). In the bioremediation step, the increase of pH may be attributed to the continuous removal of acid forming metals such as Al, Fe, Mn and Zn (Wang *et al.*, 2017). The raise of pH may also be the consequences of photosynthesis process since plants and algae use hydrogen ions thus increasing the pH (Suelee *et al.*, 2017; Chen *et al.*, 2021). The increase in pH may also be attributed to the biochemical processes occurring in the substrate since plant reduces the hydrogen ion activity during photosynthesis process leading to an increase of pH (Pavinato *et al.*, 2008). The bioremediation results further revealed that in the series of CWs, the different types of wetland contributed at different percentage to the pH increase. The finding further revealed that the pH increased gradually from the first wetland (SSVF-CW) to the third wetland (SSHF-CW) and the contribution of each wetland in pH increase was in the following order: SSVF-CW (28%), FWS-CW (30%) and SSHF-CW (42%) thereby confirming previous studies (Comino *et al.*, 2013; Gonzalo *et al.*, 2020) (**Figure 7.3**). This difference may be attributed to the type of wetland and the biochemical process occurring within each wetland. In addition, the type of water flow in wetland influences the oxido-reduction conditions and affect metals removal thereby affecting the increase of pH (García *et al.* 2010). Overall, the hybrid system contributed to raise the pH of AMD water from 2.6 to 10.4 which is within the required standard of 12.0 set by the Department of Environmental Affairs (DEA) in the republic of South Africa for effluent discharge but slightly above the required compliance of 9.7 by the Department of Water and Sanitation (DWS) in the republic of South Africa for drinking water quality guidelines standard.

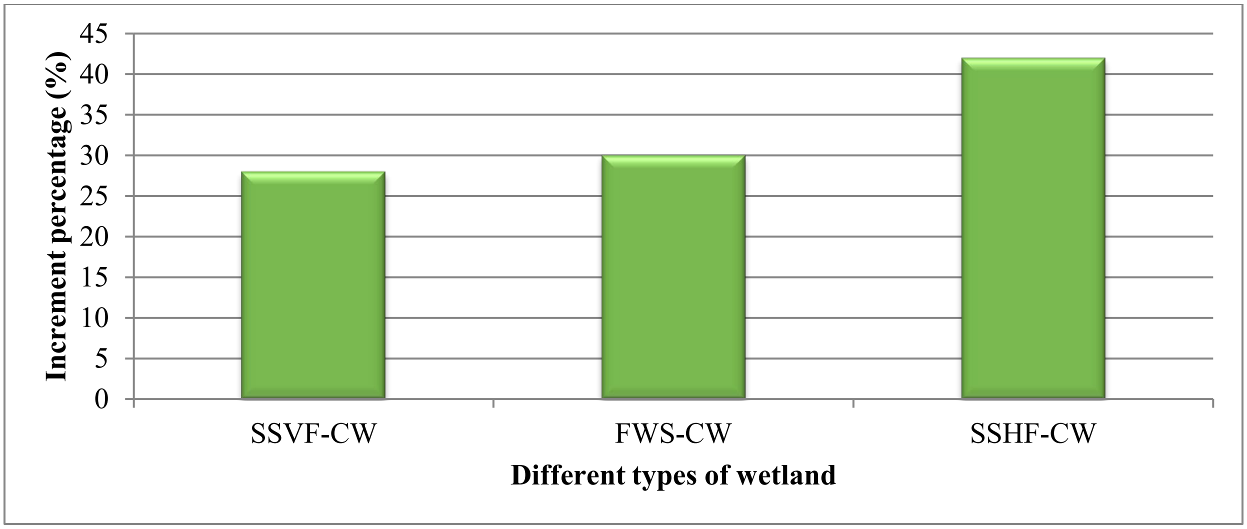

Figure 7.3: Variation in pH as a function of different types of wetlands subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW)].

7.3.1.2 Effect of the hybrid system on electrical conductivity (EC)

The effect of neutralisation and bio-remediation on EC has been evaluated over a period of 30 days. The obtained results are shown in **Figure 7.4**.

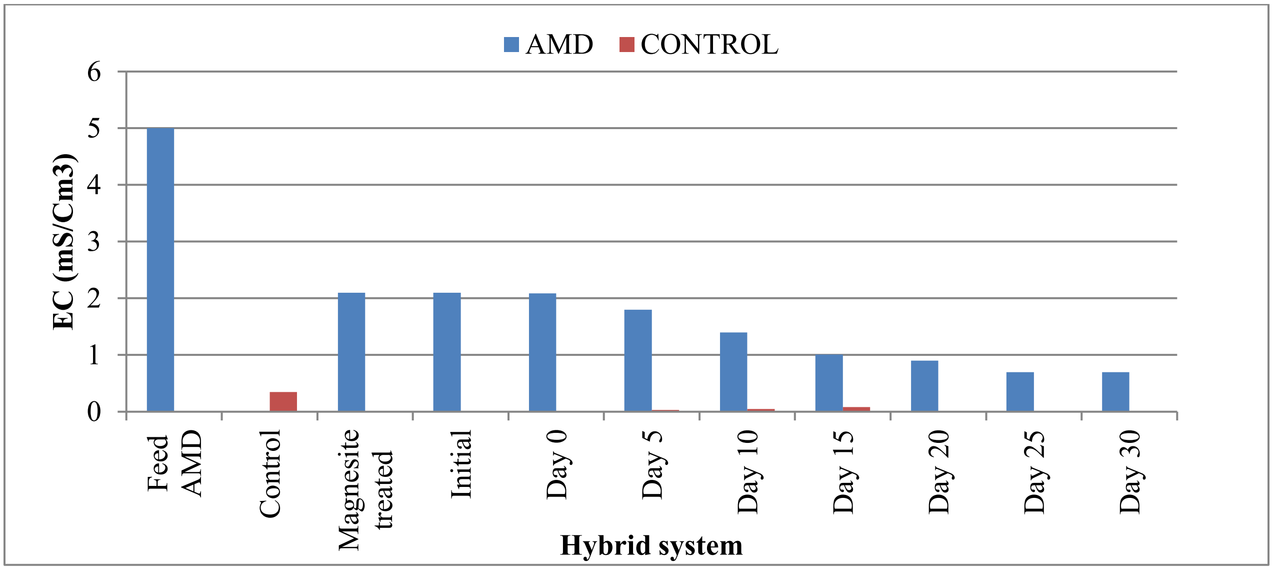

Figure 7.4: Variation in EC as a function of neutralisation with activated magnesite and bio-remediation using a series of constructed wetland planted with *Vetiveria zizanioides*

As reported by the **Figure 7.4**, the results revealed that the hybrid system was able to reduce EC of AMD water from 5 mS/cm to 0.7 mS/cm corresponding to 86% of overall removal efficiency. The chemical treatment step using activated magnesite reduced EC of real AMD from 5 mS/cm to 2.1 mS/cm corresponding to a reduction efficiency of 58% while the bioremediation step reduced the EC of pre-treated AMD water from 2.1 to 0.7 mS/cm translating to 28% of overall EC reduction (**Figure 7.4**). The reduction of EC of mine water after contact with magnesite may be attributed to the reduction of SO_4^{2-} ions and other ions present in mine water. In fact, after a one hour contact of feed AMD water with magnesite, most ions sulphate and other ions in AMD water were reduced (Masindi *et al*., 2015) thereby leading to the reduction of EC. During the bioremediation step of the hybrid technology, the findings revealed that the EC was gradually reduced from Day 1 to Day 30 (**Figure 7.4**). The reduction of EC in a series of CWs may be attributed to the filtration and absorption role of staged wetland thereby leading to the reductions of ions in AMD water (Borges *et al*., 2008; Kaseva and Mbuligwe, 2010). This may also be the results of wastewater filtration by plants since one of the major role of plants in wetland is to filter wastewater thereby promoting the precipitation/sedimentation of pollutants (Klomjek and Nitisoravut, 2005; Stefanakis, 2019). In the meantime, in the control wetland, the neutralisation of tap water with magnesite led to a reduction of EC of control water from 0.347 mS/cm to 0.00 mS/cm in one hour. However, in the bioremediation step, the EC of control wetland increased slightly in the beginning of the experiment but followed by the decrease to below detection limit (**Figure 7.5**). The slight increase of EC in control water may be attributed to the presence of pollutants such as lead, chromated copper arsenate and creosote arising from wetland substrate which contributed to slight increase observed (Adhikari *et al*., 2011). However the effects of those pollutants were insignificant in treatment wetland. The result of bioremediation step further revealed that electrical conductivity was gradually reduced in the staged wetland with SSVF-CW, FWS-CW and SSHF-CW contributing to 38%, 33% and 29% respectively of overall EC reduction within the staged wetland (**Figure 7.5**).

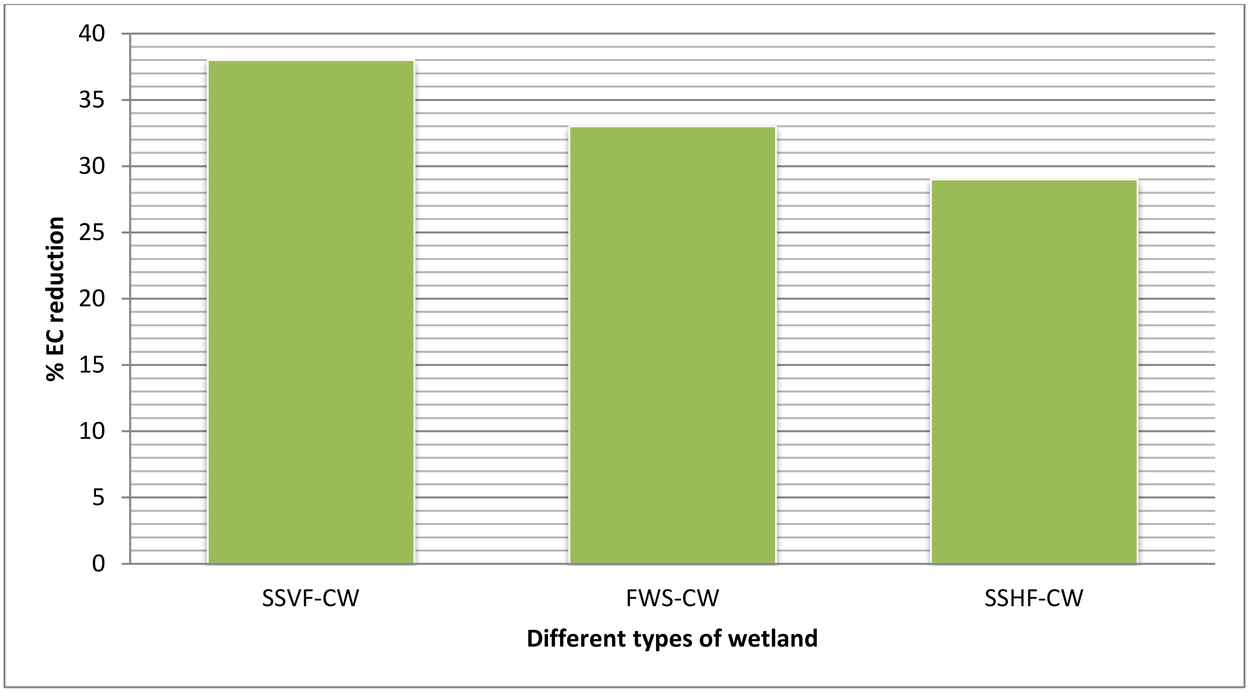

Figure 7.5: Variation in electrical conductivity as a function of different types of wetlands [subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW)].

7.3.1.4 Effect of retention time on the concentration of sulphate

The effect of retention time on SO_4^{2-} level has been evaluated over a period of 30 days and the results are reported in **Figure 7.6**.

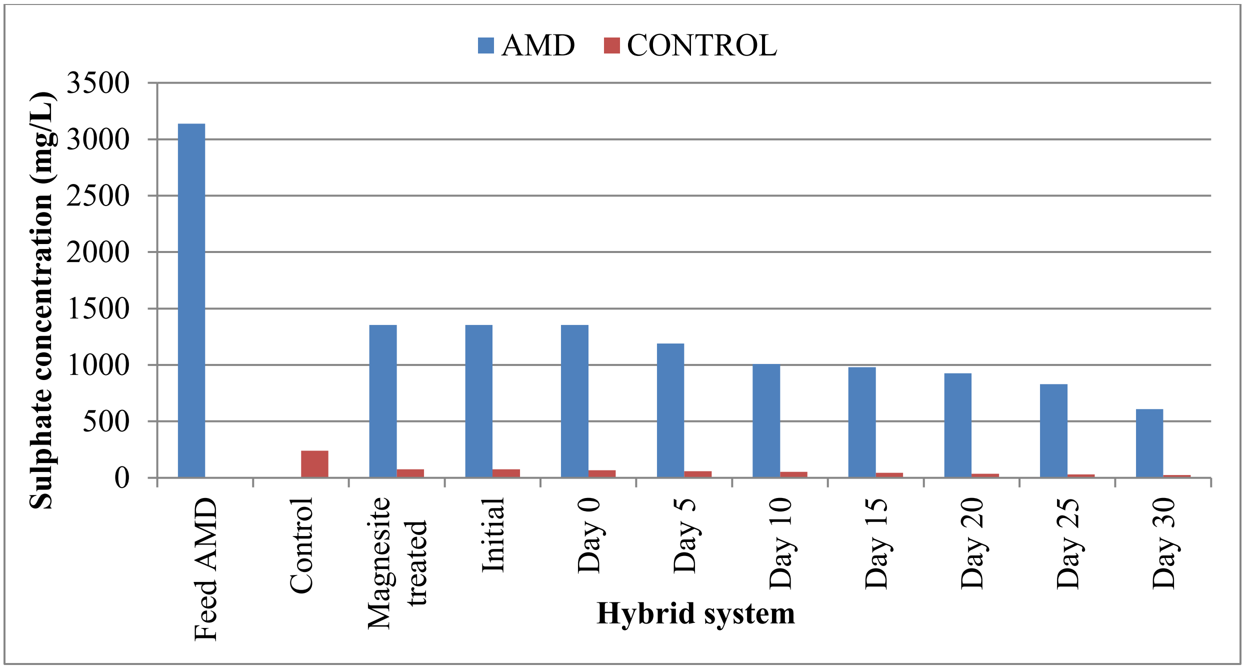

Figure 7.6: Variation in sulphate concentration as a function of neutralisation and bio-remediation.

Figure 7.6 clearly portrayed that the hybrid system (activated magnesite and a series of constructed wetlands) was able to reduce SO_4^{2-} concentration in AMD water from the initial concentration of 3137 mg/L to 608.7 mg/L corresponding to an overall reduction efficiency of 80.59%. The chemical treatment step using activated magnesite reduced SO_4^{2-} concentration of real AMD water from 3137 mg/L to 1354.8 mg/L corresponding to 56.81% of overall SO_4^{2-} reduction while the bioremediation step using a series of CWs further reduced the SO_4^{2-} of magnesite treated AMD from 1354.8 mg/L to 608.7 mg/L translating to 23.78% of overall SO_4^{2-} reduction in 30 days retention time (**Figure 7.6**). The obtained findings corroborates findings reported in our previous studies (Masindi *et al*., 2015; Masindi *et al*., 2018b; Magagane *et al*., 2019). In the control experiment, magnesite reduced SO_4^{2-} in tap water from 250 mg/L to 75 mg/L and the bioremediation step further reduced it from 75 mg/L to 33.75 mg/L. The result of activated magnesite step was in line with the study of Masindi *et al*. (2015), which revealed that the treatment of AMD water with magnesite at 1:100 (1g/100 ml) ratio led to 58.83% (≈60%) of SO_4^{2-} reduction. The reduction of SO_4^{2-} may be attributed to the formation of sulphate bearing minerals such as gypsum and oxy-hydrosulphates. This was further confirmed by

PHREEQC simulation results. Thenceforth, the reduction of SO_4^{2-} in a series of CWs may be attributed to plant accumulation since SO_4^{2-} plays a huge role in plant metabolism (Kiiskila *et al.*, 2019)**.** More explicitly, plant reduces SO_4^{2-} and sulphur dioxide to form which are essential for the formation of chlorophyll, plays an active role in the synthesis of oil by plant (Takahashi, 2019) and contribute to many functions in plant metabolism such as production of proteins and hormones required for plant metabolism (Bloodnick, 2020). The results further revealed that the series of CWs contributed to SO_4^{2-} reduction at different percentages. The observed reduction percentage obeyed the following order: SSVF-CW (59.79%) > FWS-CW (20.97%) > SSHF-CW (19.24%) **(Figure 7.7).**

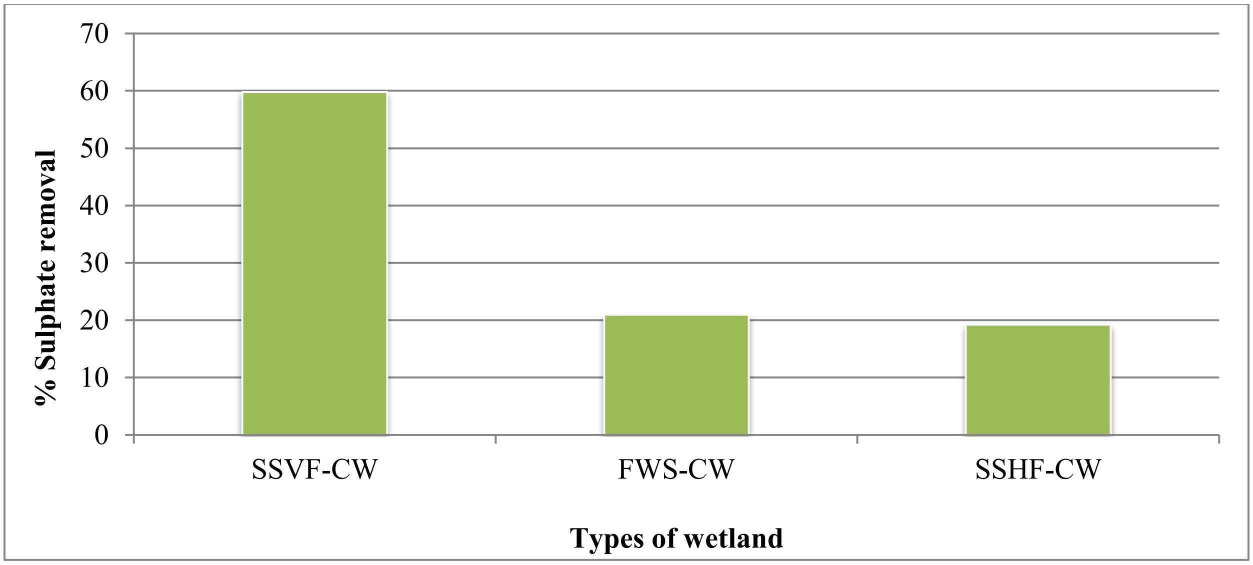

Figure 7.7: Variation in sulphate concentration as a function of wetlands type [subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW)].

Overall, the hybrid system allowed reducing SO_4^{2-} concentration in AMD water from 3137 mg/L to 608.7 mg/L which is within acceptable standard set by the DEA but slightly above the 500 mg/L as set by the DWS.

7.3.1.5 Effect of retention time on the levels of metals

The effect of retention time on the levels of metals has been evaluated over a period of 30 days and the results are reported in **Figure 7.8**.

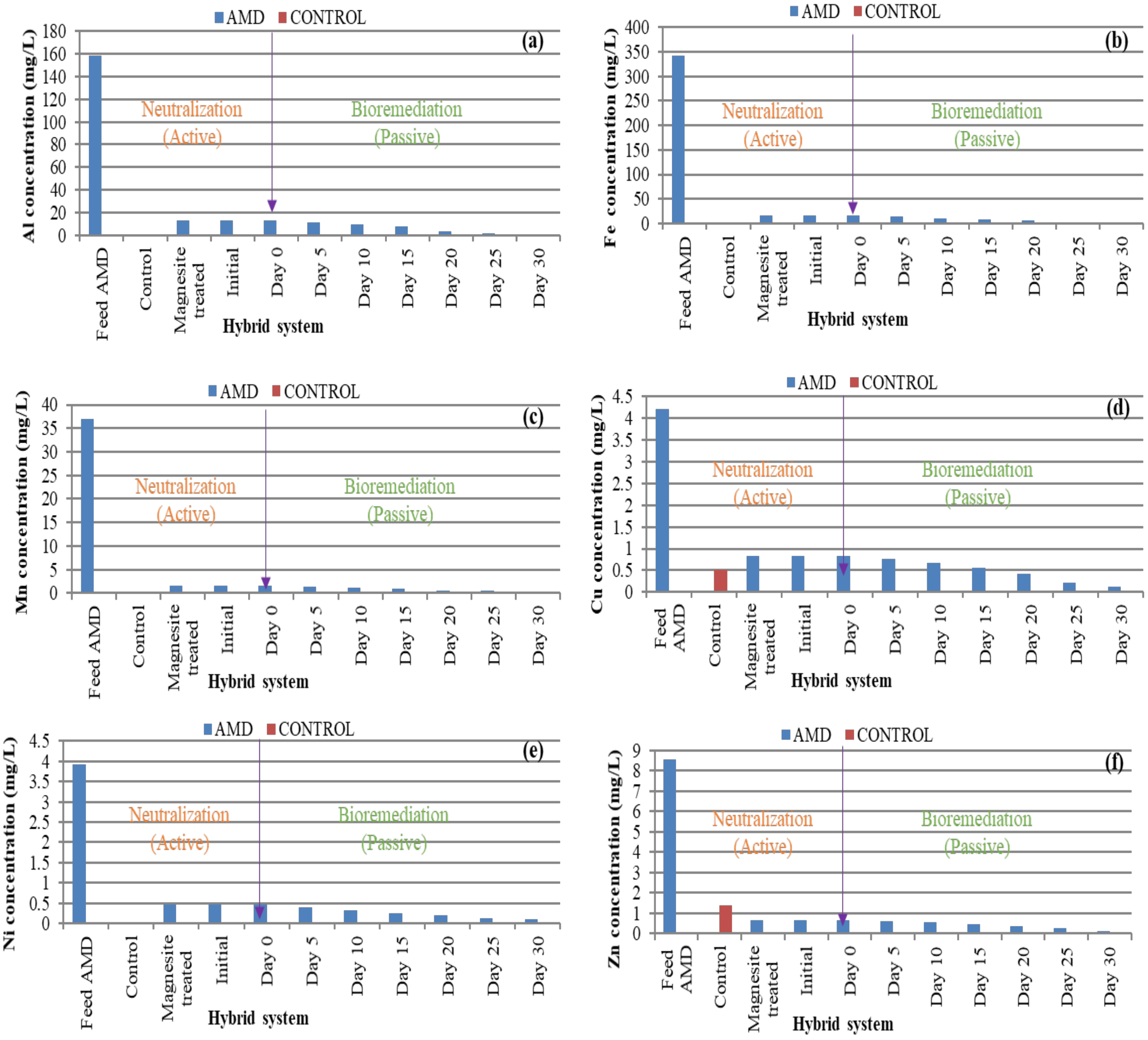

Figure 7.8 (a-f): Variation in metals level as a function of neutralisation and bio-remediation.

As shown in **Figure 7.8(a),** the results revealed that 99.5% of Al was removed by the hybrid system with cryptocrystalline magnesite treatment contributing to 92% of overall Al removal within one hour and a series of CWs contributed to 7.5% of Al removal within 30 day retention time. The treatment of feed AMD with magnesite increased the pH of AMD water thereby leading to precipitation, adsorption and co-precipitation of metals with Al precipitated at pH > 4 (Hart, 2002; Wei, Viadero and Buzby, 2005). Similarly, the results were confirmed by XRD and PHREEQC geochemical model. The elevated removal percentage of Al in the activated magnesite treatment step is the result of Al precipitation, and co-precipitation with other metals. The bioremediation (series of CWs) accounted only for a small fraction of Al removal and the results showed that Al removal followed the same trend for all the three wetlands with Al removed gradually from one wetland to the next one from the beginning to the end of the experiment. Furthermore, the Al removal in the bioremediation step (series of CWs) may be attributed mainly to different types of biological processes such as precipitation and plant uptake controlling pollutant removal in constructed wetland. Al plays a huge role in plant growth and as consequence, it is needed by plant for some metabolic function such as promotion of roots growth, improvement of plant's nutrient uptake, increasing enzyme activity, assistance in the development of defence pathogen, increasing metabolism and antioxidant activity as well as modulation of colours flowers (Schmitt *et al*., 2016; Bojórquez-Quintal e*t al.*, 2017) and alleviation of the toxicity of metals such Fe and Mn and hydrogen ion in acidic soil (Muhammad *et al*., 2019). The multiple role of Al in plant may have had a huge contribution in Al removal in staged wetland. Overall the contribution of each step of the hybrid system in Al removal denoted 0.5% which corresponds to 0.71 mg/L, which is within the department of environmental affairs (DEA) guidelines standard for effluent discharge but above the 0.3mg/L required for drinking water as set by department of water and sanitation (DWS).

As shown in **Figure 7.8(b),** the hybrid system fostered the reduction of Fe from 341mg/L to 0.66 mg/L. Interestingly, the activated magnesite treatment step reduced Fe concentration from 341mg/L to 17.05mg/L translating to a removal efficiency of 95% within one hour duration while the bioremediation step further reduced Fe concentration from 17.05 to 0.66mg/L in 30 days retention time. Contrary, the iron concentration in control experiment was reduced from 0.2 to 0.00 mg/L. The reduction in Fe may be explained by an increases of the pH which stimulates the precipitation of metals (Mbedzi *et al*., 2017). This chemical process meticulously explains the high removal efficiency of Fe after the treatment of AMD water by the activated magnesite. Subsequently, in the bioremediation step, the Fe removal followed the same trend within the three wetlands since it was gradually removed from the first day 1 to the last day (Day 30) of the experiment. The Fe removal in a series of CWs may be attributed to biochemical process such as precipitation, rhizo-filtration and the interaction between microorganism and metals occurring simultaneously in the wetland rhizosphere thereby influencing the mobility and bioavailability of Fe (Arivoli *et al*., 2015). Thenceforth, the removal of Fe may also be attributed to *Vetiveria ziznioides* accumulation since Fe plays crucial role in plant metabolism. More precisely, the deficiency of Fe in plant leads to many morphological changes such as plants chlorosis (López-Millán *et al*., 2013) since iron plays an active role in the synthesis of chlorophyll that is much needed for photosynthesis process in plant and it is also required for certain enzyme functions such as chelate reductase enzyme activity located in root plasma membrane (Hochmuth, 2011)**.** Overall, the hybrid system allowed reducing Fe concentration from 341 mg/L to 0.66 mg/L which is within the 50 mg/L set by the DEA guideline standard for effluent discharge but above the 0.01 mg/L set by DWS for drinking water quality standard

As illustrated in **Figure 7.8(c),** the hybrid system reduced Mn concentration from 37mg/L to 0.28mg/L. whilst the control experiment demonstrated ≈100% attenuation efficacy. The neutralisation step reduced Mn concentration from 37 mg/L to 1.48 mg/L while the bioremediation step further reduced Mn concentration from 1.48 mg/L to 0.28 mg/L. PHREEQC geochemical model predicted the formation of Pyrochroite and Rhodochrosite. This was further confirmed by literature (Freitas *et al*., 2011) thereby fostering the reduction of Mn in AMD water. In the bioremediation step of the hybrid system, Mn reduction followed the same patterns for all the three wetlands with Mn removed gradually from the beginning to the end of the experiment but at different removal efficiency (≈ ≥ 99%). Mn removal in the bioremediation step (CWs) may be attributed to biochemical process such as ion-exchange, sedimentation with substrate and plant uptake. Mn is needed by plants since it is important for diverse function such as photosynthesis, respiration, pathogen defence, hormone signalling, and collection of reactive oxygen species in plant growth (Fernando and Lynch, 2015; Leplat *et al*., 2016; Alejandro *et al*., 2020). Overall, this hybrid technology reduced Mn concentration from 37 mg/L to 0.28 mg/L which is within the 20 mg/L set by the DEA for effluent discharge and above the 0.05 mg/L for drinking water standard as set by the DWS.

As shown in **Figure 7.8(d),** the copper concentration was reduced from 4.2 to 0.84 mg/L translating to 80% of copper removal from AMD water using activated magnesite. Subsequently, the bioremediation step further reduced the concentration of copper from 0.84 to 0.11 mg/L translating to 17.38% of overall copper reduction. The treatment of feed AMD water with activated magnesite increased the pH and attenuate inorganic contaminant (Masindi *et al*., 2015) thereby leading to the precipitation of copper at pH varying between 3.8 and 6.5 (Porozhnyuk *et al*., 2018). The bioremediation step revealed that the removal of copper in a series of CWs followed the same trend with copper removed gradually from day 1 to day 30 and from the first treatment cell (SSVF-CW) to the last treatment cell (SSHF-CW). Furthermore, the removal of copper in CWs may be attributed to different biological process such as precipitation, ion- exchange and plants uptake occurring within the wetland. In plant, copper is an important micronutrient since it is a stimulant of photosynthesis and respiration process and also has major role in the generation of lignin in the cell wall (Printz *et al*., 2016; Pietrini *et al*., 2019). The copper needs of plants may have contributed to copper removal in CWs. The hybrid system managed to acquire close to 99% removal efficacy corresponding to the reduction of Cu concentration from 4.2 mg/L to 0.11 mg/L. Interestingly, the final water met the prescribed limit for effluent discharge as stipulated in DEA for effluent quality guidelines and drinking water quality standard as set by the DWS.

As shown in **Figure 7.8(e),** the Ni concentration was reduced from 3.92 to 0.47mg/L after one hour treatment using activated magnesite while the bioremediation step further reduced Ni concentration from 0.47 to 0.09mg/L within 30 days retention time. For the control experiment, the Ni concentration in fresh water was completely removed after treatment with activated magnesite. This led to an increase of pH thereby leading to complete nickel removal at pH > 9. In the bioremediation step, the removal of nickel occurred gradually within the series of CWs and the concentration of Ni was reduced from one treatment wetland to the next one from the beginning to the end of experiment. The Ni removal in CWs may be attributed to many factors such as filtration, sedimentation; co-precipitation with others metals and plant uptake since Ni plays a huge role in the activation of enzyme urease in plant (Kamboj *et al*., 2018; Amjad *et al*., 2020). However, elevated concentration of Ni in plant leads to adverse effects such as hindering the uptake of important macro and micronutrient as well as their translocation from roots to shoots (Amjad *et al*., 2020) and this can explain why despite the low concentration of Ni entering the bioremediation step, it was not totally removed by the constructed wetland after 30 days retention time. The hybrid system reduced Ni concentration from 3.92 mg/L to 0.09 mg/L, values within acceptable limit of 10 mg/L for effluent discharge as set by the department of environmental affairs but slightly above the 0.09 limit for drinking water quality standard as set by the DWS.

As shown in **Figure 7.8(f),** the hybrid system was able to remove huge quantity of Zn from AMD. The concentration was observed to reduce from 8.55 to 0.14 mg/L. The activated magnesite treatment step played a huge role by reducing Zn concentration from 8.55 to 0.68mg/L after one hour duration while the bioremediation step further reduced Zn concentration from 0.68 to 0.14 mg/L within 30 days retention time. The control experiment, led to complete removal of zinc from tap water, however, this is understandable since the level of Zn is trivial. In bioremediation step, the removal of Zn followed the same trend within the three wetlands since it was gradually removed from the beginning to the end of the experiment, albeit at different removal efficiency. The removal of Zn in CWs may be attributed to different biochemical processes such as precipitation, substrate adsorption and plant uptake occurring within the wetland (Almuktar *et al*., 2018). In plant Zn plays an important role such as improving seed germination, interacts with plant hormones, increases the expression of stress proteins and stimulates the antioxidant enzymes for counteracting drought effects (Hassan *et al*., 2020). Overall, the hybrid system allowed reducing zinc concentration in AMD water from 8.55 to 0.14mg/L which is complying with the DEA guideline standard but above the DWS limit of 0.5 mg/L for drinking water standard. The hybrid system allowed removing close to 99% of all metals of concern with product water complying with the DEA effluent quality specifications or standards.

7.3.1.6 The removal efficiencies of water quality indicators

The removal efficiencies of water quality indicators over a period of 30 days is demonstrated as percentage removal and the results are reported in **Figure 7.9**.

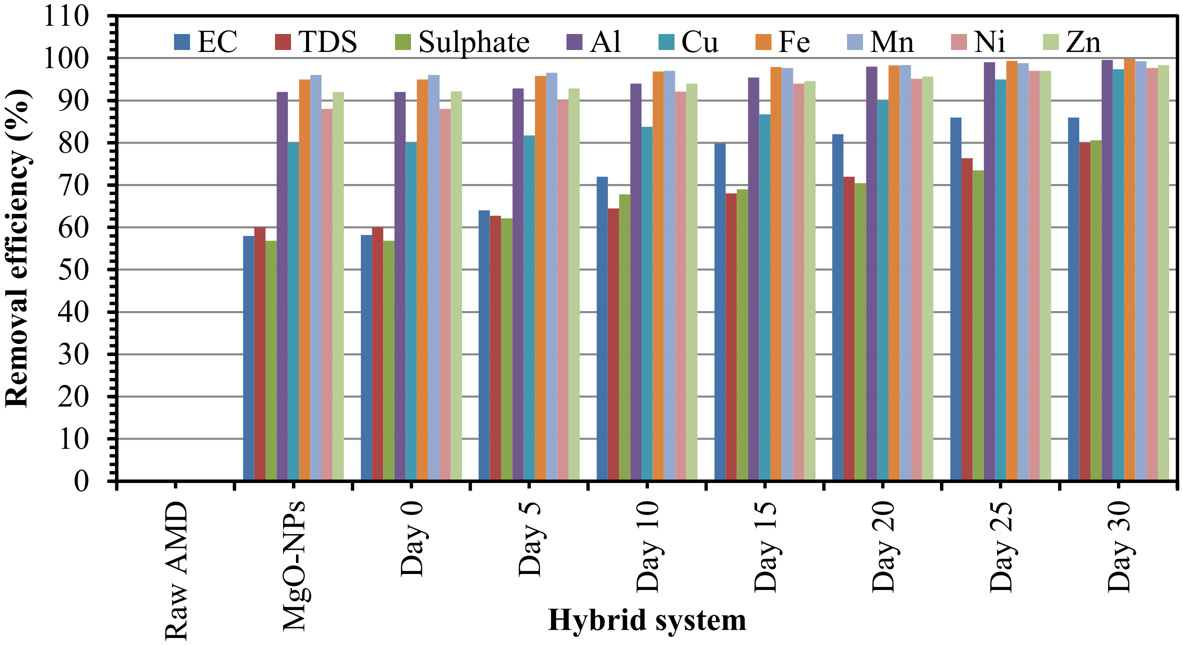

Figure 7.9: The percentage removal efficiencies of water quality indicators.

As shown in **Figure 7.9**, the removal efficiency of hybrid system to pollutants removal from AMD was in in the following order: Fe (99.8%) > Al (99.5%) > Mn (99.24) > Zn (98.36%) > Ni (97.7%) > Cu (97.38%) > EC (86%) > $SO4^{2-}$ (80.59%) > TDS (80%). This was significantly high in relation to the required standard. The activated magnesite treatment step significantly contributed to the overall removal of pollutants with the removal efficacies obeying the following order: Mn (96%) > Fe (95%) > Zn (92.04%) > Al (92%) > Ni (88.01%) > Cu (80%) > SO_4^{2-} (56.81%) within the one hour duration while the constructed wetland step accounted for a smaller fraction of pollutants removal in 30 days of retention time with removal efficiency as follows: EC (28%) > $SO4^{2-}$ (23.78%) > TDS (19.88%) > Cu (17.38%) > Ni (9.7%) > Al (7.55%) > Zn (6.3%) > Fe (4.8%), Mn (3.25%), while the percentage of pollutants which were not fully removed were: TDS (20%) > $SO4^{2-}$ (19.41%) > EC (14%) > Cu (2.62%) > Ni (2.3%) > Zn (1.64%) > Mn (0.76%) > Al (0.45%) > Fe (0.2%). The elevated removal of pollutants in the activated magnesite step may be attributed to precipitation, co-adsorption and/or co-precipitation (Masindi *et al*., 2015; Masindi, 2016). The precipitation, co-adsorption and co-precipitation of metals is the result of pH increase following the treatment of AMD water by magnesite which led to precipitation of Fe at pH > 3 (Coetzee *et al*., 2017; Mbedzi *et al*., 2017) , Al at pH > 4

(Wei *et al*., 2005) , Ni at pH > 4.5 (Escudero *et al*., 2017), Cu at pH > 6.5 (Porozhnyuk *et al*., 2018)**,** Zn at pH >8.7 (Olds *et al*., 2013)**.** This was further confirmed by PHREEQC geochemical model.

7.3.1.7 Fate of heavy metals in the staged constructed wetland

The partitioning of metals between subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW) mode are shown in **Figure 7.10**.

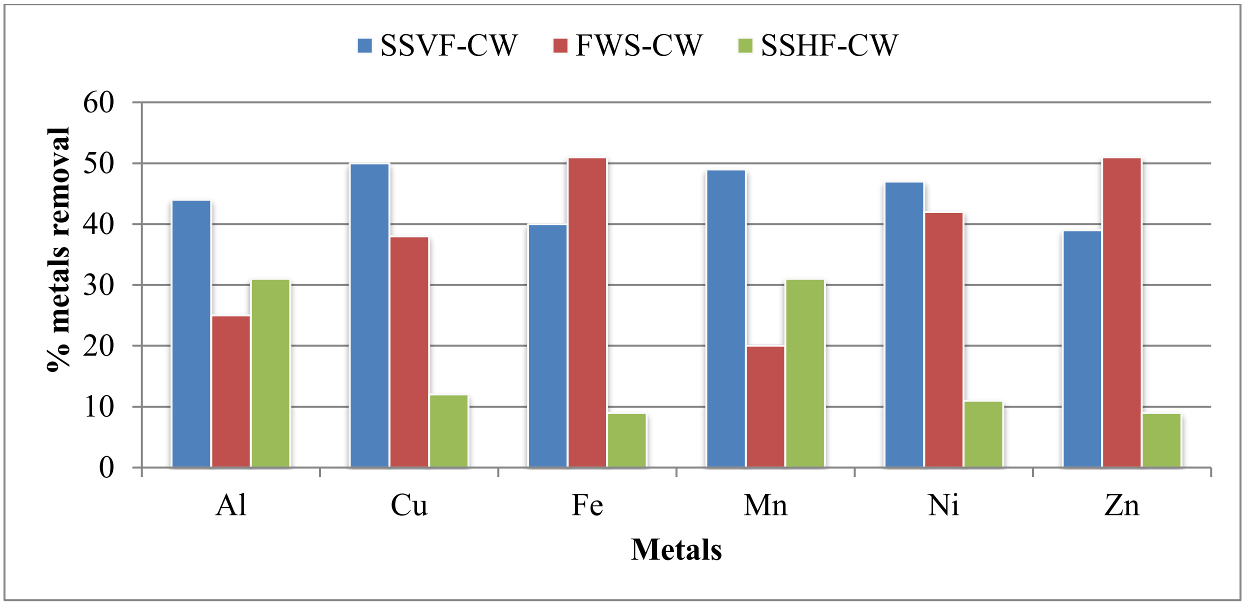

Figure 7.10: Partitioning of metals between subsurface vertical flow (SSVF-CW), free water surface flow (FWS-CW) and subsurface horizontal flow (SSHF-CW) mode.

The **Figure 7.10** clearly portrayed that the fate of metals removal in the bioremediation (constructed wetland) showed different trends and this may be attributed mainly to different types of biochemical process occurring within each type of wetland. Specifically, metals removal in wetland is mainly linked to the interaction of metals with substrate which includes filtration, interception and absorption-desorption, convective-diffusion, complexation, redox reaction, precipitation, ion-exchange, neutralisation and plant uptake occurring simultaneously (Kumar and Dutta, 2019; Yuan *et al*., 2020). It may also be contributed by the importance of metals in plant metabolism. For instance, 98.82% of iron entered the staged wetland were removed in and this may be attributed to the need of plant for its metabolic processes such as DNA synthesis, respiration and photosynthesis (Rout and Sahoo, 2015; Connorton *et al*., 2017). The results of constructed wetland step further revealed that metal removal varied according to the type of wetland**.** More precisely, Al, Cu, Mn and Ni were more removed in SSVF-CW and SSHF-CW while Fe was more removed in FWS-CW. The removal of Zn in the constructed wetland was moderate with 39%, 35% and 26% by SSVF-CW, FWS-CW and SSHF-CW, respectively, thereby confirming the studies of Sheoran. (2017) and Pat-Espadas *et al*. (2018). The variation of metals removal according to the type of wetland may be due to the water flows regime within each wetland since the type of flow in wetland influence the redox conditions thereby affecting metals removal process (García *et al.*, 2010). High Mn removal in SSVF-CW and SSHF-CW may be attributed to the adsorption process resulting from the presence of iron oxide in the substrate (Marín-Rivera *et al*., 2019)**.** The presence of iron oxide and Mn promote the development of Mn and iron oxidizing bacteria thereby contributing to Mn removal (Aziz *et al*., 2020)**.** The results of metals removal in a series of CWs further revealed that SSVF-CW contributed to 44%, 45%, 31%, 49%, 46% and 39% removal of Al, Cu, Fe, Mn, Ni and Zn, respectively. This may be attributed to the position of SSVF-CW (First) in the staged wetland since the pre-treated AMD water first entered the SSVF-CW where it went through the first polishing stage. High removal efficiency of metals in SSVF-CW may also be the result of metals precipitation with oxy-hydroxides since the phenomena is typical to vertical subsurface flow wetland (Marín-Rivera *et al*., 2019; García-Ávila, 2020). The results of bioremediation step were in line with the finding of study conducted by Yeh and Wu. (2009) which revealed that metals were gradually removed within a staged hybrid constructed wetland made of FWS-CW and SSHF-CW treating wastewater.

7.3.2 Overall water quality

Chemical composition of AMD before and after treatment using a hybrid technology were compared to the DEA guidelines standard for effluent discharge and DWS guidelines standard for drinking water and the results are shown in **Table 7.1.**

Table 7.1: Concentrations of chemical species for untreated and treated AMD against the DEA effluents discharge limits and DWS drinking water quality standard (all units in mg/L except pH and EC)

Parameters	Feed AMD	Treated Final water	Removal efficiency (%)	DEA guidelines for effluent discharge	DWS guidelines for drinking water
pH	2.6	10.4	8.8 (increment)	6-12	5.5-9.7
TDS	3380(mg/L)	780.3	79.88	2400	0-1200
EC	5000μS/cm	0.7	86	150μS/cm	0-700μS/cm
Al	158 (mg/L)	0.71	99.5	20	0-0.9
Cu	4.2 (mg/L)	0.11	97.59	20	0.1
Fe	341 (mg/L)	0.66	99.80	50	0-0.1
Mn	37 (mg/L)	0.28	99.24	20	0-0.05
Ni	3.92 (mg/L)	0.09	97.3	10	0-0.07
Zn	8.55 (mg/L)	0.14	98.36	20	0-0.5
Sulphate	3137(mg/L)	608.7	80.59	2400	0-500

As shown in **Table 7.1**, the parameters of concern in raw AMD were pH, TDS, EC, major ions (Al, Fe, Mn and ions sulphate) and trace ions (Cu, Ni and Zn). After the treatment of AMD with hybrid technology (A combination of neutralisation with activated magnesite and a series of constructed wetland), the product water had an increased pH, with reduced TDS, EC, metals species and SO_4^{2-} concentration. The reported results are similar to what has been reported in literature for chemical treatment (Masindi *et al*., 2015; Masindi, 2016). The values of pH, TDS, EC, metals (Al, Fe, Cu, Mn. Ni, Zn) and SO_4^{2-} were within the DEA for effluent quality guidelines standard. The combination of neutralisation with a series of CWs significantly improved the quality of AMD water by increasing the pH, removing a huge quantity of metals, SO_4^{2-} thereby reducing TDS and EC to acceptable effluent quality guidelines. The findings further revealed that, drinking water standard as set by the DWS cannot be directly reclaim from this technology taking into account the hydraulic retention time (one hour for chemical treatment and 30 days for bioremediation) since the pH (10.4) and some metals concentration [Al (0.71 mg/L), Fe (0.66 mg/L), Mn (0.28 mg/L), Ni (0.09 mg/L), Zn (0.14 mg/L) and SO_4^{2-} (608.7 mg/L) were slightly above the required limit for drinking water. However, to reclaim product water that can meet drinking water standard, the hydraulic retention time of the bioremediation step can be extended since plants were still healthy after 30 days retention time. Pollutants were mostly removed by the chemical treatment step while the CWs accounted for a small portion of overall pollutants removal hence indicating it as polishing stage. The presence of Fe and sulphate in elevated amount denotes that this AMD is from the oxidation of pyrite. PHREEQC confirmed the chemical species existed as divalent, trivalent, oxyanions, and oxycations.

7.3.3 Variation of metal concentrations in the substrate

The media plays an important role in wetland since it provides a suitable condition for pollutant removal. Pollutant reduction in wetland is accomplished through various mechanisms which include sedimentation, filtration, precipitation, adsorption, microbial interactions, and plants uptake through absorption (Ventura *et al*., 2019, 2021). The analysis of substrate for metals concentration was done and the results are shown in **Table 7.2.**

Table 7.2: Variation in metals content in wetland substrate

Metals	HRT	SSVF-CW		FWS-CW		SSHF-CW	
		Treatment	Control	Treatment	Control	Treatment	Control
Al	Initial concentration	20.02	20.02	20.02	20.02	20.02	20.02
	Day 0	20.6	20.02	20.02	20.02	20.3	20.02
	Day 5	20.62	19.85	20.41	19.23	20.87	19.84
	Day 10	21.84	19.25	20.01	18.84	19.08	19.43

	Day 15	21.55	19.02	19.32	18.21	18.58	18.87
	Day 20	21.34	18.95	19.24	17.75	18.39	18.23
	Day 25	21.27	18.79	19.13	17.46	18.23	17.76
	Day 30	20.28	18.70	19.08	17.12	18.11	17.38
Cu	Initial concentration	0.5	0.50	0.5	0.5	0.5	0.5
	Day 0	0.53	0.50	0.52	0.5	0.5	0.5
	Day 5	0.58	0.50	0.56	0.4	0.47	0.39
	Day 10	0.60	0.49	0.49	0.4	0.41	0.25
	Day 15	0.51	0.48	0.37	0.2	0.33	0.17
	Day 20	0.46	0.27	0.28	0.19	0.17	0.06
	Day 25	0.38	0.17	0.26	0.08	0.11	0.00
	Day 30	0.22	0.07	0.16	0.01	0.08	0.01
Fe	Initial concentration	60.5	60.5	60.5	60.5	60.5	60.5
	Day 0	60.5	60.50	61.59	60.5	60.2	60.2
	Day 5	61.2	60.20	62.23	60.01	59.27	58.23
	Day 10	62.16	59.70	62.07	59.76	58.11	57.88
	Day 15	61.13	58.61	60.23	58.85	57.55	57.35
	Day 20	59.89	58.48	57.48	56.41	57.26	56.58
	Day 25	58.07	56.89	56.12	55.36	56.03	55.33
	Day 30	57.20	56.37	55.29	54.30	56.34	55.17
Mn	Initial concentration	10.1	10.1	10.1	10.1	10.1	10.1
	Day 0	10.2	10.1	10.1	10.1	10.1	10.28
	Day 5	10.51	10.1	10.21	09.75	09.75	10.16
	Day 10	10.45	9.83	10.14	09.43	09.23	08.95
	Day 15	10.13	9.56	9.37	08.89	08.87	08.68
	Day 20	10.05	9.22	9.21	08.51	09.09	08.33
	Day 25	9.79	9.01	9.05	08.14	08.91	07.85
	Day 30	9.24	8.95	8.66	07.79	08.16	07.42
Ni	Initial concentration	0.07	0.07	0.07	0.07	0.07	0.07
	Day 0	0.08	0.07	0.07	0.07	0.07	0.07
	Day 5	0.10	0.07	0.08	0.06	0.05	0.03
	Day 10	0.18	0.068	0.06	0.05	0.01	0.01
	Day 15	0.24	0.06	0.05	0.03	0.008	0.00
	Day 20	0.15	0.064	0.02	0.01	0.003	0.00
	Day 25	0.09	0.064	0.02	0.00	0.001	0.00
	Day 30	0.04	0.061	0.02	0.00	0.001	0.00
Zn	Initial concentration	4.1	4.1	4.1	4.1	4.1	4.1
	Day 0	4.13	4.1	4.11	4.1	4.1	4.1
	Day 5	4.28	4.21	4.18	3.96	3.96	2.99
	Day 10	4.19	3.97	4.29	3.54	3.54	2.67
	Day 15	3.42	3.25	3.26	3.21	3.11	2.34
	Day 20	3.17	2.89	3.01	2.98	2.98	2.18
	Day 25	3.11	2.43	2.91	2.67	2.17	2.15
	Day 30	3.07	2.37	2.98	2.11	2.31	2.09

As shown in **Table 7.2**, the results of metals analysis in the substrate revealed that in SSVF-CW and FWS-CW, there was an increase in metals concentrations in the beginning of the experiment followed by a decrease of metals content and in some cases (Fe, Cu, Mn, Ni and Zn) below the initial concentration but at different dates (**Table 7.2**). Substrate plays a huge role in wetland acting as catalyst to improve the metals removal process (Batool and Saleh, 2020). The slight increase of metals content in substrate may be attributed to the continuous load of metals in the system while the decrease of metals content in substrate may be attributed to plants uptake, rhizofiltration, precipitation and sedimentation within the wetland (Vandenkoornhuyse *et al*., 2015). However, in the SSHF-CW, the fate of Al and Mn in the substrate followed the same trend as in SSVF-CW and FWS-CW while the concentrations of Cu, Fe, Ni and Zn decreased gradually from the beginning to the end of the experiment. The fate of Al and Mn in SSHF-CW may be attributed to load of metals from SSVF-CW and FWS-CW since the results revealed that the contribution of SSHF-CW in Al and Mn removal was up to 31% for each metal. The gradually decrease of Cu, Fe, Ni and Zn in SSHF-CW may also be attributed to the role played by SSVF-CW and FWS-CW in the removal of above mentioned metals since the concentrations of above cited metals were very low in the AMD water entering the SSHF-CW and as result for their metabolisms, plants (*Vetiveria zizanioides*) growing in SSHF-CW accumulated metals from substrate. In the control staged wetland, the concentration of all metals in the substrate were below the initial concentration in the substrate and this may be attributed to the role played by magnesite since the neutralization of potable fresh water allowed to completely remove metals in tap water and as results plant growing in control wetlands accumulated metals from substrate for their metabolism. These results demonstrated that heavy metals were retained in the substrate of each wetland thereby suggesting that sedimentation could act as sink for heavy metals (Batool and Saleh, 2020; Ventura *et al*., 2021). Metals retention in the substrate of each wetland may also be attributed to the association occurring between metals and particulate matter which settles in the bottom of the wetland (Wang *et al*., 2017). The lower concentrations of Cu and Ni from SSHF-CW substrate may be attributed to the initial concentration of the aforementioned metals in substrate which were very low (Cu = 0.5 mg/L and Ni =0.07 mg/L) and plant uptake since they play important role in plant metabolism; For instance, nickel regulates urea level in plant by transforming urea nitrogen into usable ammonia within the plant and the nickel deficiency in plant leads to elevated level of urea which become toxic to plant (Fabiano *et al*., 2015**)** while copper is involved in many enzymes productions and plays huge role in the photosynthesis process, plant respiration and metabolism of carbohydrates and proteins (Pietrini *et al.*, 2019). Overall, the substrate plays a huge role in constructed wetland since it is suitable medium for sedimentation, rhizofiltration and precipitation of pollutants which are later accumulated by plants.

7.3.4 Metals contents in plants

At the end of the experiment (30 days retention time), one plant was removed from each wetland without damage to the roots, rinsed with distilled water and air dried for two weeks. Plants were later separated into roots and shoot following by metals content determination as previously mentioned in **section 7.3.5** and the results of metals content in plants portion are presented in **Table 7.3**.

Table 7.3: Metals contents in different portal of plant

Metals	Plant portal	SSVF-CW		FWS-CW		SSHF-CW	
		Treatment	Control	Treatment	Control	Treatment	Control
Al	Root	1.31	0.29	1.28	0.04	0.08	0.03
	Shoot	0.04	0.03	0.00	0.02	0.00	0.01
Cu	Root	0.62	0.03	0.05	0.01	0.03	0.00
	Shoot	0.00	0.00	0.06	0.00	0.02	0.00
Fe	Root	2.47	1.08	3.96	1.40	3.09	3.18
	Shoot	1.04	0.05	1.25	0.81	1.07	1.15
Mn	Root	0.07	0.09	0.58	0.67	0.56	1.05
	Shoot	0.13	1.06	0.86	1.69	0.98	0.63
Ni	Root	0.01	0.004	0.01	0.00	0.00	0.00
	Shoot	0.00	0.001	0.00	0.00	0.00	0.00
Zn	Root	0.07	1.02	0.83	1.33	1.26	1.10
	Shoot	0.01	0.71	0.28	0.66	0.53	0.61

The results in **Table 7.3 showed** that metals concentration in roots were very low. The low concentration of metals in roots may be attributed to the neutralization role cryptocrystalline magnesite since the removal efficiency of metal after treatment by cryptocrystalline magnesite was very high (92%, 80%, 95%, 96%, 88% and 92%) for Al, Cu, Fe, Mn, Ni and Zn, respectively. The findings also revealed that the concentration of metals in roots from treatment wetland decreased gradually from SSVF-CW (1st wetland to received magnesite treated AMD) to SSHF-CW (3rd wetland to received magnesite treated AMD) thereby confirming findings of EDS analysis which showed that the percentage of metals in roots decreased gradually following the disposition of wetlands in the staged hybrid system. In the control staged system, the findings revealed that metals concentration in plants portal were very low for all metals of concern except and follow the same trend as compared to treatment staged wetland. These results thereby confirming that metals were completely removed after treatment with magnesite and the low level of metals in plant portal may come from wetland substrate.

7.4 Characterisation of the samples

This section will discuss mineralogical composition of activated cryptocrystalline magnesite, water reacted magnesite, AMD-reacted magnesite,

7.4.1 Mineralogical compositions

The mineralogical compositions of feed and product materials after the treatment of AMD are shown in **Figure 7.11 (a-f)**.

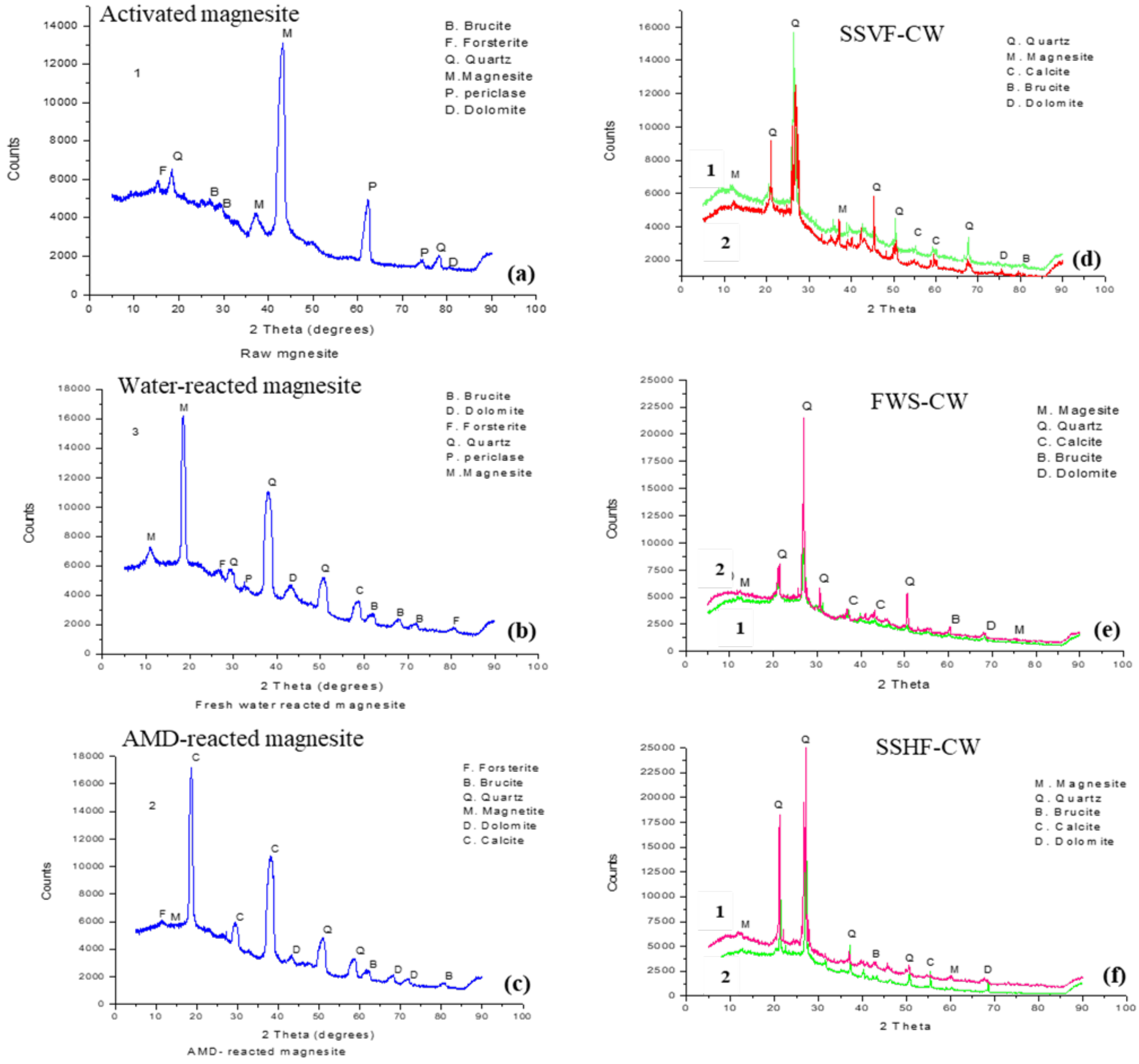

Figure 7.11 (a-f): XRD patterns of feed and product materials.

As shown in **Figure 7. 11 (a),** the XRD analysis revealed that raw magnesite contains magnesite, periclase, brucite, dolomite, forsterite and quartz. Following the reaction of magnesite with AMD water, calcite, brucite, magnetite were found to be present (**Figure 7.11b**). The presence of calcite, dolomite, brucite and magnetite in AMD-reacted magnesite indicates appropriate conditions for the precipitation of calcium, magnesium and iron bearing chemical species. In the AMD-reacted magnesite, periclase was absent and this may be attributed to the dissolution of magnesium oxide (MgO). In the fresh water reacted magnesite, the XRD analysis revealed the presence of periclase, brucite, dolomite, forsterite, quartz and dolomite which are found in activated magnesite (**Figure 7.11c**). Although some of the minerals will be amorphous in nature as predicted by PHREEQC, there are new mineral phases which will be formed from the interaction of activated magnesite and AMD. This could also be explained by the water quality, FTIR, and SEM-EDS results.

As shown in **Figure 7.11 (d - f)**, the X-ray diffraction of substrate from the staged wetland (treatment and control) showed several peaks spread over the range 2Θ from 10° to 68° however, at different intensity. The peaks at 2Θ= 20.6°, 28°, 45° and 50° may correspond to Quartz (SiO_2) which is the major elemental composition of compost (Biyada *et al.*, 2020). The peak at 2Θ = 43° may correspond to calcite (Lee *et al*., 2010) while the peak at 2Θ = 68° may correspond to dolomite (Yu *et al*., 2017). The difference in intensity may be attributed to the chemical composition of aqueous solution fed into the wetland: AMD treated with cryptocrystalline magnesite for treatment wetland and fresh water reacted with cryptocrystalline magnesite for control wetland. Furthermore, XRD analysis revealed the inorganic composition of substrate and it follows that the presence of calcite may originated from calcium carbonate ($CaCO_3$) (Sharma *et al*., 2019) which is the most inorganic components of compost soil used as wetland substrate while dolomite and brucite may originate from magnesite (Masindi *et al*., 2015)**.**

7.4.2 Elemental composition

The elemental composition of raw magnesite, tap water reacted magnesite and AMD-reacted magnesite is shown in **Table 7.4**.

Table 7.4: Elemental composition of raw magnesite, tap water reacted magnesite and AMD-magnesite.

Sample (wt%)	Raw magnesite	Tap water- reacted magnesite	AMD- reacted magnesite

SiO_2	6.08	6.10	5.23
Al_2O_3	0.68	0.71	0.59
Fe_2O_3	0.52	0.54	2.44
MnO	0.01	0.03	0.46
MgO	82.10	78.02	60.25
CaO	2.37	2.40	4.23
Na_2O	0.11	0.09	0.10
K_2O	0.04	0.01	0.03
SO_3	0.19	0.5	8
LOI	7.31	11.20	18.02
Total	99.37	99.6	100.35
H_2O	1.61	1.82	2.01
As	< 4	< 2	8
Ba	< 5	< 3	8.4
Br	2.5	< 0.5	< 2
Co	< 1	< 0.3	5.9
Cr	3.7	3.7	14
Cu	6.01	6.03	10.1
Ga	1.1	1.1	1.8
Hf	8.6	8.7	10.5
Nb	155	155	222
Ni	10	10.8	132
Pb	3.7	3.7	19.3
Se	10	10.1	19
Sr	5.2	5.7	81
Ta	4.1	4.2	20
Y	2	2.1	29
Zn	1	1.2	37
Zr	< 2	0.05	4.9

As shown in **Table 7.4**, the results revealed that after reaction of magnesite with tap water, there was a significant variation of Al_2O_3, As, Ba, CaO, Cu, H_2O, K_2O, LOI, MgO, Na_2O, Nb, Ni, Se, SiO_2, Ta, Y, Zn and Zr with raw magnesite for major and trace element. The results obtained with activated magnesite are in line with the results from study by Masindi *et al.* (2015). In their study, they reported that magnesite was characterised by 82 wt% of MgO and few others impurity. However, after reaction with AMD, there were significantly increase of Fe, Ca, Mn and S indicating possible formation of new phases. The level of major element such as Al and Si decreased and this may indicate possible dissolution. The elemental composition of trace element revealed the presence of As, Ba, Co, Zr in

AMD-reacted magnesite while others elements such as Br, Cu, Ni, Pb, Ta, Hf, Se, Sr, Nb were found to increase in AMD-reacted magnesite and this may indicate the formation of new phases thereby confirming the results revealed by in SEM-EDS analysis. PHREEQC geochemical model also confirmed the formation of new mineral phases. At the end of the experiment, soil samples (substrate) were collected from each wetland and X-ray fluorescence analysis was performed on each sample and the elemental composition result are shown in **Table 7.5.**

Table 7.5: Elemental composition of initial substrate, AMD-reacted substrate, and tap-water reacted substrate.

Samples (wt%)	Initial soil	Soil from staged treatment wetland			Soil from control staged wetland		
		SSVF-CW	FWS-CW	SSHF-CW	SSVF-CW	FWS-CW	SSHF-CW
Fe	84.9026	86.8821	85.3174	84.9888	84.5621	84.2078	84.1258
Ti	4.6132	3.1235	2.5821	2.3402	2.6512	1.7438	1.2478
Rb	3.24	5.0512	4.4900	3.3801	2.2341	2.4789	2.5318
Sr	2.70	3.5951	3.0956	2.6804	3.7852	1.2681	2.2586
Mn	2.29	3.4015	2.6897	2.97	2.0360	1.0984	1.0398
Mg	--------	4.2589	3.2578	1.2583	2.5861	--------	--------
Zn	0.68	0.6885	0.7479	0.7123	0.0039	0.0021	0.0028
Si	0.1393	0.0591	0.0364	0.0281	0.3214	0.0645	0.0654
Al	---------	1.0825	0.0396	---------	0.0189	0.1131	0.0326
Na	---------	0.4430	0.1832	0.0421	0.0278	---------	0.0145
Ni	5.4505	0.2570	0.8845	0.1457	0.1486	0.3108	0.0189
Zr	----------	1.0527	0.0984	0.0256	1.0035	2.1532	0.1247
Cl	----------	0.1246	0.0085	---------	0.0589	---------	--------
Cr	----------	1.0231	0.0541	---------	---------	0.2589	0.0126
Ca	----------	0.1178	0.0052	0.0289	---------	0.0893	0.0754
Cu	----------	0.0531	0.0089	0.0021	---------	0.089	0.0458

As shown in **Table 7.5**, the XRF results of soil samples from different wetland revealed that element such as Fe, Mn, Rb and Zn were found to increase in the soil samples from staged treatment wetland and almost stable in soil samples from control staged wetland. The increase of these elements in soil samples from staged treatment wetland may originate from AMD water. Magnesium (Mg) was absent in initial soil samples but was found to be present in soil samples from both treatment and control staged wetland and it may be originated from magnesite. Nickel was found to decrease in soil samples from both staged wetland and the decrease of nickel may be attributed to possible dissolution. Copper, calcium and chrome were absent in initial soil samples but were found to be present in soil samples from staged treatment wetland and their presence may be originated from AMD water.

7.4.3 Elemental spectra by EDS analysis

To understand the interaction approach of AMD with magnesite, AMD-reacted magnesite, and the roots (SSVF, FWS, and SSHF constructed wetland), The SEM-EDS of feed and product materials were done and the results are shown in **Figure 7.12**.

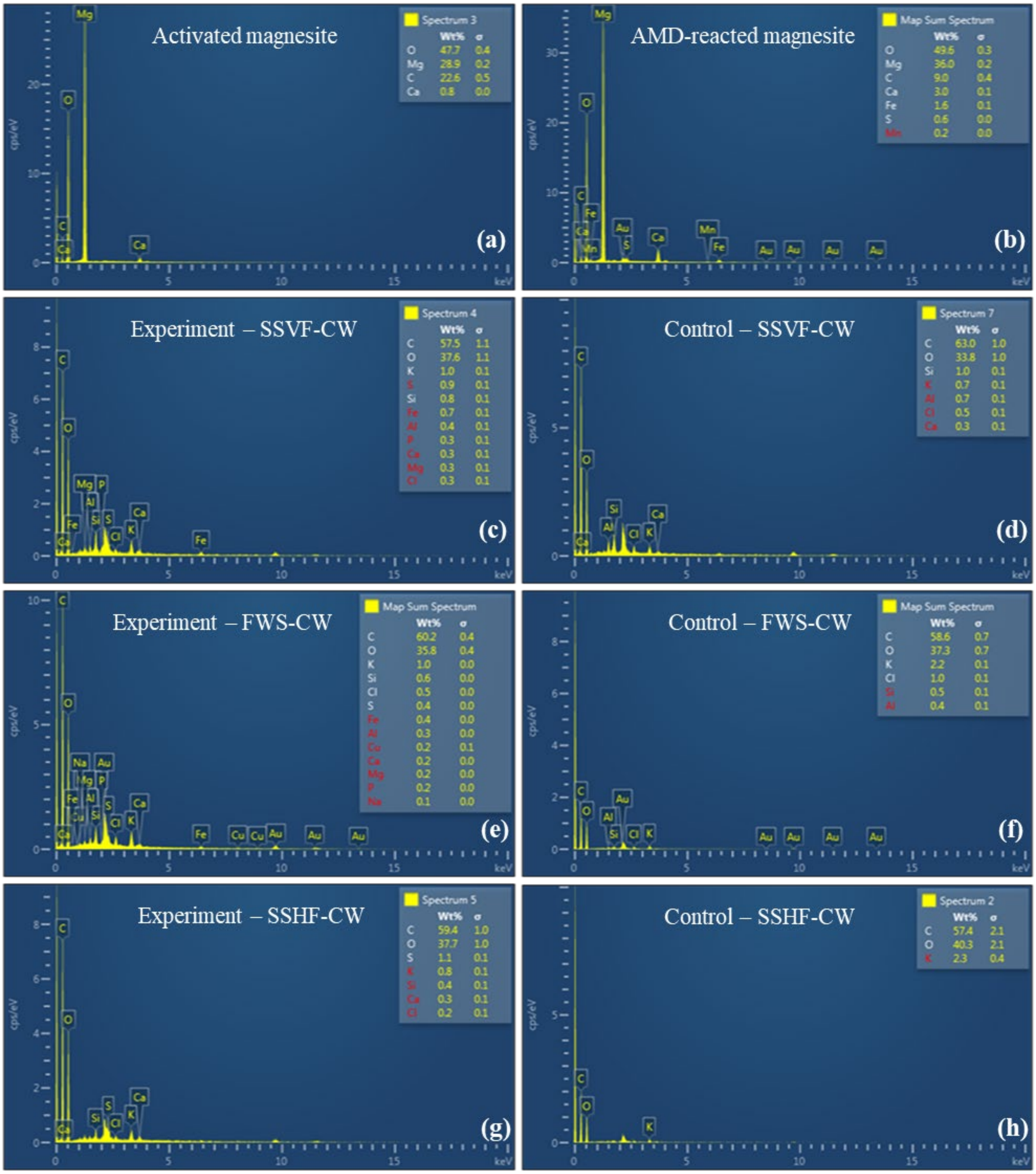

Figure 7.12: The EDS analysis of roots from treatment staged wetland (A, C and E) and from control staged wetland (B, D and F).

As shown in **Figure 7.12 (a-h),** raw magnesite contained oxygen, magnesium and carbon with percentage composition of 47.7%, 28.9%, 22.6 and 0.8 % respectively and impurity of calcium at 0.8% concomitantly observed (**Figure 7.12a**). High level of carbon, oxygen, magnesium and low level of calcium confirm the heterogeneous structures thereby indicating the material is magnesite. The EDS spectra for AMD-reacted magnesite indicated an increase in oxygen, Magnesium, and calcium. However, iron, sulphur and manganese were observed to be present hence confirming their removal from AMD to the product sludge. The formation of mineral phases with iron, manganese and sulphur can be translated to the reduction of metals and sulphate in neutralized product AMD water. The presence of Magnesium and calcium may be attributed to the presence of brucite and calcite as shown in the XRD results. Furthermore, the obtained results were confirmed using PHREEQC geochemical model.

The EDS of roots from SSVF-CW treatment and control staged wetland revealed high level of Carbon, Oxygen (**Figure 7.12b-c**) and the presence of silica in control of SSVF-CW. In the treatment staged wetland, the level of Fe, Al, S, P, Ca, Mg and Cl in root were observed to decrease gradually from the first wetland (SSVF-CW) to the third wetland (SSHF-CW) where Fe and Al were not present thereby confirming the water quality results. The same trend was observed in root of the staged control wetland where Fe, S, P and Mg were not present in the root from all wetlands [**Figure 7.12(c-f)**]. Al, Cl, Ca and Si were present in root from SSVF-CW control wetland **(Figure 7.12c**), but their level gradually decreased in root from FWS-CW control wetland (**Figure 7.12d**) and finally to below detection limit of 0.0001 mg/L in root from SSHF-CW control wetland (**Figure 7.12h**). The low level of some elements in roots from SSVF-CW treatment wetland and the absence of the same element in root from SSVF-CW control wetland can be attributed to the neutralization action of magnesite which removes a huge quantity of metals. The gradually decrease in the level of element from both treatment and control staged wetland can be explained by the polishing role of each wetland since pollutants in neutralised AMD water were removed gradually as long as it passed throughout the system.

7.4.4 Morphological properties of activated magnesite and the roots

To understand the interaction approach of AMD with magnesite, AMD-reacted magnesite, and the roots, the SEM images of feed and product stocks were done and the results are shown in **Figure 7.13**.

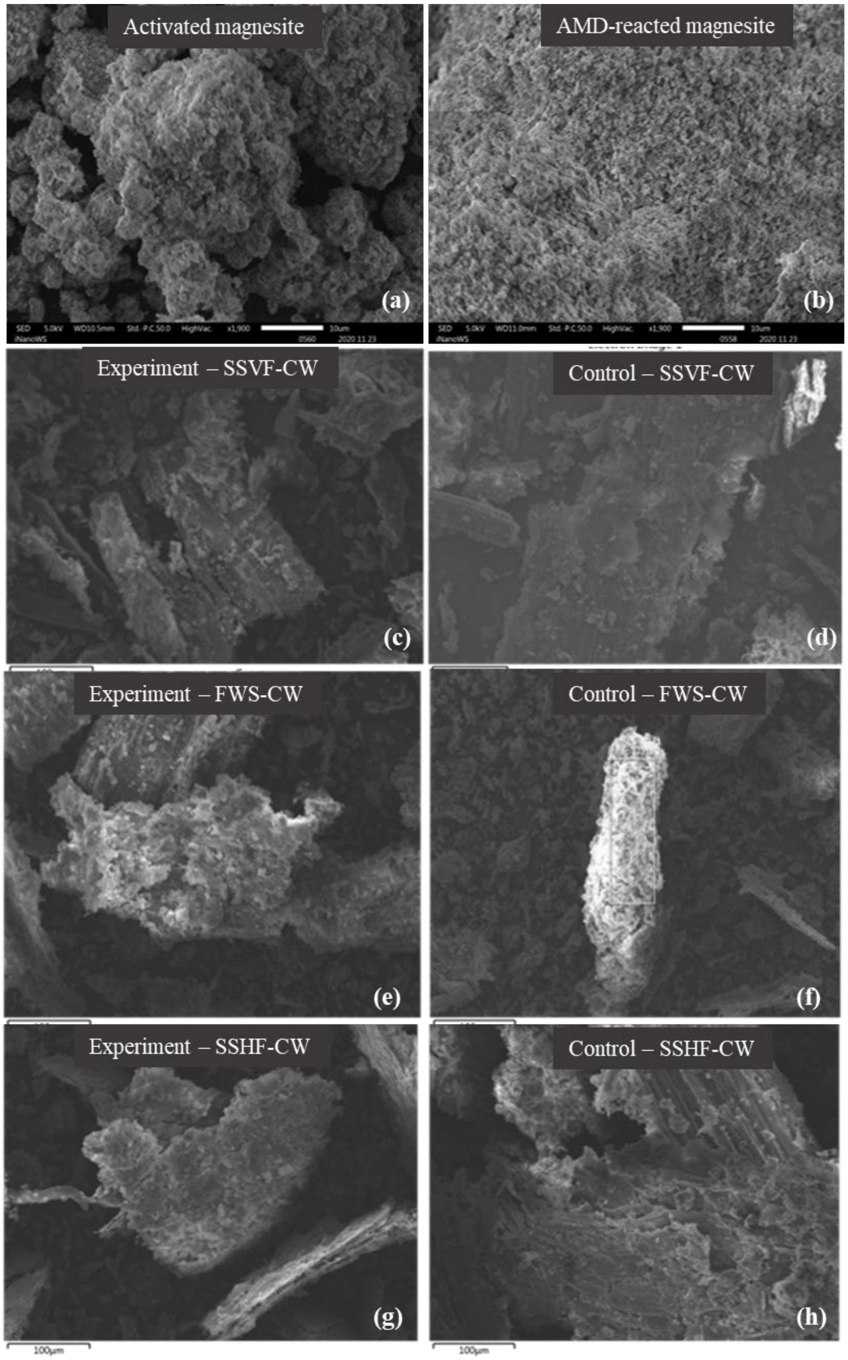

Figure 7.13: The SEM image of the feed and product materials.

As shown in **Figure 7.13(a-b),** the morphological properties of activated magnesite comprised cylindrical, foliage like and shaped structures indicating a heterogeneous material (**Figure 7. 13a**). After the contact of AMD with magnesite the spaces between the cylindrical form foliage like and shaped structures clustered together to form a complete mass as observed in **Figure 7. 13b** and this may be attributed to the precipitation of metals from AMD after contact with magnesite.

Furthermore, **Figure 7.13(c-h),** the results revealed that SEM images of roots from both treatment and control staged wetland show very few noticeable changes and this may be attributed to vital role played by magnesite in removing a huge quantity of pollutants after treatment of AMD water by cryptocrystalline magnesite. However, SEM images of roots from staged treatment wetland show a sort of palettes spread out within the surface while the SEM images of roots from SSVF-CW and FWS-CW in staged control wetland show a dense mass surrounded by an aggregate of small palettes whereas the SEM image of root from SSHF-CW control show a dense mass occupying the whole surface.

7.4.5 Metals functional groups of activated magnesite and the roots

To understand the interaction between activated magnesite and the roots before and after contacting AMD, the metals functional groups were determined using FTIR analysis and the results are shown in **Figure 14 (a-d).**

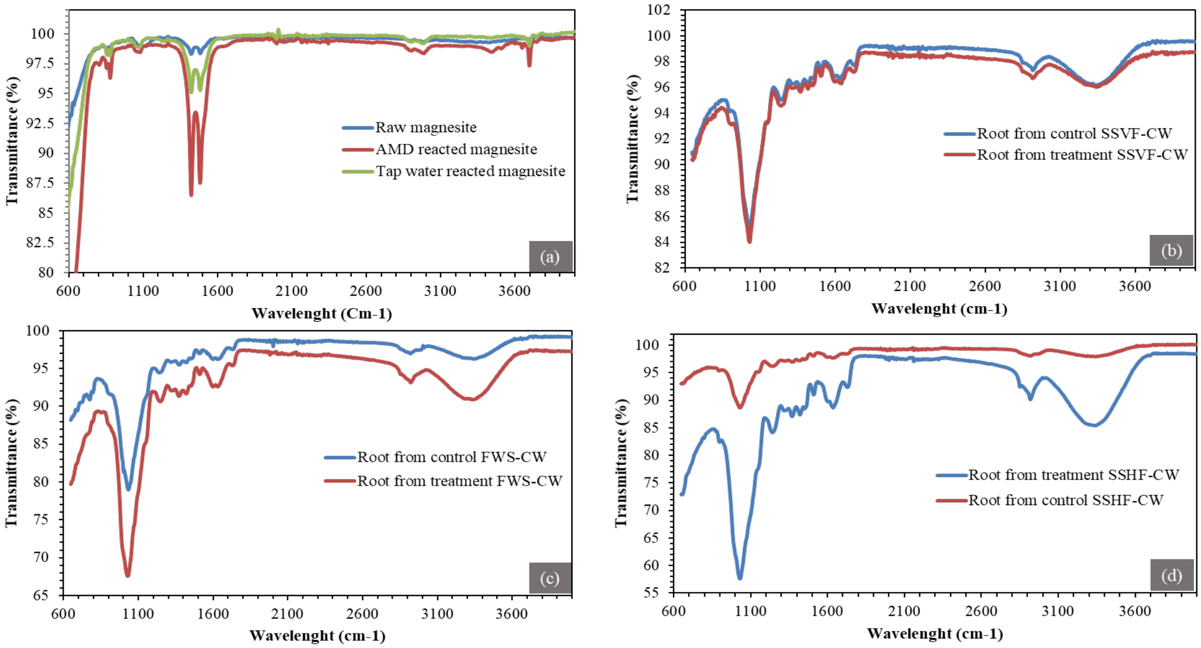

Figure 7.14 (a-d): Metal functional groups for raw and reacted magnesite and roots.

As shown **Figure 7.14(a),** the results showed a system of bands in raw magnesite and it may be characteristic of brucite since it is a mineral form of magnesium hydroxide and it correspond to band 902 cm^{-1} (Makreski and Jovanovski, 2003). Brucite alters commonly with periclase and the bands at 1100 cm^{-1} may correspond to periclase stretching vibration (Gopinath and Gunasekaran, 2018) while the doublet at 1330 and 1588 cm-1 correspond to asymmetric elongating of carbonate likely due to the formation of calcium carbonates ($CaCO_3$) and magnesium carbonates ($MgCO_3$) (Ivashchenko *et al*., 2016). In tap water reacted magnesite, the system of band at 900, 1038 and 1192 cm^{-1} is characterised of brucite and may be associated with OH group adsorbed water after reaction with tap water (Gopinath and Gunasekaran, 2018). In the AMD-reacted magnesite, the bands at 878 and 1072 cm^{-1} is characterised of brucite while band at 1432 and 1492 cm^{-1} may be associated with OH group adsorbed water (Masindi, 2016). The system of bands for reacted magnesite at 3700, 3702 and 3706 cm^{-1} is characterising of the stretching of brucite (Masindi *et al*., 2015). The presence of these metals was confirmed by XRF and PHREEQC.

As shown **Figure 7.14(b),** the results indicated complex accumulation of pollutants from both SSVF-CW treatment and control wetland **(Fig. 14b**). The spectrum of the root grown in control wetland shows a band with peak at 856 cm^{-1} which shitted to a peak at 890 of the root grown in treatment wetland. The **Figure 7.14b** shows a stretching vibration of root from control wetland at 1209, 1402, 1558 and 1871 cm^{-1} which shifted to strong banding elongating vibration at 1351, 1516, 1689 and 1949 cm^{-1} respectively and it may be attributed to the presence of carboxyl group (C=O) (RoyChowdhury *et al*., 2020) from water in both SSVF-CW (treatment and control). The signal at 2770 cm^{-1} for root from control and at 2912 cm^{-1} for root from treatment wetland may be attributed to the OH group which was shifted to form a broad band between 3000 cm^{-1} and 3510 cm^{-1} for root from control wetland and between 3227 cm^{-1} and 3614 cm^{-1} for root from treatment wetland (Yu *et al*., 2017).

As shown **Figure 7.14(c),** the spectrum shows a band with a peak at 825 and 842 cm^{-1} for root grown in control and treatment wetland respectively corresponding to Si-O functional group (Yu *et al*., 2017). Those peaks were followed by strong vibration corresponding to band between 867 and 965 cm^{-1} for roots grown in control wetland and between 984 and 1089 for root grown in treatment wetland corresponding to SO_4 functional group (Tabelin *et al*., 2018). Both spectra showed a series of vibrations with bands at 1198, 1295 and 1489 cm^{-1} for both root corresponding to C=O functional group (McDonagh and Chinga-Carrasco, 2020) and 1683 cm^{-1} for root grown in control wetland and 1697 cm^{-1} for root grown in treatment wetland corresponding to C=O functional group (RoyChowdhury *et al*., 2020) and may indicate the accumulation of pollutants by the roots. The spectrum also shows in signal at 2842 cm^{-1} and 2915 cm^{-1} for root grown respectively in control and treatment wetland. These signal may correspond to O=H functional group (Yu *et al*., 2017). However those signals were shifted to form a broad band between 3165 and 3459 cm^{-1} for control root and between 3169 and 3473 cm^{-1} for treatment root (McDonagh and Chinga-Carrasco, 2020).

As shown **Figure 7.14(d),** the spectrum of root from SSHF-CW shows a peak at 836 cm^{-1} for root grown from both treatment and control wetland followed by stretching vibration between at 981 and 995 cm^{-1} for control root and between 1000 and 1100 cm^{-1} for treatment corresponding to SO_4 functional group (Tabelin *et al*., 2018). The spectra of root from both treatment and control wetlands showed a series of vibration between 1150 and 1800 cm^{-1} followed by straight band and a lightly vibration between 3187 and 3448 cm^{-1}. The series of vibration may correspond to C=O and O=H functional group (Kannappan *et al*., 2017; McDonagh and Chinga-Carrasco, 2020) while the vibration between 2914 cm^{-1} and 3492 cm^{-1} may correspond to O=H functional group (Yu *et al*., 2017; McDonagh and Chinga-Carrasco, 2020).

7.5 Conclusions and recommendations

This study successfully confirmed the use of a hybrid system for the treatment of acid mine drainage (AMD). This study has shown that the combination of activated magnesite and a series of constructed wetland was efficient to treat AMD. Contact of magnesite with AMD for one hour duration led to an increase of pH from 2.6 to 9.8 and significant reduction of EC, TDS, $SO4^{2-}$ and metals (Al, Cu, Fe, Mn, Ni and Zn). The application of staged constructed wetland equipped with *Vetiveria zizanioides* further increased the pH from 9.8 to 10.4 leading to more pollutants removal with an overall removal efficiency of 86%, 79.88%, 80.59%, 99.5%, 97.38%, 99.8%, 99.24%, 97.7% and 98.36% for EC, TDS, sulphate, Al, Cu, Fe, Mn, Ni and Zn respectively. The finding revealed that the removal efficiency of hybrid system to pollutants removal from AMD was in in the following order: Fe (99.8%) > Al (99.5%) > Mn (99.24) > Zn (98.36%) > Ni (97.7%) > Cu (97.38%) > EC (86%) > SO_4^{2-} (80.59%) > TDS (80%). This was significantly high in relation to the required standard. The activated magnesite treatment step significantly contributed to the overall removal of pollutant with the removal efficacies obeying the following order: Mn (96%) > Fe (95%) > Zn (92.04%) > Al (92%) > Ni (88.01%) > Cu (80%) within one hour duration while the constructed wetland step accounted for a smaller fraction of pollutants removal in 30 days of retention time with removal efficiency as follows: EC (28%) > SO_4^{2-} (23.78%) > TDS (19.88%) > Cu (17.38%) > Ni (9.7%) > Al (7.55%) > Zn (6.3%) > Fe (4.8%), Mn (3.25%). This study revealed the efficiency of activated magnesite in neutralising AMD and remove huge quantity of pollutants and the polishing role of a series of constructed wetland. However this technology presents some disadvantage such as large land area for the construction of staged wetland and the release of contaminated sludge after treatment with cryptocrystalline magnesite but which can be further decontaminated by phytoremediation process using suitable plants. The PH REdox EQuilibrium (in C language) (PHREEQC) geochemical model confirmed that metals existed as di-and-trivalent complexes in solution. Furthermore, the metals were precipitated as hydroxides and oxy-hydrosulphates. The product water conformed to prescribed standards, specifications, and guidelines for effluent discharge. In light of the findings, this study proved that a synergy of chemical and phytoremediation treatment could potentially yield the desired results in mine water management and this could be deployed to mines to curtail the ecological impacts of AMD.

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CHAPTER EIGHT

General conclusion and recommendations

8.1 Conclusion

The aim of this study was to develop a hybrid approach consisting of an integration of neutralisation and a series of constructed wetland for the treatment of AMD. Several research questions were answered and therefore, the conclusion was drawn in accordance with the research questions and objectives.

Acid mine drainage was collected from Sibanye gold mine in Krugersdorp, Gauteng province, South Africa. The AMD water was characterised by ICP-OES and the selected parameters revealed that the AMD from Sibanye gold mine contained an average of 3137 mg/L of sulphate, very acidic pH, TDS of 3880 mg/L, EC of 5000 μS/cm, 341 mg/L of Fe, 158 mg/L of Al, 37 mg/L of Mn, 8.55 mg/L of Zn, 4.2 mg/L of Cu and 3.92 mg/L of Ni thereby proving that AMD water is highly polluted water that can have severe environmental impact.

The passive remediation of AMD using types of constructed wetland (FWS-CW, SSVF-CW and SSHF-CW) planted with *Vetiveria zizanioides* revealed that constructed wetland planted with *Vetiveria zizanioides* was able to improve the quality of AMD water by a significant reduction of EC, TDS, SO_4^{2-} and metals (Al, Cu, Fe, Mn, Ni, Zn) and slightly increase of pH after 30 days hydraulic retention time. The results also revealed that wetland substrate played a huge role in metals removal since it accounted for high percentage of overall metals removal and this could justify the XRF and XRD analysis results where there was an increase in percentage of metals in elemental and mineralogical composition of substrate from AMD treatment wetland compared to elemental and mineralogical composition of initial substrate and substrate from control wetland. The SEM analysis of roots from treatment and control wetland showed a noticeable difference in morphology with SEM image of root from treatment wetland showing an aggregate of crystal like deposit whereas SEM image of root from control wetland showed a smooth surface while EDS analysis of roots from AMD treatment wetland showed high level of metals compared to EDS analysis of root from control wetland. The FTIR analysis of roots from treatment and control wetland showed a series of vibration at different wave length revealing the quantity of pollutants accumulated by *Vetiveria zizanioides* plant. Overall the constructed wetland (FWS-CW, SSVF-CW and SSHF-CW) using compost soil as substrate and planted with *Vetiveria zizanioides* improved the quality of AMD. However none of the constructed wetland was able to treat AMD water to meet the water quality guidelines standard as set by the Department of Water and Sanitation (DWS) and the Department of Environmental Affairs (DEA) for effluent discharge thereby proving that the wetland (FWS-CW, SSVF-CW and SSHF-CW) operating individually are not suitable for AMD treatment within 30 days hydraulic retention times.

The treatment of AMD using a hybrid technology (neutralisation with magnesite and staged constructed wetland using compost soil as substrate and planted with *Vetiveria zizanioides*) raised the pH of AMD from 2.6 to 10.4 and significantly reduced chemical species (EC, TDS, SO_4^{2-} and metals) to acceptable standard for effluent discharge as set by the DEA and DWS. The study revealed that in the neutralisation step, many biochemical processes such precipitation, adsorption, co-precipitation and ion-exchange were observed to govern the treatment of AMD using magnesite while in the staged hybrid constructed wetland, biochemical processes including sedimentation, filtration, interception and absorption-desorption, convective-diffusion, complexation, redox reaction, precipitation, ion-exchange, neutralisation and plant uptake occurring simultaneously within the system contributed to polish AMD water to acceptable level.

Overall, this study proved that hybrid technology consisting of neutralisation using magnesite and bioremediation using a series of constructed wetland planted with *Vetiveria zizanioides* is better candidate to manage AMD water compared to neutralisation and constructed wetland operating individually. The hybrid technology treated AMD water to acceptable standard as stipulated by the water quality guidelines thereby making it a good candidate to replace conventional AMD treatment methods. Neutralisation using magnesite and different type of constructed wetland operating individually showed significant performance however, they were not fully efficient. This suggests that neutralisation can be used as a pre-treatment step and staged constructed wetland used as polishing step. This successfully proved the hypothesis which state that the integration of neutralisation and constructed wetland planted with *Vetiveria zizanioides* can neutralise AMD and attenuate the enshrined chemical species. However, more studies need to be conducted using AMD from different sources and also investigate the microbial diversity of product water in order to test the efficiency of this hybrid technology (neutralisation and staged hybrid wetland) to recover drinking water from acid mine drainage.

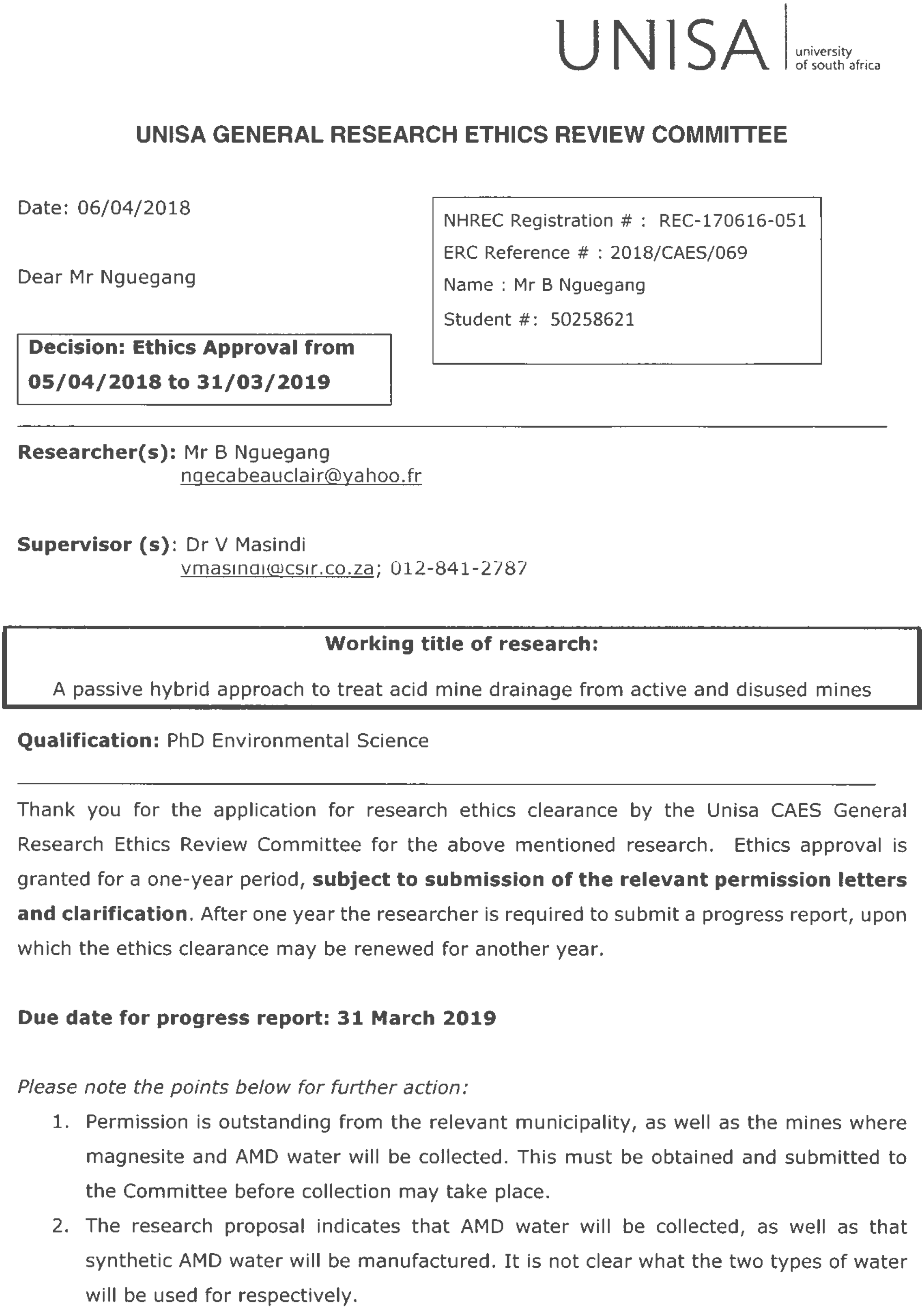
.8.2: Recommendations

The application of hybrid technology (neutralization and staged constructed wetland) showed the potentiality for AMD treatment and product water very close to DWS water quality guidelines standards. However, further research should be conducted in this area in order to consider this technology efficient for the AMD treatment. As such the following research areas could be investigated

- Ability of hybrid technology (neutralisation with magnesite and staged constructed wetland) for the treatment of AMD from different sources including neutral/alkaline mine drainage.
- This study focused on laboratory experiment for neutralisation and semi-scale field experiment for bioremediation. Since AMD formation is natural process that can take place in active and abandoned mine, studies that can mimic natural process under the force of gravity need to be conducted in order to assess the effectiveness of neutralisation and staged constructed wetland for AMD treatment.
- Magnesite and constructed wetland have been used individually for the treatment of AMD. However, the main focus was on physicochemical qualities. Combination of both technologies should also take into consideration the microbial diversity of product water since the microbial diversity is very important parameter in drinking water.
- This hybrid system proved to produce product water close to potable drinking water standard. However, the neutralisation step releases contaminated sludge and in a hybrid technology including, neutralisation and staged constructed wetland, the sludge-waste rock mixture could be used to reduce metals load in the effluent column.

- Techno-economic evaluation, environmental impact assessment, and lifecycle assessment of the proposed hybrid technology towards the treatment of acid mine drainage should be considered in future research initiatives and interventions.

APPENDIX A



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UNISA GENERAL RESEARCH ETHICS REVIEW COMMITTEE

Date: 06/04/2018

NHREC Registration # : REC-170616-051
ERC Reference # : 2018/CAES/069
Name : Mr B Nguegang
Student #: 50258621

Dear Mr Nguegang

Decision: Ethics Approval from 05/04/2018 to 31/03/2019

Researcher(s): Mr B Nguegang
ngecabeauclair@yahoo.fr

Supervisor (s): Dr V Masindi
vmasindi@csir.co.za; 012-841-2787

Working title of research:

A passive hybrid approach to treat acid mine drainage from active and disused mines

Qualification: PhD Environmental Science

Thank you for the application for research ethics clearance by the Unisa CAES General Research Ethics Review Committee for the above mentioned research. Ethics approval is granted for a one-year period, **subject to submission of the relevant permission letters and clarification**. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2019

Please note the points below for further action:

1. Permission is outstanding from the relevant municipality, as well as the mines where magnesite and AMD water will be collected. This must be obtained and submitted to the Committee before collection may take place.
2. The research proposal indicates that AMD water will be collected, as well as that synthetic AMD water will be manufactured. It is not clear what the two types of water will be used for respectively.

3. What is the exact location of the sampling sites? The researcher must also identify the mine from which magnesite will be obtained.
4. How will the AMD water be transported to the Unisa Science Campus? How and where will it be stored once on campus?
5. The permission requests submitted as part of the application is poorly written and unprofessional in appearance. The applicant is reminded that as a student of Unisa his actions reflect on the university, and is urged to have such communications edited before sending them to authorities/institutions.

The ***medium risk application*** *was* ***reviewed*** *by the CAES General Research Ethics Review Committee on 05 April 2018 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:
*The reference number **2018/CAES/069** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

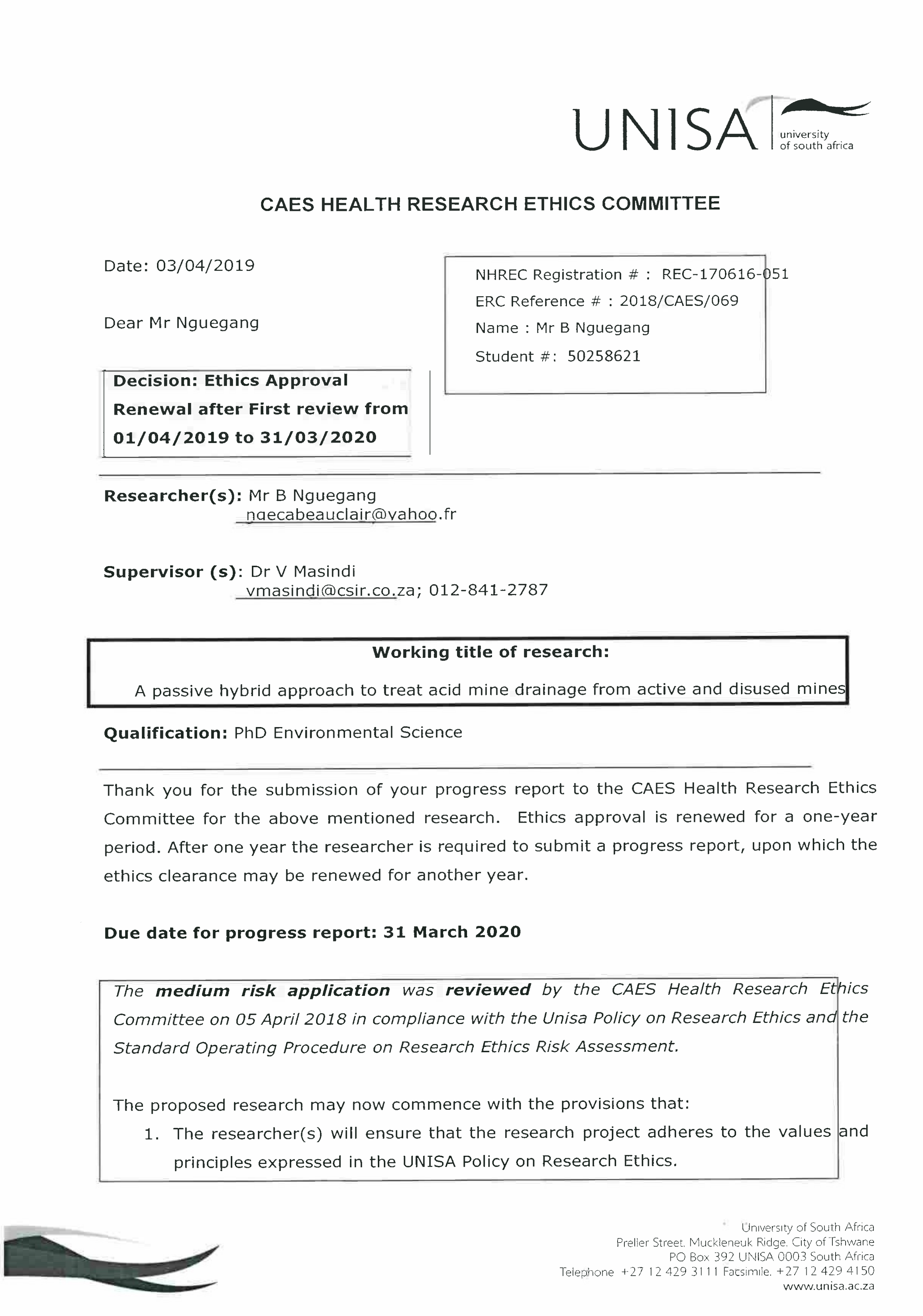
Yours sincerely,

DM Madeso

Prof EL Kempen
Chair of CAES General Research ERC
E-mail: kempeel@unisa.ac.za
Tel: (011) 471-2241

Prof MJ Linington
PP **Executive Dean : CAES**
E-mail: lininmj@unisa.ac.za
Tel: (011) 471-3806

APPENDIX B



UNISA university of south africa

CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 03/04/2019

NHREC Registration # : REC-170616-051
ERC Reference # : 2018/CAES/069
Name : Mr B Nguegang
Student #: 50258621

Dear Mr Nguegang

Decision: Ethics Approval Renewal after First review from 01/04/2019 to 31/03/2020

Researcher(s): Mr B Nguegang
ngecabeauclair@yahoo.fr

Supervisor (s): Dr V Masindi
vmasindi@csir.co.za; 012-841-2787

Working title of research:

A passive hybrid approach to treat acid mine drainage from active and disused mines

Qualification: PhD Environmental Science

Thank you for the submission of your progress report to the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is renewed for a one-year period. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2020

*The **medium risk application** was **reviewed** by the CAES Health Research Ethics Committee on 05 April 2018 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.

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3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:

*The reference number **2018/CAES/069** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof EL Kempen

Chair of CAES Health REC

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Tel: (011) 471-2241

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Executive Dean: CAES

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