

POTENTIAL OF SELECTED MICROALGAE IN BIOREMEDIATION OF
WASTEWATER POLLUTED DAM WATER: A CASE OF GOREANGAB DAM IN
WINDHOEK, NAMIBIA

A RESEARCH THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
(BIOLOGICAL SCIENCES)

OF
THE UNIVERSITY OF NAMIBIA

BY

JENNIFER NDURA

201203121

OCTOBER 2022

MAIN SUPERVISOR: Dr Timothy Sibanda (The University of Namibia)

CO-SUPERVISOR: Dr Earl Lewis (The University of Namibia)

ABSTRACT

The study was aimed at determining the efficacy of freshwater microalgae in the treatment of polluted dam water with respect to the reduction of pathogen loads, as well as the concentrations of heavy metals and nutrients. Microalgae collected to treat water samples from the Goreangab Dam were collected from the Klein Windhoek River, Avis Dam as well as the Goreangab Dam. Identification of the microalgae species revealed the presence of the *Microcystis* species in the Goreangab Dam (control), *Anabaena* species in the Klein Windhoek River (treatment 1) and the *Scenedesmus* species as well as the *Anabaena* species in the Avis Dam (treatment 2). The water samples in 12 L conical flasks each containing 1.5 L of Goreangab Dam water were treated in a greenhouse facility at the University of Namibia for a period of 12 days. Triplicate 100 ml samples were collected from the flask at day 0, day 6 and day 12 and quantitatively assessed for faecal and total coliform bacteria, heavy metals (Pb and Cd) as well as nutrients (phosphorous and nitrates). A one-way analysis of variance (ANOVA) indicated that there were no statistical differences between the mean values of Pb ($p = 0.346$) and Cd ($p = 0.940$) concentrations after treatment with microalgae. The Kruskal Wallis tests indicated statistical differences between the mean values of the nitrate levels ($H(3) = 8.597$, $p = 0.035$) after treatment with microalgae. And the Pair Wise Comparisons indicated significant differences in nitrate uptake between treatment 1 and treatment 2 ($p = 0.039$) and also between treatment 2 and treatment 3 ($p=0.005$). However, the ANOVA indicated that there were no significant differences between the mean values of total phosphate uptake ($p = 0.052$) after treatment with microalgae. The results for pathogen loads in the wastewater samples indicated that there were statistical differences between the mean values of total coliform ($H(3) = 10.352$, $p = 0.015$) after treatment with microalgae. Further, the Pair Wise Comparisons indicated significant differences in total coliform removal between the control and the consortium ($p = 0.040$), between the control and treatment 2 ($p=0.002$) and between treatment 1 and treatment 2 ($p = 0.040$). Additionally, for faecal coliform presence the results indicated statistical differences between the mean values ($H(3) = 10.421$, $p = 0.015$) after treatment with microalgae and the Pair Wise Comparisons indicated significant

differences in faecal coliform removal between the control and treatment 2 ($p = 0.002$), between the control and the consortium ($p=0.041$) and also between treatment 1 and treatment 2 ($p = 0.041$). Given this evidence the use of microalgae in bioremediation is effective in the removal of pathogen loads and excessive nutrients from wastewater.

Keywords: *Microcystis* species, *Scenedesmus* species, *Anabaena* species, bioremediation, pathogens, heavy metals, phosphate, nitrate, Goreangab Dam.

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LIST OF ABBREVIATIONS

ANOVA	One-way analysis of variance
BNR	Biological Nutrient Removal plants
CoW	City of Windhoek
EAEC	Enterogaagregative <i>E. coli</i>
EMB	Eosin methylene blue agar
EPEC	Enteropathogenic <i>E. coli</i>
GWWTTP	Gammams Wastewater Treatment Plants
ICP-OES	Inductively coupled plasma - optical emission spectrometry
MoHSS	Ministry of Health and Social Services (MoHSS)
NGWRP	Goreangab Wastewater Reclamation Plant
OGWRP	Old Goreangab Wastewater Reclamation Plant
PE	Polyethylene
PSTs	Primary Settling Tanks
PTFE	Polytetrafluorethylene
RO	Reverse Osmosis
SPSS	Statistical Package for the Social Sciences
UNAM	University of Namibia

UNESCO	United Nations Educational, Scientific, and Cultural Organization
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
UWTP	Ujams Wastewater Treatment Plant
WHO	World Health Organization

ACKNOWLEDGEMENTS

I would firstly like to thank our heavenly Father for the opportunity that he has granted me with. I take guidance from Psalm 121:2 which states that “my help comes from the Lord, the maker of heaven and earth”.

I further express sincere gratitude to my supervisors Dr. Timothy Sibanda and Dr. Earl Lewis for offering me motivation and helping me complete this research by providing necessary corrections and guidance where necessary, without difficulty.

I would also like to show gratitude to the Namibian Chamber of Environment (NCE) for partially funding my tuition fees for two consecutive years. I am also grateful to the University of Namibia, Biological Sciences laboratory for providing the necessary equipment needed to conduct my research. I would also like to thank Mr. Augustinus Mbangu for his continuous assistance in the laboratory.

DEDICATION

I dedicate this work to my family, Hendrik and Hannse Shali for being my emotional support and motivators; and to my beloved mother (Prieska Ndura) for always praying for me.

DECLARATIONS

I, Jennifer Ndura, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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October 2022

Name of Student

Signature

Date

CHAPTER ONE: INTRODUCTION

1.1. General Introduction

Multiple environmental and anthropogenic stressors such as pollution and climate change are putting water security under threat, especially in arid and semi-arid regions (Kahil et al., 2015). Pollution of water limits the different ways of water usage since its availability is linked to its quality (UNESCO, 2017). Notable sources of water pollution include discharge of effluents from urban wastewater treatment plants into surface water bodies, including rivers and dams (Abdel-Razek et al., 2019), as well as anthropogenic activities such as urban development, industrialization and agricultural activities (Zhao et al., 2012). Wastewater discharges contain inorganic compounds that often result in water eutrophication and ecosystem imbalance, as well as pathogenic microorganisms and heavy metals that render such water bodies a threat to both public and ecological health (Abdel-Razek et al., 2019).

The pollution of water resources exacerbates water scarcity challenges in countries like Namibia that are already regarded as water scarce, the country is the driest in sub-Saharan Africa, with a very hot and dry climate characterised by low and highly variable annual rainfall patterns (Scott et al., 2018). Temperatures in Namibia have been on an increasing trend since the 1970s (Dirkx et al., 2008). The country experiences high evaporation rates with losses of up to 97% of rainfall through evaporation. Windhoek, the country's capital city, experiences an average rainfall of only 360 mm (Namibia National Weather Bureau, 2003). In recent times, the extreme droughts of 2015 and 2017 placed a severe strain on the City of Windhoek's (CoW) capacity to provide clean,

fresh drinking water to its inhabitants (Scott et al., 2018). The authors also stated that water pollution has been identified by CoW as one of the driving forces contributing to limited water supplies. A case in point is the decommissioning of the Goreangab Dam in 2010. The dam used to serve as one of the sources of abstraction water for the drinking water treatment plant that supplies the city but has since been decommissioned owing to its unprecedentedly high pollution loads, with organic matter, nutrients, and heavy metals as the primary pollutants (Faul et al., 2013).

However, green microalgae are known to play an essential role in the bioremediation of wastewater as well as polluted water (Kshirsagar, 2013). Bioremediation is the process in which living organisms such as microalgae are used to eliminate toxic contaminants (Kensa, 2011). Microalgae facilitates bioremediation through its photosynthetic activity which allows it to convert solar energy into biomass, assimilating nutrients such as nitrogen and phosphorus which are responsible for eutrophication. Besides, microalgae mediated water treatment is advantageous in terms of better pathogen and heavy metal removal, as well as generation of energy rich algal biomass (Kshirsagar, 2013).

1.2. Statement of the problem

The Goreangab Dam water is heavily polluted due to the discharge of effluents rich in organic matter and heavy metals from the City of Windhoek (Faul et al., 2013). Due to the mentioned pollutants, the quality of the dam water fails to meet the national standard criteria (Du Pisani, 2006). Studies indicate that the dam is eutrophic and causing fish kills and low macrophyte abundance (Nangolo, 2018). In its current polluted state, the Goreangab Dam also represents a public health concern because of some potential waterborne pathogens, including bacteria, viruses and protozoa (Shilikomwenyo, 2019).

However, despite its polluted state, some local fishermen used to use the dam as a livelihood resource, catching fish for sale, mostly to other poor people who are only happy to buy these affordable fish. Handling and consumption of such fish enhances people's chances of contracting both waterborne and foodborne diseases, which can be acute or chronic, with costly long-term effects such as degenerative heart disease and stomach ulcer (Akpor and Muchie, 2011). Additionally, Lehmann (2010) stated that the water scarcity stress on the CoW is worsened by the current water pollution of the Goreangab Dam. Hence, there is a high need to explore innovative clean-up methods such as bioremediation in order to eliminate the public and ecological health risk posed by the polluted water, as well as to restore the dam's status as one of the CoW's supply dams. To my knowledge, there are currently no scientific studies directed towards the rehabilitation of the Goreangab Dam, hence this study.

1.3. Objectives

The aim of the study was to determine whether treatment of the polluted Goreangab Dam water with various species of freshwater microalgae could significantly reduce its faecal indicator bacteria loads, as well as the concentrations of heavy metals and nutrients.

The specific objectives of the study were:

- i. To collect, isolate and identify microalgae species from various freshwater bodies, including the Avis Dam, Klein Windhoek River, as well as the Goreangab Dam.

- ii. To collect polluted water from Goreangab Dam and determine its faecal indicator bacteria loads, as well as its heavy metal and nutrient concentrations before and after the microalgal bioremediation assay.
- iii. To assess the potential of the selected algal species in the bioremediation of the dam water samples.

1.4. Research hypotheses

The current study tested the following research hypotheses:

1.4.1. H_0 : There is no significant difference in faecal indicator bacteria loads present in the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

H_1 : There is a significant difference in faecal indicator bacteria loads present in the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

1.4.2. H_0 : There is no significant difference in the heavy metal concentrations in the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

H_1 : There is a significant difference in the heavy metal concentrations in the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

1.4.3. H_0 : There is no significant difference in the nutrient levels of the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

H₁: There is a significant difference in the nutrient levels of the wastewater samples from the Goreangab Dam after treatment with three different microalgae species.

1.5. Significance of the study

This study has the potential to improve the water quality of the Goreangab Dam by decreasing the level of hazardous contaminant of the water and potentially eliminating the public health threat currently posed to the Windhoek community by the polluted dam. Further, bioremediation offers advantages such as lower costs and less disruption of the contaminated environment when compared to other cleanup methods. It is a natural process which uses microorganisms to degrade hazardous contaminants, whilst producing harmless products, including carbon dioxide, water, and cell biomass. Contaminants are not transported from one environmental medium to another as complete pollutant degradation can occur *in situ* hence, eliminating threats to human health and the environment that might be posed by transportation of contaminated water from one area to another. Additionally, the study offers a prospect of restoring the status of Goreangab Dam as a supply dam for the City of Windhoek, thereby increasing supply while also lessening demands on the current water resources. This research is also significant in that it will provide information on certain microalgae species in Namibia and their performance in the bioremediation of polluted water.

1.6. Limitation of the study

Since it was an *ex-situ* experiment, there was an initial challenge in growing the microalgae species outside of their natural environment. Another limiting factor was

financial resources as it was a self-funded study and costly tests for heavy metal and nutrient analysis were not done on a daily basis but rather on a pre and post treatment basis.

1.7. Delimitation of Study

The experiment only focused on green microalgae species isolated from freshwater bodies around Windhoek, and the result may not be reproducible when green microalgae species either from the marine environment or from other freshwater bodies are used.

CHAPTER TWO: LITERATURE REVIEW

2.1. Background

Surface and underground water pollution is an ongoing problem that threatens the integrity and availability of freshwater resources the world over (Varol et al., 2012). The criticality of this issue lies in the fact that although water occupies 71% of the earth's surface, yet only 2.8% of that water is available for human consumption, which renders it a critical resource for sustenance of life (Karikari and Ansa, 2006; Kumar, 2007; Abdul-Razak et al., 2010). In Windhoek, Namibia, a mismatch between rates of urbanisation and industrialization and development of sanitary infrastructure has been blamed for the pollution of both surface and underground water sources from the discharge of untreated industrial and domestic effluents (Cashman et al., 2014). In rural Namibia, lack of sanitation facilities has been blamed for the contamination of underground water sources (Lewis and Claasen, 2018). Elsewhere, Tajuddin et al. (2012) observed that water quality in major rivers in Bangladesh has deteriorated in recent years due to the discharge of poorly treated sewage effluents, while industrial effluents have been blamed for heavy metals and other physico-chemical pollutants. Water is considered polluted if there is a presence of excessive amounts of a hazard in such a way that the water is no longer suitable for consumption (Qadri and Faiq, 2020). Another study has indicated that due to pollution, approximately one-third of the world's population is facing water scarcity issues at a time when the consumption of freshwater has significantly increased (He et al., 2021).

Hence, this chapter reviews literature on the various causes of water pollution, and the various types of water pollutants ranging from microbial pollutants of water that are

responsible for various diseases that impact human life to chemical pollutants which include heavy metals and other physicochemical pollutants whose presence in the water is hazardous to the environment, as well as to humans. Focus is also placed on potential methods and solutions that can be used in reclaiming water to states which are acceptable. However, the main focus of this research is on the use of green microalgae in the bioremediation of wastewater.

2.2. Water pollution

2.2.1. Microbial pollution loads

The discharge of inadequately treated wastewater effluents into surface water bodies is the main source of microbial contaminants in these water systems, as well as in underground water (Giljanovic–Stambuk, 2005; Naidoo and Olaniran, 2014). Of the most common microbial contaminants of surface and underground water sources, various classes and types of viruses, bacteria and protozoa present in polluted water are known to cause human diseases and are mostly associated with drinking water as they spread via the faecal-oral route (Ashbolt, 2004; Schwarzenbach et al., 2010; Chaudhry and Malik, 2017). For instance, in 2017 the Ministry of Health and Social Services (MoHSS) declared an outbreak of Hepatitis E in Windhoek, as well as in the Erongo, Omusati, Oshana, Ohangwena, Oshikoto and Kavango East regions of the country (Shilikomwenyo, 2019). Most of these cases were linked to the polluted state of the Goreangab Dam which was deemed as the epicentre of the outbreak of this disease. In another study done in South Africa, occurrence of pathogenic bacteria, protozoa, and enteric viruses in eight rivers was detected over a one-year survey (Potgieter et al., 2020). These prevalent strains were identified as enteropathogenic *E. coli* (EPEC),

followed by enteroaggregative *E. coli* (EAEC) which are both severe infections in immunocompromised individuals and children (Chen and Frankel, 2005; Samie et al., 2007). Other studies have also emphasized that in the last decade, infectious diseases have emerged, caused by newly identified or known microorganisms in polluted water systems and these infections have increased worldwide (Sherchand, 2012; Kot et al., 2015).

Furthermore, WHO (2002) reported that out of 132 countries that experienced infectious disease outbreaks from 1998 to 2001, waterborne diseases were recorded at the top of the list with cholera as the most frequent disease, followed by acute diarrhea, legionellosis, and typhoid fever. These types of diseases are a major concern in developing and transition countries with a lack of proper sanitation, for instance cholera and typhoid fever are acute diarrheal infections that are caused by the ingestion of food or water contaminated with the bacterium *Vibrio cholerae* or *Salmonella typhi*, respectively, and in both cases are very hazardous to health (Schwarzenbach et al., 2010). Furthermore, hepatitis A and E viruses, rotaviruses, and the parasitic protozoa *Giardia lamblia* are also often found associated with inadequate water supply and hygiene (Ashbolt, 2004).

Only a few researchers have previously reported on the microbial contamination of Goreangab Dam, which is the subject of this thesis. Of those that did, Chinsemu et al. (2010) reported the presence of *Legionella pneumophila* in the Goreangab Dam, these are gram negative bacilli, that cause Legionnaires' disease which is a severe form of pneumonia. Furthermore, a knowledge gap exists in relation to microbial contamination of the Goreangab Dam.

2.2.2. Chemical pollution

Chemical water pollutants are divided into two categories, macropollutants which typically occur at the milligram per liter level, and the micropollutants, which are typically detected in the environment at trace concentrations (Musolff, 2009). Macropollutants include nutrients such as nitrogen and phosphorus as well as natural organic elements (Jorgenson, 2009). When in excessive amounts, nitrogen and phosphorus lead to an increase in primary production of biomass, oxygen depletion, and toxic algal blooms (Lohse et al., 2009). On the other hand, micropollutants may exert toxic effects even at low concentrations. Sources of micropollutants are pesticides from inputs from agriculture, oil and gasoline spills and from the human-driven recruitment of naturally occurring geogenic toxic chemicals, such as heavy metals and metalloids (Schwarzenbach et al., 2010). Although studies are scarce, Goreangab Dam has been reported to be contaminated with various species of heavy metals including Pb, As, Fe, Zn, and Hg (Moisès et al., 2020). In this study, heavy metal concentration was higher in the sediments than it was in the water column. However, whenever there is sediment disturbance, such as when the dam receives heavy inflows, such sediment bound pollutants may be re-suspended into the water column and pose health hazard by direct intake. Elsewhere, other researchers have reported that heavy metals are amongst the most harmful of the elemental pollutants (Boran and Altınok, 2010). The metallic elements such as arsenic, lead, cadmium,mercury are documented to being very toxic to the aquatic environment at any concentration and are not required to be taken into cells even at ultra-trace level (Baysal et al., 2013). Water quality analysis for heavy metals is very crucial for ensuring the provision of healthy water for the environment.

2.2.3. Water quality impact on human health

Approximately one billion people living in developing countries lack access to healthy drinking water, which is important to sustain human life, amplifying the issue of water scarcity (The United Nations, 2010). These drastic water conditions enable water resources to act as important sources of a variety of infectious diseases in both animals and humans (Praveen and Loh, 2016). Water pollution affects humans directly through consumption or bathing in polluted water sources and also indirectly through the consumption of vegetables irrigated with contaminated water and water pollution also affects fish and other animals that live in the polluted water or consume animals grown in the polluted water (Li and Wu, 2019). Generally, polluted water causes a transmission of various diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio (WHO, 2018).

Further to the above, as one of the driest countries in sub-Saharan Africa, Namibia has limited surface water resources. Hence, most of its water is obtained from underground aquifers (Kahil et al., 2015). And according to Wanke et al. (2014), this can pose as a health concern because limited access to potable water is connected to many infectious diseases. The authors further reported that diarrhea causing agents in Namibia are linked to the consumption of contaminated water. This contaminated water contains feces that have a large range of pathogens like *Salmonella*, *Giardia*, *Cryptosporidium*, *Entamoeba*, and *rotavirus* (Fererr et al., 2012). In 2008, there was a cholera epidemic in Zimbabwe that resulted in 4 287 deaths and 98 585 reported cases. It was reported to being one of the largest and deadliest outbreaks in the history of Zimbabwe (Cuneo et al., 2017). This disease is caused by bacteria present in polluted water, and its symptoms such as

stomach ulcers, severe dehydration, rapid diarrhea are severe, sometimes fatal (Fazal-ur-Rehman, 2019). In other studies, in sub-Saharan Africa it was documented that 10.7% of all mortality was due to poor water supply and improper sanitation (Cashman et al., 2014). Devastating water borne diseases account for 6 million deaths per year in developing countries (Njemanze, 2009). In a study done in Kano State, Nigeria on the effects of water pollution on public health and the environment, it was determined that there were high incidences of poor waste disposal practices, resulting in a variety of health problems for the local community and impacting the environment negatively (Dharwal et al., 2020). Another study done in urban Dhaka, the capital city of Bangladesh indicated that polluted water is one of the main environmental issues in the area which has caused health problems in the community, with the locals affected with skin diseases, diarrhea, gastric ulcers, and respiratory illnesses (common cold, asthma) (Halder and Islam, 2015). The local community comes in contact with this polluted water while fishing or using it for agricultural purposes. Besides water-borne diseases, chemicals in polluted water also impact human health negatively, pesticides are able to damage the nervous system and can cause cancers because they contain carbonates and organophosphates, while chemicals such as chlorides may cause reproductive and endocrinal damages (Baker-Austin et al., 2017).

Furthermore, WHO (2019) reported that at least 2 billion people worldwide use a drinking water source contaminated with faeces hence, if water related issues are not addressed, half of the world's population will be living in water-stressed areas by 2025 and more people in the years to come.

2.3. Water Reclamation

Arid to semi-arid regions are classified as receiving less than 166 mm average annual rainfall (Al-Ansari et al., 2014). Therefore, water scarcity in these regions represents an essential troubling factor in economic development and prosperity as water is a strategic resource for socio-economic development and environmental protection (Al-Ansari, 2013). The situation is predicted to become more severe in the future due to worsening climatic conditions (Al-Ansari et al., 2014). Climate change is the main contributor to the current and future water shortages. For instance, it is projected that in arid regions, the mean temperatures are expected to increase by 3°C to 5°C whereas precipitation will decrease by about 20% by the end of this century (Boko et al., 2007). Water run-off is projected to be reduced by 20% to 30% and water supply by 10% or greater by 2050 (Milly et al., 2005). The mentioned climatic conditions are stressing water availability in some parts of the world including the Mediterranean area, some parts of Europe, Central and Southern America, and Southern Africa (Estrela et al., 2012).

Due to both the importance and scarcity of water resources, researchers have explored various means for water reclamation to ensure the availability and sustainable use of water resources, as discussed in the sections that follow.

2.3.1. Membrane Filtration Methods

The use of advanced treatment technologies such as the membrane technology, particularly microfiltration, ultrafiltration and reverse osmosis (RO) which are both cost-effective and reliable in removing harmful bacteria and pathogens have been documented as having essential roles in water reuse and purification (Li et al., 2011;

Alzahrani and Mohammad, 2014). Membrane-based technologies are mostly used in reverse osmosis processes (Skuse et al., 2020). Membranes for pressure driven processes such as microfiltration, ultrafiltration, nano filtration and reverse osmosis are made from man-made organic polymers which include polyethylene (PE), polytetrafluorethylene (PTFE), polypropylene, and cellulose acetate among others (Aliyu et al., 2018). Reverse osmosis membrane technology has in several studies proven to be the most advanced and most energy-saving separation technology., This process as a physical technique only needs pressure and it can be employed at room temperature without phase change (Wang & Wang, 2019). Furthermore, developed countries like China have successfully employed reverse osmosis technologies to curve water crisis (Zhou et al., 2019).

However, the impurities associated with use of synthetic organic polymers and the by-products used in membrane technologies cause harm to human health (Ezugbe and Rathilal, 2020). And, although membrane technology is progressively revolutionizing water and wastewater treatment, there is still an urgency for improvement as these processes are associated with major issues such as fouling and high energy demands. Researchers need to introduce cheaper pre-treatment processes or for the development of fouling resistant membranes to find a long-lasting solution to wastewater treatment (Ezugbe and Rathilal, 2020).

2.3.2. Wastewater Reclamation Plants - Windhoek as a case study

There are three main wastewater treatment plants in the city of Windhoek, namely Gammams, Otjomuise and Ujams wastewater treatment plants. The Gammams wastewater treatment plant treats about 80% of the city's domestic discharge whilst the Otjomuise wastewater treatment plant treats the remaining 20% and the Ujams

wastewater treatment treats industrial wastewater (du Pisani, 2006). The Ujams Wastewater Treatment Plant is a relatively new plant in Windhoek which uses the latest technologies to treat industrial wastewater to standards that meet the National and the US EPA standards (Wapoteka, 2016). The plant replaced the old Ujams Wastewater Treatment Plant that had difficulties with treating the industrial wastewater and was releasing effluent of poor quality into the environment. The new UWTP treats wastewater by passing the waste through the latest generation membrane bioreactor, a UV disinfection stage, sludge treatment and odour treatment process before being discharged into the surrounding Klein Windhoek River. A study done in 2016 which focused on the effects of the effluent from the new Ujams wastewater treatment plant on the quality of the Klein Windhoek River has indicated that the new UWTP complies with most effluent standards and is improving the quality of the water in the Klein Windhoek River (Wapoteka, 2016).

The Gammams and Otjomuise wastewater plants treat wastewater through an activated sludge process with nitrogen and phosphorus removal, and the two plants are therefore referred to as Biological Nutrient Removal (BNR) plants (Lahnsteiner and Lempert, 2007). The plants treat water in three stages as follows: primary treatment stage - 50% of organic matter is removed from the sewage (Sibeya, 2016). Grit is also removed followed by primary settling tanks (PSTs) where suspended solids are settled and removed; secondary treatment stage - 85% of the remaining organic matter is removed by biological processes where bacteria biodegrade the organic matter in the effluent into simpler compounds such as carbon dioxide and water. The final phase is the tertiary treatment stage in which the secondary settling tank at GWWTP is further cultured in

maturation ponds with a retention time of approximately three days to reduce the pathogen load of the final effluent.

The final effluent is then directed to the Goreangab Wastewater Reclamation Plant (NGWRP) and old Goreangab Wastewater Reclamation Plant (OGWRP) where it is further treated to drinking standards (Sibeya, 2016) as part of the City of Windhoek's water reclamation strategies. The rest of the water that is not channeled towards the reclamation plants is fed into the river downstream of Goreangab Dam. However, Sibeya (2016) determined that the effluent being discharged into Goreangab Dam is significantly contributing towards its eutrophication as it still contains high amounts of orthophosphate, as well as other pollutants. As a result, the water in this dam has become hyper polluted to the extent that the dam has been de-commissioned as one of the City of Windhoek water supply dams. This study was therefore aimed at determining the efficiency of microalgae in the removal of pollutants from this dam as a potential water reclamation strategy that could see this dam's water being re-usable again. The following sections are dedicated to the review of microalgae as potential water reclamation or bioremediation tools.

2.3.3. Bioremediation

Bioremediation technologies have received a lot of attention and interest since the 1960s. This process is both environmentally friendly and economically beneficial (Iye & Iye, 2015). Bioremediation is an ecological process that uses natural biological methods to completely remove pollutants from a contaminated environment (Kensa, 2011). This process involves using microorganisms like fungi, green plants and their enzymes to return a contaminated environment to its original state. In-situ bioremediation takes

place in the environment and ex-situ bioremediation takes place when the contaminated material is removed from the environment and treated elsewhere (Das and Dash, 2014). Bioremediation was also described as the use of microorganisms to destroy or immobilize waste materials (Shanahan, 2004). A study conducted by Adams et al. (2015) that reviewed technologies that are used to carry out bioremediation have observed that biotechnological approaches for remediation have received a great deal of attention in recent years. For instance, research based on the application of microalgae for wastewater treatment is evolving and focusing on joining bioremediation to treatment processes which are economically practical to even developing countries. Bio-products from the use of microalgae contribute to a circular economy which presents a multipurpose solution (Pacheco et al., 2020). Another factor that supports bioremediation of wastewater with the application of green microalgae is that the species are commonly found in a variety of freshwater sources, and they have a very high growth rate which easily survive on light, temperature and nutrients which are obtained from the organic pollutants in wastewater (Pacheco et al., 2020).

Furthermore, there has been research and reviews on the two types of bioremediation processes, namely biostimulation and bioaugmentation. Biostimulation is the process in which limiting nutrients are added to the environment to support the growth of microbes that are capable of bioremediation (Tyagi, et al., 2011). During this process foreign microorganisms do not have to be introduced to the water site as there are native microorganisms already present which are suitable for bioremediation, but a challenge of this process is that it has a high dependency on environmental factors that controls its potentiality (Adams et al., 2015). On the other hand, bioaugmentation is defined as the

addition of cultured microorganisms that are capable of bioremediation to the environment (Herrero and Stuckey, 2015), however the technical aspects of these technologies are few if available at all.

For this research, since the Goreangab Dam has been polluted with the increase of organic matter, the resident microorganisms have been stressed out due to repeated exposure to sewage contamination (Haindongo, 2010). Hence, the bioremediation process of *ex situ* bioaugmentation was employed for the treatment of the wastewater samples.

2.3.4. Application of microalgae

Microalgae are photosynthetic microorganisms found mostly in aquatic habitats and that can convert light energy and inorganic carbon sources into biomass while releasing oxygen to the atmosphere (Dessy et al., 2011). The term microalgae usually include both cyanobacteria (blue-green algae) as well as microalgae and they are both found in microalgal-based systems for wastewater treatment (Molinuevo-Salces et al., 2019). In this research, the term microalgae will be used to refer to both groups. Green microalgae are characterised by a strong tolerance for pollutants and fix water pollution through accumulation and degradation, as they have the ability to assimilate nutrients and when algae grow, pH increases, phosphorus precipitates and ammonia strips to the air, facilitating the disinfection of wastewater (Molinuevo-Salces et al., 2019). Microalgae also have the ability to remove organic contaminants, nutrients and heavy metal (As, Cd, Pb, Hg) from wastewater and contaminated water (Zarazúa-Ortega et al., 2013). Moreover, some species of microalgae has also indicated a great ability to remove 87% of toxicant from polluted water (Adamia et al., 2018).

Microalgae biotechnology is used for different commercial applications. In recent years, development of microalgae-bacteria consortia for wastewater treatment has received more attention as an efficient alternate to conventional wastewater treatment plants, decreasing energy costs and recovering nutrients as valuable biomass (Hernández et al., 2013). For treatment of wastewater, the microalgae need to be cultivated in apparatus used for growing phototrophic microorganisms under controlled conditions or in open ponds and channels (Grima et al., 2003). Open and closed apparatuses present both advantages and disadvantages. According to Gouveia (2011), open systems are associated with higher water loss through evaporation and poor efficiency of gas transfer and light utilization. Also, the harvesting costs in open systems are high with a high contamination by other microorganisms. On the contrary, closed systems make it easier to control growth conditions even though operating cost are high but with low harvesting costs. Also, scale-up technology for commercial level is more difficult than in open systems. In wastewater treatment, mixed open ponds are the only large-scale implemented technology due to the high-energy costs to operate closed photobioreactors.

The microalgal species used in wastewater treatment systems are especially tolerant to pollution, and species such as *Chlorella* sp., *Nitzschia* sp. and *Scenedesmus* sp. are reported as being the most tolerant genera that are abundant in wastewater systems (Muñoz and Guieysse, 2006; de Godos et al., 2009). Furthermore, species such as *Ankira* sp., *Microspora* sp., *Chroococcus limneticus*, *Cyanophyta coccal*, *Dactylococcopsis* sp., *Phormidium* sp. and *Stigeoclonium* sp. have been reported in microalgal-based systems treating fish processing wastewater (Riaño et al., 2011) while

Chlamydomonas subcaudata, *Teilingia* sp., *Anabaena* sp., *Phormidium tergestinum*, *Pinnularia* sp. and *Nitzschia* sp. have been reported in open ponds for slaughterhouse wastewater (Hernández et al., 2013).

However, there is still a gap in knowledge that exists about the application of microalgae as a form of wastewater treatment (Pacheco et al., 2020). For this research, the following microalgae were selected to treat the wastewater from the Goreangab Dam:

i. *Scenedesmus* species

Scenedesmus is a green algae genus which is also considered as common freshwater plankton which has 70 colonial species from the family *Scenedesmaceae* (Rath, 2012). The species are commonly used experimentally to study pollution and photosynthesis and are a potential source of biodiesel. *Scenedesmus* species provide oxygen for the bacterial breakdown of organic matter and thereby help to destroy other harmful substances. Furthermore, the algal species is widely used for nitrogen and phosphorus removal (Shi et al., 2007).

ii. *Anabaena* species

Anabaena is a genus of nitrogen-fixing blue-green algae with beadlike or barrel-like cells and interspersed enlarged spores (heterocysts), found as plankton in shallow water and on moist soils (Agnihotri, 2014). The genus *Anabaena*, is from the family *Nostocaceae* and contain photosynthetic pigments in their cytoplasm to perform photosynthesis. The *Anabaena* species consists of free-living cells that are investigated for their ability for organic matter biodegradation and heavy metal removal from the

effluent, the species has been previously documented for removing 70.88% of heavy metals from wastewater (Dubey et al., 2019).

iii. *Microcystis* species

The *Microcystis* is a genus from the family *Microcystaceae* (da Silva Malone et al., 2014). This algal species is known to form harmful algal blooms of economic and ecological importance. They are the most common toxic cyanobacterial blooms in eutrophic fresh water. However, some members of the *Microcystis* community show high tolerance and strength for removal of lead and cadmium

CHAPTER THREE: MATERIALS AND METHOD

3.1. Description of study area

The study was conducted in Windhoek, Khomas Region, Namibia. Algal samples were obtained from three sites, namely Goreangab Dam (22.5286° S, 17.0087° E), Avis Dam (22.5741° S, 17.1288° E) and Klein Windhoek River (22.3471° S, 17.0509° E). The polluted water for the bioremediation assays was obtained from Goreangab Dam. The Goreangab Dam is located in the north-western suburbs of Windhoek which is the capital city of Namibia (Ogunmokun et al., 2000). The dam once served as a source of raw water for Windhoek's drinking treatment plant since its commissioning in 1958 until it was decommissioned in 2010 due to its polluted state (Du Pisani, 2006). The dam receives effluent from the Gammams Wastewater Treatment Plant. The Avis Dam, on the other hand, is Windhoek's first dam, and is situated approximately 9 km east of Windhoek. It was constructed in 1933 for the supply of water to the City of Windhoek (Crovello et al., 2010). The dam, however, failed to supply enough sustainable water due to its small catchment area (Hamata and Chinsebu, 2012). And lastly, the Klein Windhoek River is a seasonal river that originates in Ludwigsdorf from where it then flows in a general north western direction from Windhoek until it empties into the Otjiseru River which is located between Windhoek and Okahandja.

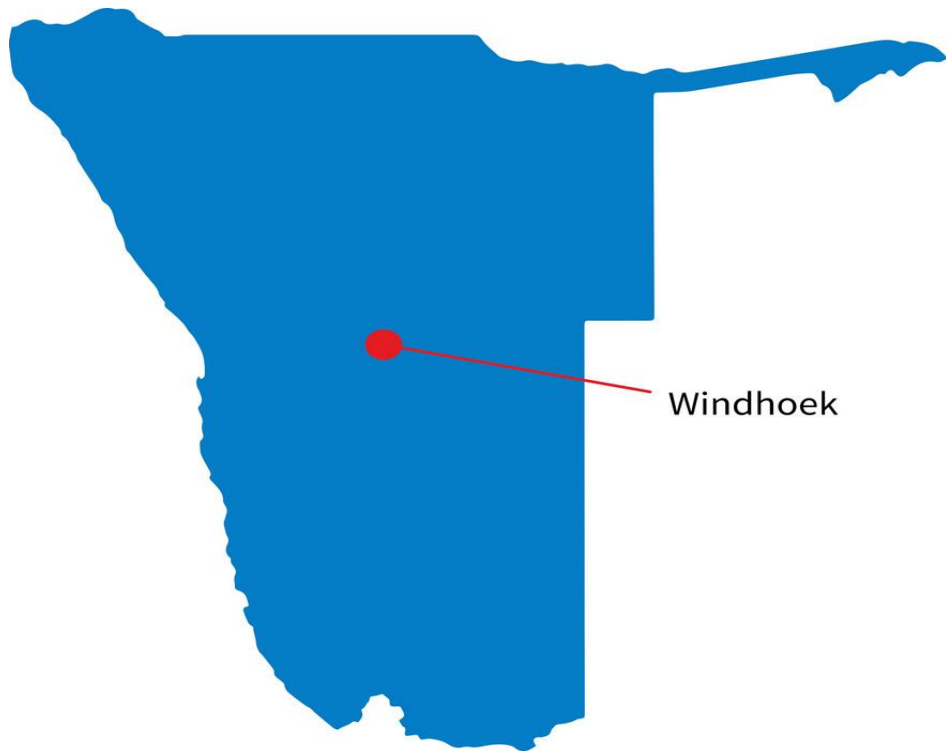


Figure 1. The sampling sites Goreangab Dam (22.5286° S, 17.0087° E), Avis Dam (22.5741° S, 17.1288° E) and Klein Windhoek River (22.3471° S, 17.0509° E) are located in Windhoek, Namibia.

The City of Windhoek is home to about 318,700 people (Pendleton and Nickanor, 2016). The average rainfall for Khomas Region ranges from about 200 mm to 350 mm per year. As a whole, the country generally experiences summer (November to April), spring (September to November) and winter seasons (May to August) (Mendelsohn and El Obied, 2005).

3.2. Sampling

3.2.1. Wastewater sampling

Water from the Goreangab Dam was collected using 25 litre plastic water containers. These containers were first washed with disinfectant followed by double rinsing with Goreangab Dam water before sample collection, as described by Cornman et al. (2018).

The grab sampling method was employed by submerging the containers to collect water directly into the containers at knee depth.

3.2.2. Microalgae sampling

Prior to site visits for microalgae sampling, BG-11 (Blue Green) media was prepared and readied for use as enrichment for the growth of green freshwater microalgae as described by Ilavarasi et al. (2011).

Microalgae samples were collected by suctioning them into appropriately labelled 1 L sterile bottles together with about 500 mL of freshwater. Samples were taken to the laboratory at the University of Namibia for further processing.

3.3. Research Procedure

A quantitative research design was employed. The experimental design included 9 experiments and their controls. Experiments were carried out in Erlenmeyer flasks containing 2000 mL of water from the Goreangab Dam in which the microalgae species from the Avis Dam and Klein Windhoek River were inoculated whilst; no external microalgae species were inoculated for the control.

The flasks were placed on a laboratory shaker which was placed in the greenhouse (Appendix 6) located in the Department of Biochemistry, Microbiology and Biotechnology (formerly the Department of Biological Sciences) at the University of Namibia. The shaker was used for a period of 12 days from 21 April 2021 to 03 May 2021. It provided microalgae turbulence which is an important factor in wastewater treatment (Larsdotter, 2006). Furthermore, the greenhouse provided essential physical

factors such as light and moderately steady temperatures which are needed for microalgal growth (Larsdotter, 2006). The water samples were analyzed for nutrient levels, heavy metal concentrations and for the presence of coliform bacteria before and after treatment.

3.4. Microalgae identification

Some of the microalgae samples from the Avis Dam, Klein Windhoek River and the Goreangab Dam were grown in BG 11 medium for identification. These algae samples were first kept in the greenhouse for a week for optimum growth before they were sent to the NamWater Laboratory (Windhoek) for identification (Appendix 7). NamWater's analytical laboratory identifies algae based on national and international accepted protocols as described by Vo et al. (2019). Briefly, microalgae were isolated using the membrane filtration technique. The membrane filters were then allowed to dry, after which they were placed on microscope slides with immersion oil and observed under a microscope. The microalgae were then identified by comparison to a phytoplankton identification book for microscopic algae in Southern African freshwaters (van Vuuren et al., 2006).

3.5. Water Quality Analysis

3.5.1. Temperature

Temperature influences the physical, chemical and biological processes of water bodies (Nangolo, 2018). Therefore, the temperature of the wastewater samples was monitored using a thermometer three times daily.

3.5.2. Heavy metal analysis

Water samples were analysed for lead, arsenic and cadmium using the ICP-OES (Inductively coupled plasma - optical emission spectrometry) technique following the descriptions of Chochorek et al. (2010). For this technique, the water samples were filtered followed by acidification to a pH less than 2 with ultrapure nitric acid (HNO_3) 16 hours before the ICP-OES analysis. Water samples were analysed without dilutions since the concentrations were not above the highest calibration standards.

3.5.3. Nutrient analysis

Nitrate, nitrite / total nitrogen determination of the water samples was based on the hydrazine reduction method as described by Bower and Holm-Hansen (1980). This method reduces nitrate to nitrite using hydrazinium sulfate. The reduced nitrate was determined by diazotisation with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form a highly coloured azo dye which was then measured.

The phosphate cell test was used to analyse the phosphorus content for the water samples as described by (Stalder and Zumbuehl, 2013). Briefly, 50 ml of each water sample was separately mixed with 11 N sulphuric acid and 4 ml of ammonium molybdate-antimony potassium tartrate and thorough mixed before 2 ml of ascorbic acid were added to the solution and further mixed. After 5 minutes, the absorbance of the solution was determined.

The Continuous Flow Analyzer (Appendix 8) is used at the NamWater laboratory as a multichannel peristaltic pump used to mix samples and chemical reagents in a continuously flowing stream to automate colorimetric tests for nutrient analysis.

3.5.4. Daily coliform observation

Eosin methylene blue agar (EMB) was used to grow faecal coliforms. This is a differential medium used for the isolation of faecal coliform bacteria (Lal and Cheeptham, 2007). Exactly 100 μ l of each sub sample were spread on the agar plates and placed in the incubator at a temperature of 45.0 ± 0.2 °C for 24 hours and prepared and observed daily. Sub-sampling for coliform observation was done daily. While m-Endo agar was used to grow total coliforms in the incubator at a temperature of 37 ± 0.5 °C for 48 hours, prepared and observed every second day.

Furthermore, a marker was used to indicate counted colonies to prevent double counting of colonies.

3.6. Data analysis

Statistical tests were done to test the data for normality of distribution using the Shapiro-Wilk test (Razali and Wah, 2011). A one-way analysis of variance (ANOVA) was used to detect significant differences between the different algae treatments by determining whether the type of treatment used had a significant impact on the mean values of the lead concentration and total phosphate levels of the water samples. And the Kruskal-Wallis H Test was used to determine whether the type of algae treatment used had a significant impact on the mean values of the cadmium concentration, nitrate levels and the coliform presence of the water samples. Post hoc tests were then used to specifically

determine which of the treatment groups differed from each other. All these tests were conducted using the SPSS statistics version 27 software.

3.7. Research Ethics

Laboratory testing was conducted in an ethical manner and adherence to the fundamental principles of scientific integrity was employed. Furthermore, guidelines for water collection and sampling were followed. And the ethical clearance was obtained from the UNAM Research Ethics Committee and research permission from the Centre for Postgraduate Studies.

CHAPTER FOUR: RESULTS

4.1. Microalgae identification

Microalgae identification confirmed the presence of *Microcystis* species, *Anabaena* species and *Scenedesmus* species in the water samples as indicated in the table below (Table 1).

The Goreangab Dam sample consisted of naturally occurring *Microcystis* species. These control samples that were not inoculated with external microalgae were still analysed and monitored for the performance of the *Microcystis* species in bioremediation (the constant of the experiment). The Klein Windhoek River sample (Treatment 1) consisted of the *Anabaena* species. The Avis Dam sample (Treatment 2) consisted of the *Anabaena* species as well as the *Scenedesmus* species. The consortium of this experiment consisted of water samples inoculated with all the microalgae species.

The *Microcystis* and *Anabaena* species belong to the phylum Cyanophyta commonly known as blue green algae that obtain energy through photosynthesis (Nweze, 2009). The *Scenedesmus* species belong to the phylum Chlorophyta commonly known as green algae (Baudelet et al., 2017).

Table 1. Algae identification report

Species	*Division/ Phyla of algae and effect	Sample Site		
		Goreangab Dam (control)	Klein Windhoek River (treatment 1)	Avis Dam (treatment 2)
<i>Anabaena</i>	BG; FC, TO, TOX		✓	✓
<i>Microcystis</i>	BG; TO, TOX	✓		
<i>Scenedesmus</i>	GA; TO			✓

BG = Cyanophyta, FC = Filter Clogging, TO = Taste and Odour, TOX = Toxin, GA = Chlorophyta

4.2. Heavy Metals

4.2.1. Cadmium

The Cadmium (Cd) level of the water samples from the Goreangab Dam was 0.02 mg/L on the first day of the experiment. After the microalgae species were inoculated into the water samples, a decrease in the Cd level of the wastewater was observed as depicted in the graph below (Figure 2). The Shapiro Wilk test ($P < .05$) showed that the data for Cd concentration were not normally distributed.

The Cd content of the water samples were way above the stipulated limits set by WHO. Treatment 1, treatment 2 and the consortium all had the same levels of Cd content from the beginning to the end of the experiment. The Cd levels in the water samples for treatment 1, treatment 2 and the consortium have shown an immediate steep decline from 0.02 mg/L on the first day to 0.01 mg/L on the sixth day, the Cd levels remained constant at 0.01 mg/L until the last day of the experiment. On the other hand, the control samples which only consisted of the natural occurring *Microcystis* species illustrated a

slower decline starting and remaining at 0.02 mg/L Cd level on the first day through to the sixth day and then steeply declined to 0.01 mg/L on the last day.

However, although there was a decline in Cd levels after treatment the Kruskal Wallis test indicated that there were no statistical differences between the mean values ($p = 0.940$) after treatment with microalgae.

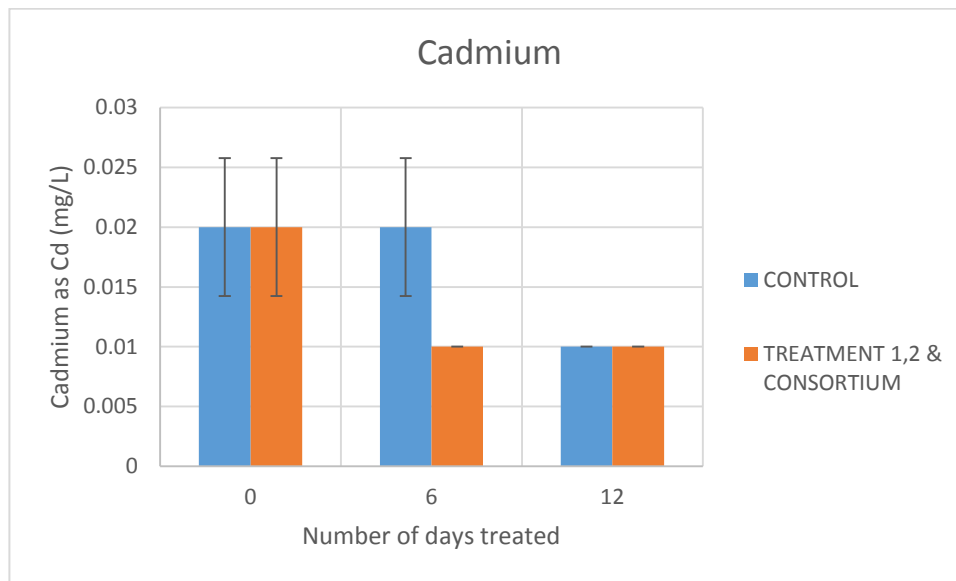


Figure 2. Cadmium content in water samples from the Goreangab Dam treated with green microalgae for 12 days.

4.2.2. Lead

The Lead (Pb) level of the water samples from the Goreangab Dam was 0.8 mg/L on the first day of the experiment. After the microalgae species were inoculated into the water samples, a decrease in the Pb level of the water was observed as depicted in the graph (Figure 3) below. The Pb content of the wastewater samples before the experiment exceeded the 0.01 mg/L stipulated limit set by WHO. However, after treatment with microalgae the Pb levels of the control water samples consisting of the natural occurring

Microcystis species indicated a great removal of Pb compared to the water samples of treatment 1, 2 and the consortium.

The Shapiro Wilk test ($P > .05$) showed that the data for Pb concentrations were approximately normally distributed. However, the one-way analysis of variance (ANOVA) indicated that there are no statistical differences between the mean values ($p = 0.346$) after treatment with microalgae. And the Welch and Brown-Forsythe test further agreed with no statistical differences between the means with the values of $p = 0.08$ and $p = 0.381$ respectively. But it is important to note that the *Microcystis* species has indicated the ability to reduce the Pb content of the water samples below 0.01 mg/L.

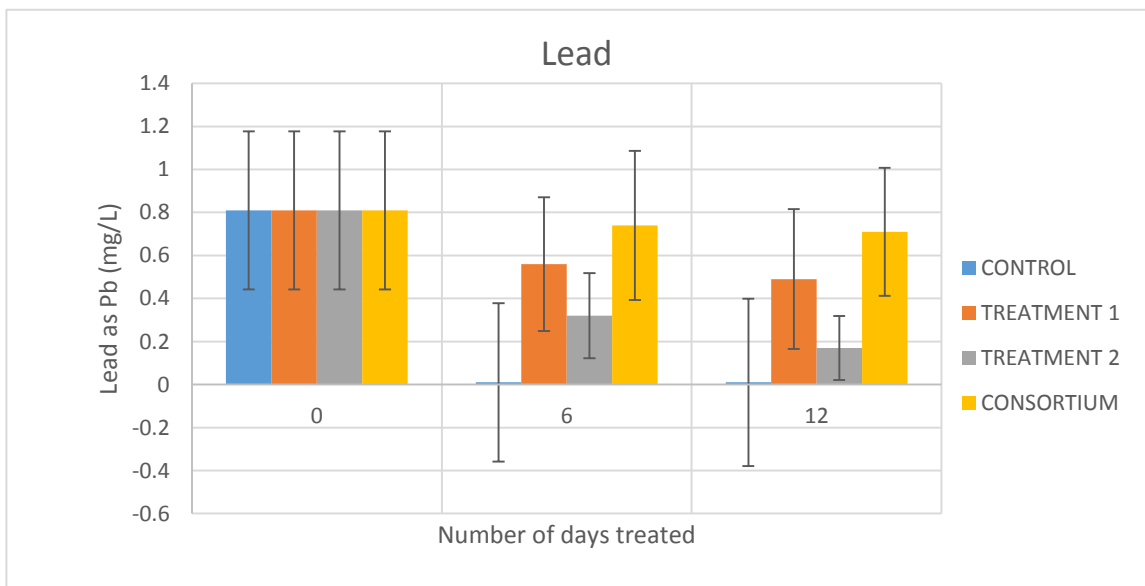


Figure 3. Lead content in water samples from the Goreangab Dam treated with green microalgae for 12 days.

4.3. Nutrients

4.3.1. Nitrate

The results in the graph (Figure 4) below show that the water samples for treatment 2 have indicated the fastest uptake of nitrates (from 2.5 mg/L to 0.5 mg/L) followed by the consortium, then the control water samples. Treatment 1 water samples showed a relatively low uptake of nitrates from the wastewater.

The Shapiro Wilk test ($P < .05$) showed that the data for nitrate levels were not normally distributed and the Kruskal Wallis test indicated that there were statistical differences between the mean values ($H(3) = 8.597$, $p = 0.035$) of the nitrate levels after treatment with microalgae. Pair Wise Comparisons indicated significant differences in nutrient uptake between treatment 1 which was the least effective in nitrate uptake and treatment 2 which was the most effective ($p = 0.005$) and there were also significant differences between the control and treatment 1 ($p = 0.039$). No significant differences were detected between the other groups.

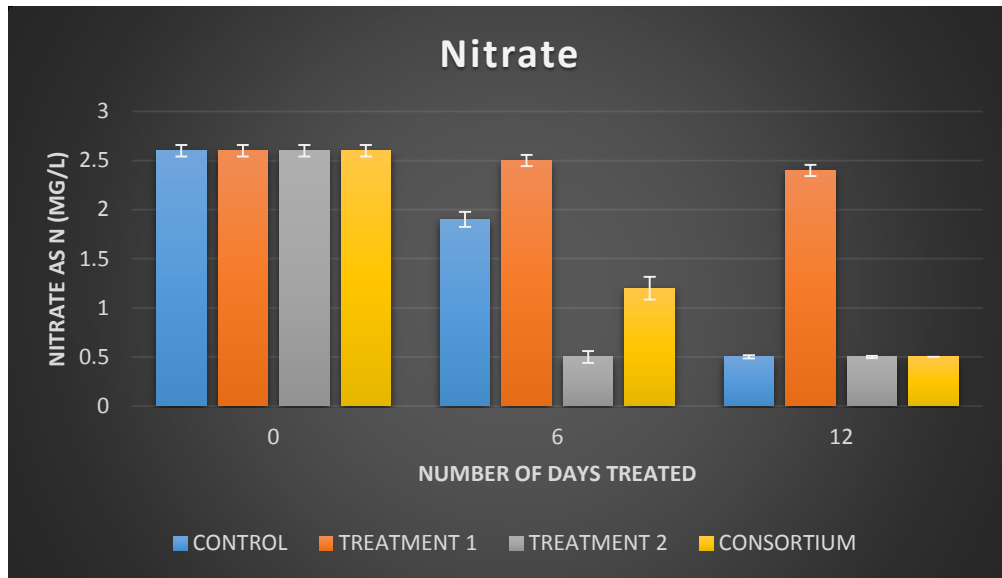


Figure 4. Nitrate content in water samples from the Goreangab Dam treated with green microalgae for 12 days.

4.3.2 Total phosphate

Phosphate uptake as illustrated by the graph (Figure 5) below indicate that the total phosphate uptake amongst the three treatments and the control are relatively similar. Ranging from a total phosphate uptake of 2.70 mg/l at the beginning of the experiment to 0.44 mg/l at the end of the experiment. Treatment 1 was the most effective group in the uptake of phosphate from the water samples from the Goreangab Dam.

However, the Shapiro Wilk test ($P > .05$) showed that the data for total phosphate levels were approximately normally distributed but the ANOVA indicated that there were no significant differences between the mean values ($p = 0.052$) after treatment with microalgae.

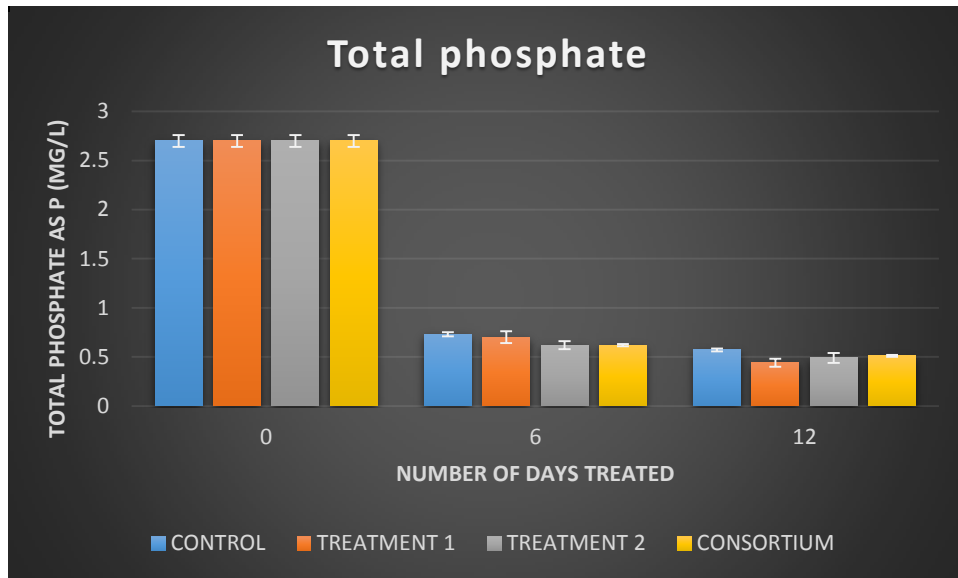


Figure 5. Total Phosphate content in water samples from the Goreangab Dam treated with green microalgae for 12 days.

4.4 Coliform presence

4.4.1. Total Coliform

The Shapiro Wilk test ($P < .05$) showed that the data for total coliform presence were not normally distributed and the Kruskal Wallis test indicated that there are statistical differences between the mean values ($H(3) = 10.352$, $p = 0.015$) after treatment with microalgae.

The Pair Wise Comparisons indicated significant differences in total coliform removal between the control which was the least effective and treatment 2 which was the most effective ($p = 0.002$), there were also significant differences between the control and the consortium ($p = 0.040$) and between treatment 1 and treatment 2 ($p = 0.040$).

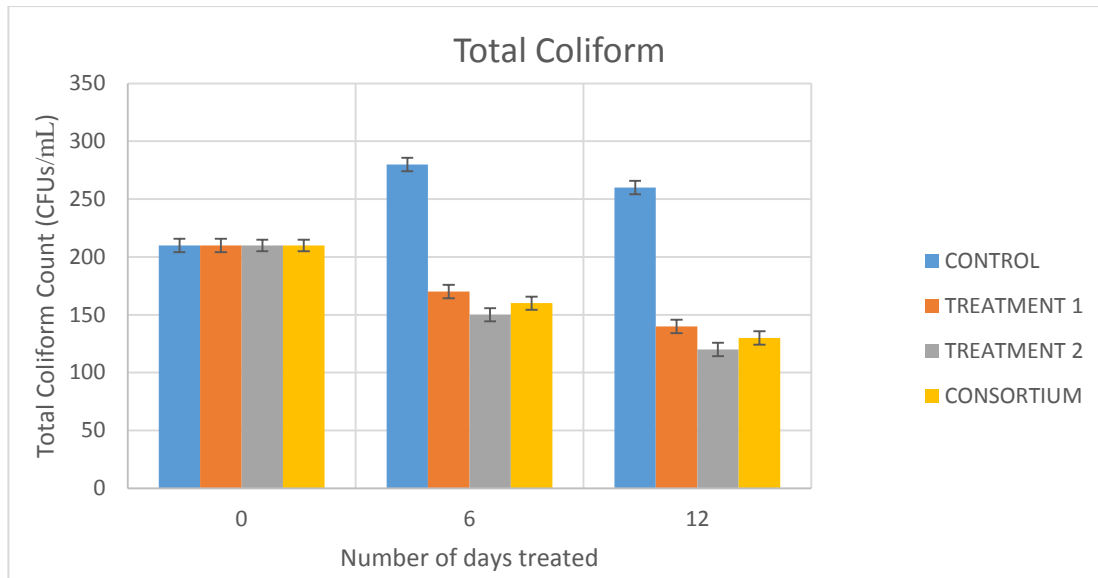


Figure 6. Total Coliform count in water samples from the Goreangab Dam treated with green microalgae for 12 days

4.4.2. Faecal Coliform

The Shapiro Wilk test ($P < 0.05$) showed that the data for faecal coliform presence were not normally distributed and the Kruskal Wallis test indicated that there are statistical differences between the mean values ($H(3) = 10.421$, $p = 0.015$) after treatment with microalgae.

For the three experiments and the controls, a decline in the concentration of faecal coliforms in the wastewater samples, have been observed (Figure 7). More in the water samples with external microalgae inoculated. Treatment 2 was the most effective in faecal coliform removal and the control the least.

The Pair Wise Comparisons indicated significant differences in faecal coliform removal between the control which was the least effective and treatment 2 which was the most effective ($p = 0.002$), there were also significant differences between the control and the consortium ($p = 0.041$) and between treatment 1 and treatment 2 ($p = 0.041$).

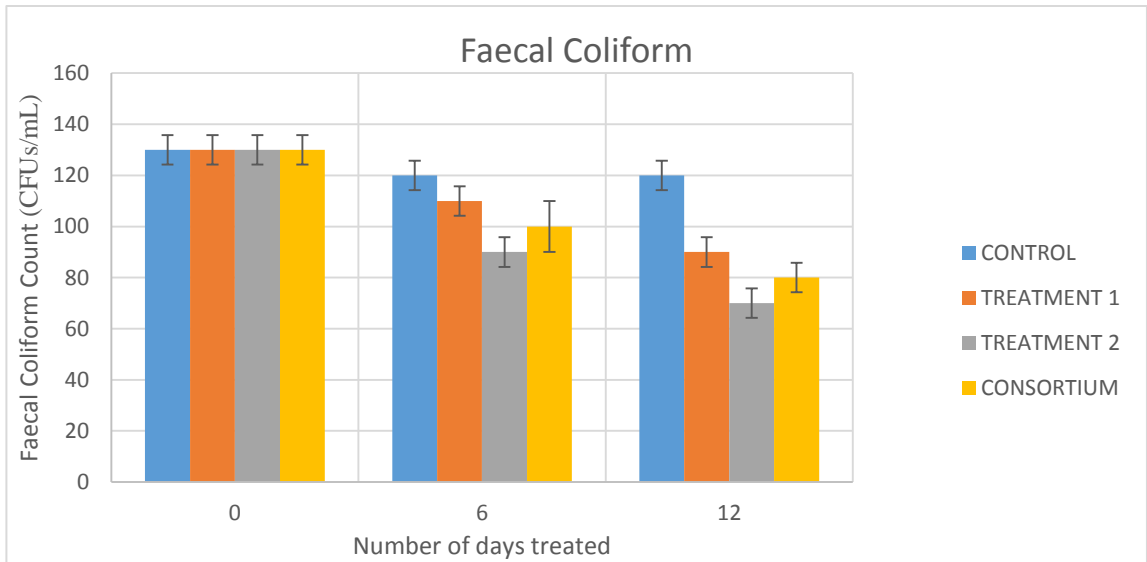


Figure 7. Faecal Coliform count in water samples from the Goreangab Dam treated with green microalgae for 12 days

CHAPTER FIVE: DISCUSSION

5.1. Heavy Metals

During this study the microalgae species did not significantly remove Cd and Pb from the water samples of the Goreangab Dam. Research has indicated that although algae such as Cyanobacteria *Microcystis* and *Anabaena* have high binding affinity when compared to other microorganisms and are generally used for the removal of heavy metals from wastewater (Abdel-Aty et al., 2013), abiotic factors and organic matter can influence the absorption of heavy metals by these algae (Torres, 2016).

Temperature influences the biosorption efficiency of the algal species (Chairat and Bremner 2016; Gupta et al., 2010). Some studies have shown that an increase in water temperature leads to a possible increase in metal ions biosorption (Khan et al., 2012; Yi et al., 2016). During this study, the water temperature decreased from 33° C on the first day of the experiment to 27° C on the last day, this can explain the poor performance in metal absorption during the experiment as other studies have presented various reasons for this. A study conducted by Zhu and Wachs (2016) indicated that decreasing water temperature causes a decrease in metal absorption by algae cells because when water temperature decreases it leads to a decrease in active sites involved in metal ions uptake and a decrease in the affinity of this sites to absorb metal ions. A decrease in temperature also causes a change in the complex formation constant with temperature. However, it is important to note that different algae species behave differently during phycoremediation in heavy metal removal from wastewater (Mantzorou et al., 2018; Chang et al., 2019; Furuhashi et al., 2019).

The optimum temperatures for the three microalgae species used in the experiment are 25 °C for *Anabaena* (Kłodawska et al., 2019), between 30 and 35°C for *Microcystis* (Imai et al., 2009) and there are conflicting results for *Scenedesmus* species as some studies state 30°C while others state 37°C (Cassidy, 2011). During the current study however, this optimum water temperatures were not present, and this might be able to highlight the importance of optimum water temperature in the removal of heavy metals by algae.

5.2. Nutrient Analysis

The *Scenedesmus* species require large amounts of nutrients for growth and has hence, been successfully used as a biological treatment to remove nitrogen and phosphorus from wastewater (Boelee et al., 2011; Gonçalves et al., 2017). In the current study, the *Scenedesmus* species was the most effective in the removal of nitrate from the water samples, and all the treatments that included this species (treatment 2 and consortium) performed well in the uptake of nitrate. The *Scenedesmus* species which is a green alga uses a diversity of nitrogen sources for growth, the algae is capable of converting a variety of inorganic nitrogen forms (nitrite, nitrate, ammonia, and ammonium-N) to organic nitrogen (Zhao et al., 2019). Several studies have indicated that nitrification and denitrification are the main processes by which algae systems remove nitrogen (Delgadillo-Mirquez et al., 2016). The study's results suggesting the successful use of the *Scenedesmus* species in the removal of nitrate agree well with the results of previous studies. A study conducted by Ansari et al. (2019) stated that nitrate from municipal wastewater was reduced by 100% by the *Scenedesmus* species. Another study also indicated that the *Scenedesmus* species was able to rapidly reduce nitrate

concentration from wastewater from 240 mg/L to 144.7 mg/L. These studies further support the abilities of the *Scenedesmus* species in the removal of nitrates.

Treatment 1 and the control which were treated with the *Anabaena* species and the *Microcystis* species separately were the least effective in the removal of nitrate, this could be because cyanobacteria have a lower tolerance to using a wider variety of nitrogen as compared to the green microalgae species *Scenedesmus* (Park et al., 2010). The *Scenedesmus* species outcompetes the *Anabaena* species and the *Microcystis* species in nitrate uptake.

On the other hand, phosphate removal from the water samples of the current study was not significant. Cyanobacteria are stated to grow best in eutrophic conditions that have high phosphorus concentrations, but they can also develop under low phosphorus conditions because a few study studies have indicated that cyanobacteria have a large storage capacity for phosphorus (Sabour et al., 2009). This explains the similar uptake of phosphate across the different treatments and control because of the presence of cyanobacteria in all the water samples. Furthermore, other studies have indicated that *Anabaena* and *Microcystis* cyanobacteria use excess phosphorus for nitrogen fixation. Phosphorus stimulates nitrification which is important for the removal of nitrogen from wastewater (Ning et al., 2021). Thus, cyanobacteria take up more phosphate compared to green algae because their cells not only store phosphorus but also use it in nitrogen fixation.

5.3. Pathogen loads

In various studies, microalgae have indicated the ability to remove pathogens when applied to wastewater. Some studies have observed pathogen removal with prolonged algal cultivation with the *Scenedesmus* species especially, which has shown a greater potential for pathogen removal (Gupta et al., 2016). This has also been the case in the current study, as all the water samples that were treated with the *Scenedesmus* species demonstrated a higher remove of pathogens as compared to the other treatments (treatment 1 and the control) which performed poorly. However, microalgae across all the treatments have indicated the ability to remove pathogens from the water samples.

Furthermore, other studies have indicated that when water sources have high pH levels, they function as antibacterial which affects the presence of pathogens and that algae also excrete a variety of metabolites which also effect pathogens (Kümmerer, 2008; Saha and Weinberg, 2019). However, in a study conducted by Gupta et al. (2016) which was aimed at determining the main reason for coliform removal, the effects of increased pH on coliforms in the absence of algae was studied, and the results indicated that increased pH in wastewaters in the absence of algae did not result in significant coliform reduction. This clarified that bactericidal effects of algal metabolites are the dominating factor for pathogen removal rather than just high pH levels. Case studies by Awuah (2006) and Ahmad et al. (2014) further shared the mechanisms by which algae are able to remove pathogens from wastewater as follows: algae out competes coliform bacteria for nutrients, hindering coliform growth; algae causes an increase in pH due to CO₂ consumption and this is lethal for pathogens; algae produces toxins of long chain fatty acids which kill pathogens; algae adds O₂ to water enhancing faecal coliform die-off

rates; and coliform bacteria attaches to the algal cells making their removal easier based on the mentioned mechanisms.

CHAPTER SIX: CONCLUSION

The study has indicated that microalgae is able to reduce the faecal indicator loads and nitrate levels of wastewater significantly. However, the study has also observed that all three species used (*Microcystis*, *Anabaena* and *Scenedesmus*) were not successful in significantly reducing the heavy metal and phosphate concentrations of the water samples.

Additionally, the study supported that the *Scenedesmus* species as indicated by other studies is a microalga that is highly effective in the treatment of organic wastes in polluted water. The algae species effectively reduced the nitrate levels and pathogen loads of the water samples of the Goreangab Dam. The study has also indicated that the *Anabaena* and *Scenedesmus* species worked well as consortia to treat nitrate concentrations and pathogen loads of the water samples. And although the *Microcystis* species produces a toxin that can negatively influence the aquatic environment, the study has shown that this species can still be used in a controlled environment to treat wastewater for phosphate concentration, as these cyanobacteria survive well in excessive phosphorus and other toxic conditions.

Furthermore, although the algae species used in the study have not reduced heavy metal and phosphate concentration significantly, there were still reductions observed as depicted by the graphs illustrated by the study's results. Therefore, the conclusion is that algae can be used to reduce heavy metal concentrations as illustrated by other studies, but more research should be conducted so that the appropriate algae species that are documented to significantly reduce heavy metals and phosphate in polluted water are used in future studies.

CHAPTER SEVEN: RECOMMENDATIONS

Based on the findings of the current study vis-à-vis the initially identified problem and the set objectives, the following recommendations are hereby proposed to guide future research in this area:

Major Recommendations

1. Engineered *in situ* bioremediation of the Goreangab Dam should be employed to reclaim the dam's water.
2. The *Scenedesmus* species is a common freshwater microalga that has effectively functioned in the removal of nutrients and pathogen loads during the study. Therefore, this species should be employed on a larger bioremediation scale by companies that function in water reclamation.
3. A variety of microalgae species need to be further investigated for their ability in the removal of organic matter from polluted water sources such as the Goreangab Dam, that can potentially serve as water supply systems which will aid in curbing the issue of water scarcity.

Minor Recommendations

1. BG 11 Media is a good growth medium for freshwater microalgae when compared to Bold's Basal Medium (BBM) which is a commonly used medium in literature but did not prove as effective during the current study. The BG 11 Media has demonstrated positively in supporting algal growth under hot and cold seasons, as the algae samples were kept and grown successfully for five months after the experiment.

2. The University of Namibia should place focus on water quality analysis tests, enabling these tests to be conducted in the laboratories by students and staff members/researchers themselves.
3. More financial support should be made available and sought out by students and researchers for water related studies such as that of water pollution and reclamation, especially in dry countries that experience low rainfall such as Namibia.
4. Globally, countries need to start adopting action plans on water pollution controls that are aimed at refining the overall water ecological environment (The State Council, 2016).

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APPENDICES

Appendix 1. Clearance Certificate



ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0105 Date: 02 June 2022

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: POTENTIAL OF SELECTED MICROALGAE IN BIOREMEDIATION OF WASTEWATER POLLUTED DAM WATER: A CASE OF GOREANGAB DAM IN WINDHOEK, NAMIBIA

Researcher: JENNIFER MEEZUU NDURA

Student Number: 201203121

Supervisor(s): DR T. SIBANDA
DR. EARL LEWIS

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:
 - i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
 - ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

A handwritten signature in black ink, appearing to read 'Z. Chiguvare', written over a horizontal line.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

A handwritten signature in black ink, appearing to read 'D. Mumbengegwi', written over a horizontal line.

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Appendix 2. Research Permission Letter

CENTRE FOR RESEARCH SERVICES

Office of the Pro-Vice Chancellor: Research, Innovation & Development

University of Namibia, Private Bag 13301, Windhoek, Namibia

340 Mandume Ndemufayo Avenue, Pioneers Park, Office F223 - Fblock, Second Floor

☎ +264 61 206 4673; E-mail:kmbulu@unam.na; URL: http://www.unam.edu.na



RESEARCH PERMISSION LETTER

Date: 06/06/2022

Student Name: Jennifer Ndura
Student Number: 201203121
Programme: Masters Science (Biological Sciences)

Approved Research Title: Potential of selected micro-algae in bioremediation of waste water polluted dam water: A case study of Goreangab Dam in Windhoek, Namibia

TO WHOM IT MAY CONCERN

I hereby confirm that the above-mentioned student is registered at the University of Namibia for the programme indicated. The proposed study met all the requirements as stipulated in the University guidelines and has been approved by the relevant committees.

The proposal adheres to ethical principles as per attached Ethical Clearance Certificate. Permission is hereby granted to carry out the research as described in the approved proposal.

Best Regards

A handwritten signature in black ink, appearing to be 'AEE Shikongo', is written over a horizontal line.

Dr. AEE Shikongo
Head: Postgraduate Support Services
Tel: +264 61 206 3129
E-mail: aeshikongo@unam.na



Appendix 3. BG 11 Medium preparation protocol

BG11 (Blue-Green Medium)

Freshwater algae and protozoa

Stocks	per 500ml
(1) NaNO_3	75.0 g
(2) K_2HPO_4	2.0 g
(3) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.75 g
(4) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1.80 g
(5) Citric acid	0.30 g
(6) Ammonium ferric citrate green	0.30 g
(7) EDTANa_2	0.05 g
(8) Na_2CO_3	1.00 g
(9) Trace metal solution:	per litre
H_3BO_3	2.86 g
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22 g
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.39 g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08 g
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	0.05 g

Medium	per litre
Stock solutions 1 - 8	10.0 ml each
Stock solution 9	1.0 ml

Make up to 1 litre with deionized water. Adjust pH to 7.1 with 1M NaOH or HCl. For agar add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

To reduce precipitation autoclave stocks 5 + 6 separately in 100ml deionized water and then add to rest of medium (autoclaved) aseptically when cool.

To make up medium from stock solutions supplied by CCAP, please refer to the labels on each stock for volume per litre.

Make up to 1 litre with deionized water. Adjust pH to 7.1 with 1M NaOH or HCl. For agar add 15.0 g per litre of Bacteriological Agar (Oxoid L11)*. Autoclave at 15 psi for 15 minutes.

Supply

*Unipath Ltd, Wade Road, Basingstoke, Hants, RG24 0PW, UK

Reference

Stanier RY, Kunisawa R, Mandel M & Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* **35**: 171-205.

Appendix 4. Heavy metals test report

Date reported: 09/June/21

e-mail: ndurajennifer@gmail.com
Tel: 081-802 5702

Client Reference no.: verbal
Quotation no.: QU-5846
Lab Reference: I210999
Enquiries: Ms Manuela Mayer

Sample description	Sample date	Analytical Parameter			#
		Cadmium as Cd mg/L	Lead as Pb mg/L	Arsenic as As mg/L	
Day 1: control	-	0.02	0.55	<0.01	1
Day 1: control	-	0.01	0.12	<0.01	2
Day 1: control	-	0.02	1.07	<0.01	3
Day 6: control	-	0.01	0.52	<0.01	4
Day 6: control	-	<0.01	0.08	<0.01	5
Day 6: control	-	<0.01	1.04	<0.01	6
Day 6: river	-	<0.01	0.34	<0.01	7
Day 6: river	-	<0.01	<0.01	<0.01	8
Day 6: river	-	<0.01	0.78	<0.01	9
Day 6: Avis dam	-	<0.01	0.24	<0.01	10
Day 6: Avis dam	-	<0.01	<0.01	<0.01	11
Day 6: Avis dam	-	<0.01	0.52	<0.01	12
Day 6: Consortium	-	<0.01	0.46	<0.01	13
Day 6: Consortium	-	<0.01	0.03	<0.01	14
Day 6: Consortium	-	<0.01	0.95	<0.01	15
Day 12: control	-	<0.01	0.53	<0.01	16
Day 12: control	-	<0.01	0.08	<0.01	17
Day 12: control	-	<0.01	1.10	<0.01	18
Day 12: river	-	<0.01	0.26	<0.01	19
Day 12: river	-	<0.01	<0.01	<0.01	20
Day 12: river	-	<0.01	0.72	<0.01	21
Day 12: Avis dam	-	<0.01	0.07	<0.01	22
Day 12: Avis dam	-	<0.01	<0.01	<0.01	23
Day 12: Avis dam	-	<0.01	0.28	<0.01	24

Enquiries: ms manuela mayer

Sample description	Sample date	Analytical Parameter			#
		Cadmium as Cd	Lead as Pb	Arsenic as As	
Day 12: Consortium	-	<0.01	0.50	<0.01	25
Day 12: Consortium	-	<0.01	0.03	<0.01	26
Day 12: Consortium	-	<0.01	0.92	<0.01	27

Remark:

Sample acceptance: Sample was collected in clients' own bottle.
Sample was suitable for testing.


Approved Technical Signatory
Ms. Manuela Mayer

This test report is only valid without any alterations and shall not be published or reproduced except in full, with written consent of the laboratory.
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Appendix 5. Nutrient test results

A	B	C	D	E
DAY	CONTROL	TREATMENT 1	TREATMENT 2	CONSORTIUM
0	2.7	2.7	2.7	2.7
6	0.73	0.7	0.62	0.62
12	0.57	0.44	0.49	0.51
			TOTAL PHOSPHATE	
DAY	CONTROL	TREATMENT 1	TREATMENT 2	CONSORTIUM
0	2.6	2.6	2.6	2.6
6	1.9	2.5	0.5	1.2
12	0.5	2.4	0.5	0.5
			NITRATE	

Appendix 6. Coliforms results

The colony forming unit (CFU) was used to estimate the concentration of total and faecal coliforms in the wastewater samples.

CFUs = (# of colonies on plate) (dilution factor)

Total Coliforms					
DAY	NUMBER OF COUNTED COLIFORMS				DILUTION FACTOR
	CONTROL	TREATMENT 1	TREATMENT 2	CONSORTIUM	
0	21	21	21	21	10 ¹
6	28	17	15	16	10 ¹
12	26	14	12	13	10 ¹

Faecal Coliforms					
DAY	NUMBER OF COUNTED COLIFORMS				DILUTION FACTOR
	CONTROL	TREATMENT 1	TREATMENT 2	CONSORTIUM	
0	13	13	13	13	10 ¹
6	12	11	9	10	10 ¹
12	12	9	7	8	10 ¹

Appendix. 7 Preparation of BG 11 media in the microbiology laboratory at the University of Namibia



Appendix 8. The 12 Erlenmeyer flasks on a laboratory shaker in the greenhouse.



Appendix 9. Process of microalgae samples dried and filtered at the NamWater Laboratory.



Appendix 10. Continuous Flow Analyzer used at the NamWater laboratory for the analysis of nutrient levels of the water samples



Appendix 11. Water samples before and after treatment with microalgae

Before Treatment



After Treatment



