Assessing the microbial community structure's diversity associated with nutrients concentration in the natural wetland systems

By

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DECLARATION

I Nondumiso Petunia Buthelezi hereby declare that this thesis is the product of my own original work and to the best of my knowledge, it has not been submitted before, for any degree or examination in any other university. I further declare that all the sources that I have used have been duly acknowledged.

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ABSTRACT

Microbial communities play a major role in natural wetlands systems biogeochemical cycles. Therefore, understanding the association between their composition, diversity and environmental parameters is significant in order to understand soil microbial ecology associated with nutrients concentration. Such knowledge may enhance the improvement in the management and protection of wetland systems, as nutrients are essential for wetland plant growth. Despite their usefulness, they promote toxin-producing cyanobacteria and facilitates algal biomass growth that cause undesirable eutrophication and oxygen depletion when present in excess. Nutrient pollution therefore is one of the most widespread and challenging environmental problems in the water that usually result to serious environmental and health challenges.

For this study, water and sediment samples were collected over a period of twelve months from Lake Icubhu, which is a fresh water wetland, and Nhlabane mouth Estuary. The physicochemical properties such as temperature, potential hydrogen (pH), chemical oxygen demand (COD), total suspended solids (TSS) and nutrients were analyzed in water samples. The InoLab_IDS multi 9310 was used for the analysis of temperature and pH, while the filter technique was used for the analysis of TSS concentrations, and spectrophotometric methods were used for the analysis of nutrients and COD concentrations, using respective kits.The pH ranged between 6.5 -7 in Lake Icubhu, and 6.9 – 7.3 in Nhlabane Estuary. Nitrate and nitrite were highest in the autumn (3.2 mg/l – 0.5 mg/l),and least in the summer (0.1 mg/l – 0.01 mg/l) for both wetlands, phosphorus and ammonium were also examined. The results of nutrients

obtained in this study demonstrated a significant threat to the studied wetlands when compared to the South African standard for Water Quality Guidelines of Aquatic ecosystem throughout the study period. It has been observed and concluded from the values obtained in this study that seasonal variations contributed to nutrient cycling. Correlation coefficient of each wetland system were also analyzed in order to understand the pollution load, as well as the effect of physicochemical parameters on the nutrients. The correlation results revealed that all these parameters are interrelated with each other and should be considered together.

Specifically, the Illumina Miseq Sequencing analysis method was used in this study to identify microbial community structures. After sequencing, the most represented bacterial community was Proteobacteria in both wetlands. Therefore, the core composition of these bacterial communities consisted of: Proteobacteria, Bacteroides, Actinobacteria, Firmicutes among others. While members of Actinobacteria, bacteroidetes and Acidobacteria were observed in Lake Icubhu, members of Firmicutes, Chloroflexi and Bacteroidetes were also dominant at Nhlabane Estuary. Finally, some representatives of Thermomicrobia, Fusobacteria, Verrucomicrobia and Fusobacteria were also retrieved from some samples. Based on the results of nutrients, microbial diversity and community structuresobtained in this study, it was concluded that seasonal variations and nutrient pollution loads were related to microbial community structures.

DEDICATION

I dedicate this dissertation to my darling princess, Silondiwe Lindelwa Ilungilindumiso "Manini" Buthelezi, for all the life-threatening suffering that we have been through MaMvulane, and for the time mummy has not spent with you.

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I would like to thank God Almighty for all the wonderful works and blessings that He has constantly showered me with and those that he still going to pour unto me.

To my husband, my daughter, my parents, my siblings and niece, I say thank you for the loyal support all the support you have given me, I real appreciate.

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I would like to express my sincere gratitude to everybody who contributed to the completion of this study either directly or indirectly, and especially, the postgraduate students of Microbiology, University of Zululand.

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PRESENTATION ARISING FROM THIS WORK

Buthelezi N.P. and Mthembu M.S. (2018, November). *Nutrient concentrations and the effects of seasonal variation in Lake Icubhu and Nhlabane Estuary*.**Paper presented at the** 12th Annual FSA Postgraduate Symposium UNIZULU, Science Centre.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Microbial communities are key players in natural wetlands systems biogeochemical cycles. Therefore, understanding of the association between their composition, diversity and environmental parameters affecting these ecosystems is significant in order to understand soil microbial ecology associated with nutrients concentration. Such knowledge can enhance the improvement in the management and protection of wetland systems, and thereby leading to wastewater purification efficiency (Ligi *et al.,* 2014). Microorganisms activities transform nitrogen through denitrification, nitrification and anammox processes which involve both aerobic and anaerobic bacteria. Anammox process is an anaerobic ammonium oxidation which combines ammonia and nitrite directly into dinitrogen gas. However, denitrification is considered as the most ultimate pathway for removal of nitrogen (Despland *et al.*, 2014; Ligi *et al.,* 2014).

Excessive presence of nutrients promotes toxin-producing cyanobacteria and facilitates algal biomass growth which cause undesirable eutrophication and oxygen depletion. These situations have negative impacts to the aquatic ecosystem, and thus decreasing water quality. While the blue-green algae produce toxins that are harmful if ingested by humans and aquatic animals (Minnesota Pollution Control Agency, 2008; Liu *et al.*, 2010); eutrophication of an aquatic ecosystem can result in loss of component species and ecosystem function (Picard *et al*., 2005; Wu *et al.,* 2011; Wu and Xu, 2011).

It has been stated in a study conducted by Huett *et al. (*2005) that considerable amount of nutrients is buried into the soil where bacteria and other microorganisms break them down,

resulting in an improved quality of water. Furthermore, appropriate macrophytes in wetland systems absorb nitrogen and phosphorus effectively and the level of water eutrophication can be lowered through phytoremediation (Minnesota Pollution Control Agency, 2008; Chao *et al.,* 2014).

Natural wetland systems are valued for their function that result in wastewater quality improvement, hence considered amongst ecological infrastructure functions. Therefore, microbial diversity studies are significant in order to understand the soil microbial ecology and other ecosystems. It is a known fact that from some of those studies that wetlands receive nutrients inputs and variety of pollution by means of run-off, storm water, periodic flooding of open waters, industrial, municipal drainage, etc. (Gopal andGhosh, 2008; Wu *et al.,* 2011).

Meanwhile, most wetlands become severely degraded or destroyed largely due to anthropogenic activities such as agriculture, land development activities such as antiquated mosquito control methods, thus destroying inhabitants of the ecosystem (Zinhiva *et al*., 2014). In another view, draining and filling of wetlands are other direct ways in which humans may contribute to degradation of wetlands, whereas weathering and erosion are natural processes.

Based on the problems that both human and natural processes may result to, wetland restoration is highly important due to the environmental benefits that they provide, though it is a complicated process since wetlands vary regionally, and the actions required for their restoration into functional equivalency of natural wetland is difficult to prescribe broadly. Failure to return the biological and biochemical features to levels found in natural wetlands even after many decades has been noted in many cases (Peralta *et al.,* 2010). Therefore, restored wetlands tend to differ physically from their original conditions. In a study conducted by Yepsen *et al*. (2014), it wasdemonstrated that restored wetlands in agricultural

settings can develop diverse native wetland plant communities within a decade but they still remain very different from the original state of natural wetlands. According to Hilderbrand *et al.* (2005), many unsatisfactory restorations results from failure to recognize and address uncertainty and from a focus on inappropriate time scales. Therefore, expecting complete restoration on human time scale is unreasonable.

This study aimed at assessing the microbial community structures and their diversity in association to nutrients concentration in the natural wetland systems. The Illumina Miseq Sequencing analysis method was used in this study to identify microbial community structures. Statistical software Prism5 was used to examine the correlation between microbial communities and physicochemical characteristics of water. These analyses enabled comparison and contrast in changes of microbial community structures, diversity as well as composition (Despland *et al.*, 2014).

1.2 Aim and Objectives

1.2.1 Aim

To investigate the influence of microbial community structures and diversity on nitrogen and phosphorus concentrations in the natural wetland systems.

1.2.2 The objectives of the study

For this study, the objectives were:

- To determine the effect of physiochemical properties on nutrients concentration in the wetland systems
- To determine nutrients concentrations in water samples from wetland systems.
- To determine microbial communities in the natural wetlands.

1.3 Literature Review

1.3.1Introduction

Natural wetlands are considered as natural filters helping to improve the quality of water from for instance, urban and agricultural lands runoff by trapping pollutants and harmful substances that may be carried with water. They are particularly useful because they are generally located between land and open water. This allows them to intercept many pollutants before they enter the river systems (Kotze, 2000). Natural wetlands have a natural, innate ability to treat wastewater, thus improving the quality of water (Gert, 1997). The slow flows of wetland water act to prolong contact times between the water and surfaces, and thus resulting to sediments settling at the bottom. Gopal and Ghosh (2008) stated that increasingly, greater attention has been paid to the wetlands for their potential of improving wastewater quality as well as for the development of an energy efficient inexpensive technology for treating wastewater based on wetlands over the past decades. When most people think of wetlands they only often think of water resources and improvement of its quality, wildlife and nature conservation. Moreover, wetlands, especially the natural ones, contain cultural wealth that is often ignored (DEAT, 2015).

1.3.2 Natural wetlands categories

Natural wetlands can be divided into coastal and inland wetlands. These wetlands differ in their characteristics as ecosystem, as well as their location.

1.3.2.1 Coastal wetlands

Coastal wetlands are ideally located in areas between the marine and terrestrial environments as shown in Figure 1,to reduce impacts from the land-based sources. Various types of coastal wetlands include riparian wetlands, tidal fresh water mashes, tidal salt marshes, mangroves,

etc. These wetlands are known to retain or transform and sometimes act as sources of nutrients sediments (Bruland, 2008; Tiner, 2009). Existing literature shows that coastal wetlands have a strong intrinsic dynamism and pronounced environmental gradients such as nutrients, etc. (Benito *et al.*, 2015). However, human activities often result in drastic modifications of these natural gradients as well as the natural ecological functioning of these systems (Benito *et al.*, 2015). Whenever this happens, it results in loss or elimination of most of the original distribution of the coastal wetlands habitats (Liao, 2014; Wang *et al.*, 2014; Benito *et al.*, 2015; Mbaye *et al.*, 2015). These transitional zones between terrestrial and aquatic inhabitants provide primary nursery habitat for fish and other inhabitants. They also provide the inhabitants with the nesting, resting and feeding place. Additionally, it also acts as a refuge for inland aquatic inhabitants during the period of droughts. Therefore, tidal inundation and seasonal freshwater flooding have an influence on these wetlands (Bruland, 2008; Jiang *et al.*, 2015).

Figure 1: An example of a coastal wetland. Pictured here is Nhlabane mouth Estuary.

1.3.2 Inland wetlands

Figure 2 below shows an example of an inland (fresh water) wetland. They result from the flow of surface or even ground water from the surrounding catchments. They are known to be natural harvesters of rain water (McCartney *et al.*, 2010). They are also commonly found on floodplains along rivers and streams, along the lakes and ponds margins and in isolated

depressions where they are normally surrounded by dry land (Ornes, 2014). Inland wetlands are significantly important or are of great value to human kind (Joaquin, 2009). They are known of their high productivity that provides environmental services as well as habitat for various species of flora and fauna. Therefore, they harbor high value of biodiversity (De Gortari–Ludlow *et al.,* 2015). However, it has been documented that these types of wetlands are mostly threatened by anthropogenic activity which also alters the natural environmental gradient of the system (Drenovsky *et al.,* 2010; Herbert *et al.,* 2015). In that case, wetlands plants may not be able to tolerate high concentrations of nutrients and toxic pollutants. This may result in destruction of microbial communities, thus the treatment efficiency of inland wetland is reduced (Leung *et al.,* 2016). Microbial communities are generally recognized as important factors in biodegradation of organic matter. They play a key role in transformation of toxic compounds into harmless products, and also in removal of nutrients (Meerbergen *et al.,* 2017).

Figure 2: An example of a fresh water wetland. Pictured here is Lake Icubhu.

1.3.3 The main components of natural wetlands

Natural wetlands are natural resources that utilize natural processes to assist in water purification (Vymazal, 2006). They comprise of wetland vegetation (macrophytes), soil medium and microbial biofilm to assist in treating wastewater. The main components of the natural wetlands are explained below.

1.3.3.1 Water column

Biochemical reactions in wetlands require the presence of water. The water column also acts as a medium of transport for organic solids, nutrients, gases, etc. Water is an important factor in the establishment and maintenance of specific types of wetlands and their processes. Abiotic factors can be affected by wetland hydraulic conditions. Moreover, these factors determine the flora and fauna that develop in a wetland. In contrast, the biotic components are active in alteration of the wetland hydrology (EPA, 2006).

1.3.3.2 Macrophytes and their roles

Macrophytes are one of the components of wetlands. Their main role in natural wetland ecosystems is transformation and uptake of the nutrients for their own growth, and thereby resulting in an improved quality in wastewater (Gopal and Ghosh, 2008). Macrophytes in natural wetlands serve numerous important functions that result in an improved wastewater quality. In another words, they contribute to transformations by aiding in the physical, chemical and microbial processes. The initial mechanism in removal of some nutrient loads is by filtering and settling of inorganic and organic particulate matter. As the polluted water slowly passes through, the wetland system offers a mechanical resistance to the flow of water. This increases the retention time and facilitate the settling of suspended particulates. Macrophytes also provide a substrate for the attachment of decomposer microorganisms that breaking down dissolved organic matter. The non-rooted macrophytes such as duckweed, sphagnum, etc., obtain their nutrients directly from the water column and the incorporation of

their detritus into the soil is a net transfer of nutrients from the water to the soil (Dale, 1993). Rooted submerged macrophytes obtain their nutrients from the sediments by their root uptake as well as by means of foliar uptake from the water. They also improve the conductance of water though the soil as dead roots create spaces. Similarly, the photosynthetic organisms also contribute to the production of oxygen and the uptake of nutrients as well as the transfer to higher trophic levels (Gopal and Ghosh, 2008). With that case, natural wetlands nutrient retention are often greatest during periods of activevegetation growth but low during non-growing seasons (Dale, 1993).

1.3.3.3 Soil medium or substrate

Natural wetland substrate include soil, sand, gravel, rocks, etc. Microbial activities together with nutrients saturation cause depletion of oxygen in the soil. Biochemical processes are then promoted by this anaerobiosis (Hurt, 2013), and soil medium accomplishes several important functions in the natural wetland systems. It is an important water site and also an important source of nutrients for rooted and submerged plants. Soil also provides physical support for the above ground plants. Soil medium is a habitat for mycorrhizae, symbiotic bacteria and soil macrofauna. It also provides wetlands with the ability to improve the quality of water by acting as a locus of biological, chemical and physical processes, aiding in an improved water quality. It also acts as an important seed germination medium and as conduit in ground water recharging (Brady andRay, 2010).

1.3.3.4 Microbial biofilm

The living organisms found in a wetland, microorganisms like bacteria, fungi, protozoa, etc., play a significant role in the purification of water. These microorganisms help in biochemical reactions taking place in the wetland as part of the treatment process. Microbial biofilm similarly plays a central role in the biogeochemical transformations of nutrients (Vymazal *et*

al., 1998). Importantly, there is a close interdependence between microorganisms and vegetation. Much of the plant nutrients are the result of the mineralization of more complex compounds by the microorganisms, whereas the activity of the latter is stimulated by enzymes released in root exudates. The aerobic conditions provided by the plants in their rhizosphere will also be determinant for the type of microbial populations and bioprocesses available in this region. The conjugation of all these factors and interactions between different wetlands components and their associated processes lead to essentially different abilities to interact with water pollutants by different types of wetlands (Drenovsky *et al.,* 2010).

1.3.4 Factors affecting microbial diversity

Zhao *et al.* (2012) reported that different microorganisms within bacterial communities will perform the same processes and most probably be found in the same niches. It has been noticed that some factors tend to affect microbial communities; these can be classified broadly into two groups, namely: biotic and abiotic factors (Fakruddin andMannan, 2013).

1.3.4.1 Biotic factors

Talking about biotic factors, they include plasmids, phages and transposons which are types of accessory DNA that have a great influence on microbial diversity. They influence the genetic properties and in most cases, the phenotypes of their host (Zhao *et al.,* 2012). Moreover, Fakruddin and Mannan (2013) reported protozoans to influence the wetland systems' soil microbial diversity by regulating its size and composition.

1.3.4.2 Abiotic factors

In contrast to biotic factors, abiotic factors include both physical and chemical factors such as temperature: pH, water availability, salinity, chemical pollution, heavy metals, pesticides,

antibiotics etc. As investigated and documented, all environmental variations are known to affect microbial diversity in different ways, and to different extents, resulting in diverse profile shift (Fakruddin and Mannan, 2013). The effect of some of the abiotic factors are explained below.

i. Temperature

The overall natural wetlands functioning is influenced by temperature since it effects several biogeochemical processes that regulate the removal of nutrients from wastewater (Robert and Reddy, 2001; Ghada, 2010). Temperature also affects microbial mediated reactions. It is on record that many biogeochemical reactions are known to proceed at a faster rate when medium temperature is being increased (Robert andReddy, 2001). This includes microbial mediated reactions and the processes that regulate decomposition of organic matter and all nitrogen cycling reactions are being affected by temperature (Ghada, 2010). However, microbial processes have optimal ranges where they occur. Therefore, microbial processes are known to proceed at rates that are temperature dependent, and therefore display seasonality (Robert and Reddy, 2001). Annelies *et al.* (2010) pointed out on their study conducted in Belgium that temperature seemed to be the stirring factor rather than the season, since total nitrogen, NH_4-N and P removal from wastewater is shown to be significantly influenced by temperatures between 5 ^{0}C– 15 ^{0}C. However, removal of nutrients from wastewater relapsed at lower or higher temperatures. Temperature in that case is known to be a key influencing factor in vegetation growth. Meanwhile, plants grow well in higher water temperatures, and then result in large production of vegetation which consequently, enables consumption of more nutrients from the wetland systems sediment (Wang *et al.*, 2014). In another investigation, low water temperatures reduce or even limit the ability of the vegetation cell growth. Thus the ability of aquatic plants to purify eutrophic water is reduced (Lee *et al.*, 2009).

ii. pH

Microbial diversity is influenced by pH since many of the microbial species cannot tolerate extreme pH levels. Microbial activity may be hindered as a results of pH alterations because it denatures proteins within the cells and / or render essential microbial enzymes inactive (Sylvia, 2005). Nevertheless, there are some microbes that can withstand extreme pH environments. For instance, fungi and other acidophilic bacteria have a competitive advantage at pH bellow 5, in contrast to other bacteria that thrive at more neutral pH. Therefore, pH also affects nutrients availability in the soil (Brady and Ray, 2010). Similarly, Fierer and Jackson (2006) observed pH as an important factor influencing microbial diversity, and with value that significantly influences the growth of aquatic plants. Gobel (2011) noted in his study that there was a significant decrease in plants with drop in pH. The alkali – resistant aquatic plants becomes the dominant species in alkaline water body, thus resulting in too simplified plant species (Lee *et al.*, 2009).

iii. Salinity

Salinity results in the alteration of the clay fractions and also the degradation of the original sediment fractions (Maganhotto andFrancisconi, 2012), thus becoming easy to be transported by rain. Plants and microbial growth can be adversely affected by salinity due to the soil structure and its consequent compactivity that has been destroyed. It has been documented in the study conducted by Hu *et al.* (2016) that wetland ecosystem structure and function are considered to be highly driven by salinity. Since a wide range of abiotic and biotic processes are being directly impacted by salinity concentrations, salinity therefore may be detrimental to sensitive microorganisms and yields to a decreased activity of the surviving cells due to the metabolic load that imposed by the need or stress tolerant mechanism (Brady and Ray, 2010; Hu *et al.*, 2016). Salinity is known to have an effect on microbial community structures

which includes anammox bacteria activity in estuaries. It further has an ability to regulate availability of substrates (NH_4^+ and NO_2^-) for anammox reactions, thus shaping the bacterial community composition of anammox bacteria (Fu *et al.*, 2015; Zeng *et al.*, 2016; Jiang *et al.*, 2017).

iv. TSS

Suspended solids are removed from wastewater by means of sedimentation, filtration and adsorption on the substrate (gravel) in natural wetland systems (Brady and Ray, 2010). While suspended solids in water consist of inorganic and organic matter (DWAF, 2006);high levels of turbidity result in less light in lower levels of water, resulting in reduction in plant productivity at the bottom levels. Thus, reduces microbial growth and activities, and underwater vegetation die-off resulting to negative effects in the system. The photosynthetic processes decreases, resulting in less production of dissolved oxygen, thus reduces DO levels. The vegetation amount to feed aquatic life is reduced, resulting in population and food chain decline. This is due to the fact that underwater plants are important food sources for many aquatic organisms (Russell and Robert, 2006).

1.3.5 Wetlands and humans

The natural wetland resources used by humans include the fiber plants used for handcrafts and construction, the land for cultivation, grazing for livestock, water for domestic use and wild sources of medicinal plants (e.g. *Ranunculus multifidus)* (Kotze, 2000). Natural wetlands also provide humans with significant benefits that include: social, economic and cultural benefits. The natural wetland resources are important in maintaining good water quality in rivers, and in recharging of ground water. They also function in storing of carbon, help in stabilization of climate conditions. Aside the above, wetlands have an important function in reduction of the impacts by storm damage as well as flooding. They are important

sites for biodiversity and pest control (Gopal andGhosh, 2008). Restoration of wetland systems is highly significant in order to maintain the good health of waters, wildlife as well as the environment (Kentula, 2002).

1.3.6 Uses of harvested macrophytes

Across many rural areas in KZN, wetland sedges such as *Jancus krausii* and *Scirpus spp.* are being harvested in coastal wetlands, while *Cyperus latifolius* is being harvested inland for weaving of traditional sleeping mats. The *Cyperus marginatus* is harvested and rolled into a durable twine, and are used in the production of traditional beer strainers. Mat houses are also made from the culms of the growing matjiesgoed sedges harvested that are stitched together using a thread and a needle (DEAT, 2015). Kotze (2000) also stated that people make these mats for their own use or local sale because of their cultural value and may be used as traditional wedding gifts because of their quality.

Still on the usefulness of wetlands, it is discovered that reed screens used mostly by safari camps which helps them to create an authentic African experience for tourists staying in the camps are being harvested in wetlands. While they are also being used in some areas of the Northern Province to provide a link in communication between people and their ancestors,some areas in Northern KZN still use network reed in the lakes to trap fish (DEAT, 2015). In another view, a healing plant known as river pumpkin or Gunnera Perpensa also grows in wetlands, but mostly found in Eastern Cape. This plant is used to ease childbirth, and promote the expulsion of the afterbirth both in humans and livestock. It may also be used to treat kidney and bladder complaints along with other plants (DEAT, 2015).

Wetlands also allow for the growth of *Colocasia Esculenta* (AmaDumbe). These are traditional crops which are widely grown in most parts of KZN and Mpumalanga. They require a high soil water. They are grown mainly for their underground starchy corms which

are easily digestible after being boiled. AmaDumbe are also known for their health benefits to humans as well as their energy source (Naidoo *et al*., 2014). Their leaves are also being used as spinach, thus providing supplement to the maize. Indian communities for instance also use amaDumbe as part of their cooking ingredients when preparing meal known as puripatha (DEAT, 2015). These root crops are native to south East Asia, and were introduced to Southern Africa centuries ago (Kotze, 2000). *Aponogeton distachyos* commonly known as Cape waterblommetjie which are white flowers of an indigenous aquatic plant that also grows in wetlands is used likewise to make a very tasty traditional cape dish or a vegetable stew known as waterblommetjie bredie (DEAT, 2015). Wetland restoration is highly significant in order to accommodate human lives and their needs for developments. Restoration also assist to maintain the good benefits of natural wetland systems as well as their surroundings (Kentula, 2002).

1.3.7 Advantages and benefits of wetlands

Natural wetlands provide number of functions and values. These include: water quality improvement, ground water recharge, cycling of nutrients and other materials. Wetlands also havean important benefit of filtering harmful substances that might be toxic for human consumption and/or yielded negative impacts on wild life and wetland inhabitants. They also protect against floods and erosion, and in another way provide habitat and food for fish and other wildlife. Wetlandsare also advantageousin terms of both active and passive recreational values (Gopal and Ghosh, 2008; Ghada, 2010).

1.3.8 Threats to wetlands

Many are threats to wetlands, but human activities such as filling and draining are major threats to wetlands. These activities occur for different purposes that include provision of land for farm, recreation, and buildings. These activities therefore can result in loss of

abilities and advantages of the wetland system (Drenovsky, 2010). Filling and draining are not the only threats to wetlands, but exotic species also threaten wetlands. When these exotic species are introduced into the wetland, they grow and make it difficult for the native plants, animals and insects to survive.

Talking about the exotic species, Purple Loosestrife is one of the most threatening ones in the wetlands as it adversely affects wetland ecosystem by replacing or displacing native flora and fauna. It also eliminates food, shelter, nesting, etc. for the inhabitants. Its seeds geminate faster than many of the native wetland species, making difficult for the birds, mammals, fish, etc. since they cannot depend on it. This leads to the lost or destruction of inhabitants, with an exception of those that can migrate and get displaced. Based on evidence, Purple Loosestrife negatively affects wetlands recreational values as well, which may impact local economies. This land also negatively affects agriculture by blocking the drainage flow, irrigation and ditches, and resulting in a decreased crop yield (Blossely *et al.*, 2001).

1.3.9 Importance of wetland restoration

Wetland systems are crucial to the health of waters as well as wildlife. Therefore, their restoration is vital and it is of current interest (Kluber *et al.*, 2014). Restoration of wetlands involves the renewal of its natural and historical value that has been degraded or lost. This process of returning a degraded wetland to its preexisting naturally functioning or condition that is more close to its original as possible is complex since it requires expertise, resources and commitment from different stakeholders. Restoration of wetlands is important for water quality, loss in part of heritage, loss of flood prevention, etc. (US Environmental Protection Agency, 2001; Kentula, 2002). According to a study conducted by Peralta *et al.* (2010), there is a significantly difference in bacterial community structures between the natural and the restored wetlands. Moreover, the assemblage of denitrifying community was found to be

similar amongst the reference sites in contrast to restored wetlands, where it was highly variable throughout the mitigation bank. This demonstration concludes that the effort in restoring wetlands has not successfully restored denitrification and that the differences in potential denitrification rates between the two wetlands may be due to distinct differences in microbial assemblages observed.

1.3.10 Nutrients

Phosphorus and nitrogen are the most common nutrients found in water (Meerbergen *et al.*, 2017). Although they are essential for plant growth, their presence in excess yields undesirable eutrophication (Liu *et al.*, 2010). Eutrophication is defined as an excessive richness of nutrients in wetlands that results in depletion of oxygen. Figure 3 below shows nitrogen and phosphorus cycles that occurs in water.

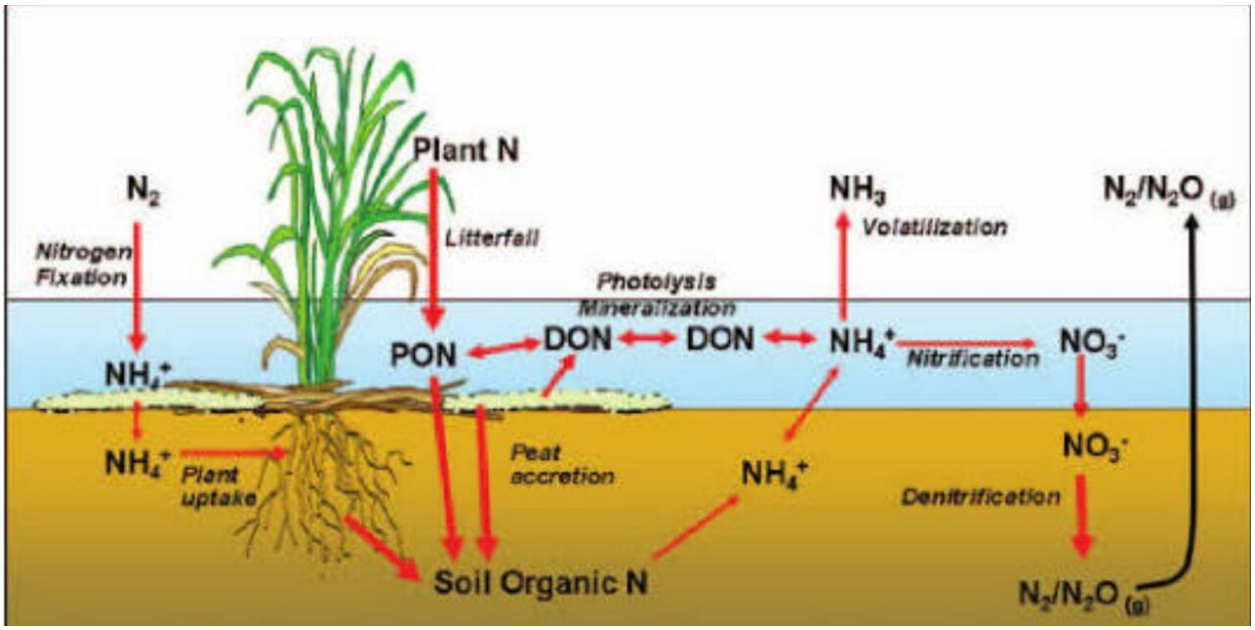

Figure 3: Nitrogen and phosphorus cycles that occurs in wetland systems

1.3.10 Mechanism of nitrogen removal in natural wetlands

Eutrophication is a major problem in natural wetlands. The presence of nitrogen in excess limits the primary production in different parts of the wetland. The removal mechanism of nitrogen in natural wetlands involves: ammonification, nitrification, denitrification, plant assimilation, etc. The total nitrogen is present in a form of both the organic and inorganic forms in water. The organic nitrogen is in a form of dissolved or particulate matter, whereas the inorganic nitrogen is either in a form of nitrates or ammonium *ions* (Gopal andGhosh 2008). While ammonium, nitric and nitrate are the most common important inorganic forms of nitrogen in the wetlands, dinitrogen, nitrous oxide, nitric oxide and ammonia exist as gaseous nitrogen (Vymazal, 2006). The mechanism of nitrogen removal from the wetlands include ammonia volatilization, ammonification, nitrification, denitrification, biodegradation, fixation, plant uptake and decomposition. The various nitrogen removal mechanisms are explained below.

1.3.10.1 Ammonia volatilization

Ammonia volatilization is a physiochemical process where ammonium – N is at equilibrium between hydroxyl and gaseous forms (Vymazal, 2006). Its losses to the atmosphere depend on climate conditions, and is significantly temperature dependent (Viero *et al.,* 2014). Both agricultural and non-agricultural ecosystems benefit from the loss of ammonia. As a result of that, they reduce the ratio of N: P in manure, allowing acceleration in P build up in soil. Ammonia presence in access also contributes to eutrophication in aquatic ecosystems, and ammonia volatilization may be represented by the following reaction (Meisinger *et al.,* 2000).

$$NH^{+}_{4} - N \leftrightarrow NH_{3g} + H^{+}$$

Serious environmental issues may result from the extensive use of nitrogen fertilizer. Emissions of ammonia from the application of agricultural nitrogen fertilizer, negatively impact both the environmental quality and human health (Yi *et al.,* 2017). However,

application of nitrogen (N) fertilizer is important in modern agriculture in order to maintaining a high rate of products.

1.3.10.2 Ammonification

Ammonification is a process through which organic nitrogen is biologically converted to ammonia. The ammonification rates are faster in the oxygenated zone and decrease as the mineralization circuit changes from aerobic to facultative anaerobic and obligate anaerobic (Lee *et al.,* 2009). When that occurs, the organic form of nitrogen is excreted from the tissues of an organisms' waste or even when it dies off. Furthermore, various prokaryotes and fungi releases the inorganic nitrogen back into the ecosystem as ammonia by decomposing the tissues. Ammonia thereby becomes available to be utilized by both the plants and other microorganisms for their own growth (Bernhard, 2010).

1.3.10.3 Nitrification

The nitrifiers such as *Nitrosomonas*, *Nitropira*, *Nitrosococus* and *Nitrobacter* perform biological nitrification, then followed by nitrification which is believed to be the pathway for ammonia removal (Bernhard, 2010). The chemolithoautotrophic oxidation of ammonia to nitrate under strict aerobic conditions is implied by nitrification and involves two sequential oxidative stages. These stages are ammonia to nitrite (ammonia oxidation), and nitrite to nitrate (nitrite oxidation). Each of these stages is performed by microorganisms of different genera's which uses ammonia or nitrite as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. The bacteria, *Nitronosomas* is most commonly recognized for the ammonia oxidation process and that of *Nitrobacter* for the nitrate oxidation process. Nitrification process is oxygen demanding and the overall equation of these reactions involved in nitrification is represented by Lee *et al.* (2009) as follows:

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+$

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$

1.3.10.4 Denitrification

The mechanism of biological denitrification uses nitrate as the terminal electron acceptor in low oxygen environments. In accomplishing these process, denitrifying bacteria decreases the inorganic nitrogen such as nitrite and nitrate into innocuous fundamental nitrogen gas. Moreover, the denitrifiers also known as denitrifying bacteria can be classified into two major species which are:*heterotrophs* and *autotrophs*. The *heterotrophs* microbes need organic substrates to obtain their carbon source for growth as well as for evolution, and get energy from organic matter. In contrast, the *autotrophs* utilize the inorganic substances as an energy sources and make use of carbon dioxide as a carbon source. Meanwhile heterotrophic denitrification process has mainly been engaged in conventional wastewater treatment plants. Under anaerobic or anoxic conditions, heterotrophic microorganisms such as:*Pseudomonas*, *Micrococcus*, *Bacillus* and *Achromobactor* are responsible for conducting denitrification. According to Lee *et al.* (2009), the proportion of total nitrogen removal by denitrification is typically 60 – 90%, in comparison to 1 – 34% assimilation by plants and algae. Therefore, denitrification process plays an important role in the removal of nitrogen in water treatment, thus improve the quality of water. An oxidized form of N, NO_2^- and NO_3^- is utilised by heterotrophic microorganisms as terminal electron acceptors and organic carbon as an electron donor under anoxic conditions. This process may be indicated by the following equation.

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Sufficient organic carbon is therefore then needed as an electron donor for reduction of nitrate, which provides energy source for denitrification microorganisms (Lee *et al.*, 2009).

1.3.10.5 Biodegradation: Anammox routes

Anammox is the anaerobic ammonium oxidation process that provides a potential alternative process for improving total nitrogen removal from natural wetland waters. Lee *et al.* (2009) reported that denitrification by anammox bacteria is now proven to be partly responsible for the transformation of ammonia into nitrogen gas within the nitrogen cycle. This process allows ammonium to be oxidized to nitrogen gas automatically while nitrite is employed as an electron acceptor under anaerobic conditions. Therefore, there is an absolutely no demand for the aeration and addition of an external carbon sources, which results in cost saving and prevention of insufficient conversion of organic substances. The anammox bacteria such *as Candidatus Brocadia anammoxidans, Planctomycetesspp.* and *Thiobacillus senitrificans* are *autotrophic*, which in contrast to classic denitrifiers that are mostly *heterotrophic* and thus needs organic carbon for their carbon and energy supply (Lee *et al.,* 2009).

1.3.10.6 Fixation

The conversion of gaseous nitrogen (N_2) to ammonia is known as nitrogen fixation. This process requires nitrogenase, an oxygen sensitive *ion*, sulphur and molybdenum containing enzyme complex which also brings about the reaction of other substances that contain triple covalent bonds, e.g. nitrous oxide and cyanides. Biological N_2 fixation in wetlands soils may occur in the flood water, soil surface, aerobic and anaerobic flooded soils, and root zone of plants and on the leaves as well as the stem surfaces of the plants. The nitrogen in the wetlands can therefore be fixed by a variety of symbiotic actinomycetes and asymbiotic heterotrophic bacteria and blue-green algae. Fixation of nitrogen in soil is greater under anaerobic conditions, although the ability to fix it is also distributed among aerobic and facultative bacteria. Under aerobic conditions in heterocysts, nitrogen may be fixed by heterocystous blue-green algae (cyanobacteria). Heterocysts are not formed when conditions

are anaerobic and fixation of nitrogen proceeds in vegetative cells of blue-green algae. Therefore, nitrogen photosynthetic bacteria require anaerobic conditions for growth, and the fixation of nitrogen is greater under reduced than oxidized conditions within the soil layer of flooded soil system (Vymazal, 2006).

1.3.10.7 Plant uptake

Macrophytes convert inorganic nitrogen forms into organic compounds as building blocks for cells and tissues. The nitrogen absorbed by various plant species differs and depends on the available N_2 form in wetlands. The rooted plants are capable of utilizing sediment nutrients. Moreover, the NH_4^- preference is mostly common in macrophytes living in environments with limited nitrification where NH_4^- is abundant. The uptake rate and storage of nutrients by plants depends on the nutrient concentration of their tissues. Therefore, plants used for nutrient assimilation and storage should have desirable features which will include fast growth, high tissue nutrient content and the ability to obtain high standing crop (Lee *et al.,* 2009).

1.3.10.8 Decomposition

The release of nitrogen in a form of ammonium (NH_4^+)results from the decomposition of organic matter by variety of microorganisms. It is then either utilized by microorganisms or diffuses back into the soil or water. While under aerobic conditions, nitrification occurs, and the NH_4^+-N is converted into nitrates by microbes such as *Nitrosomonas* and *Nitrobacter sp;*but ammonium oxidation (anammox) occurs under anaerobic conditions which utilizes the nitrates and result in a release of molecular N_2 (gas). Under alkaline conditions ammonium is transferred to ammonia (NH_3) and then get released into the atmosphere as ammonia gas. The NH_4^+ formation may also result from the microbial transformation of nitrates, known as dissimilatory nitrate reduction. The major mechanism for loss of nitrogen from the wetlands

is *denitrification*, in contrast to most nitrogen transformations which conserve nitrogen within the soil and water system. This is favoured by peat accumulation which creates anaerobic conditions. The rapid loss of nitrogen from the wetlands is being promoted by the frequent wetting and drying cycles, redox gradient along with the flow pathway in the wetland, and the occurrence of an aerobic – anaerobic interface near the wetland soil surface. The decomposition of plant litter also depends on nitrogen concentration. Under favourable conditions, various organisms particularly the cyanobacteria (blue green algae), both the green living and symbiotic, may add substantial amount of nitrogen into the wetland trough nitrogen fixation. Nitrogen compounds are readily leached out since they are labile. Therefore, further decomposition takes place only after microbial immobilization of nitrogen (Gopal and Ghosh, 2008).

1.3.11 Mechanism of phosphorus removal in natural wetland systems

Phosphorus mainly occurs as phosphate in inorganic or organic compound in the wetlands. The only form of phosphorus known to be utilized directly by algae and macrophytes is the free orthophosphate which represents a major link between inorganic and organic phosphorus cycling in wetlands. The polyphosphates that are linearly condensed and cyclic are another group of inorganic phosphorus. It should be remembered that phosphorus of organic forms can be grouped into easily decomposable phosphorus (nucleic acids, phospholipids or sugar phosphates) and slowly decomposable organic phosphate (inositol phosphate or phytin). Originally, bound phosphorus is also present e.g. in phospholipids, nucleic acids, nucleoproteins, phosphorylated sugars or organic condensed polyphosphates. The soil phosphorus and nitrogen cycle are fundamentally different as there are no valence charges during the biotic assimilation of inorganic phosphorus or even during the decomposition of organic phosphorus by microorganisms. Soil phosphorus primarily occurs in the $+_5$ (oxidized) valence state because all lower oxidation states are thermodynamically unstable

and readily oxidized to PO_4 even in high reduced wetland soils (Vymazal, 2006). Phosphorus that is loosely adsorbed is vital for plant growth and for controlling the phosphorus concentration of the overlaying water column. Phosphorus associated with oxyhydroxides is readily desorbed under most conditions, with the phosphorus associated with crystalline iron and aluminium desorbed only under prolonged anoxic conditions (Gopal and Ghosh, 2008).

Another point worthy of notice is that excess phosphorus act as a pollutant when the wetland becomes enriched with nutrients, and thus, resulting in surface water damage (Gazzetti, 2011; Leader *et al.,* 2015). Industrialised countries no doubt cause an increased water pollution especiallyfrom the acidic and metal rich mine drainages following flooding of abandoned mine workings. But natural wetlands have an ability of removing pollutants such as phosphate from the wastewater. Meanwhile, wetland plants exploit the phosphorus present in wetland systems and use it for their own growth. However, the amount of phosphorus that can be retain from the wetland system depends on the type of wetland vegetation (Gopal and Gosh, 2008).

Importantly, organic phosphorus is mineralized by alternate wetting and drying cycles. This may lead to changes in substrate pH and increased microbial activity, and thereby leading to mineralization of organic phosphate. Removal of such phosphate in natural wetlands from wastewaters occurs through several pathways. These pathways include: sedimentation in the water column, adsorption on the organic or mineral sediments, co-precipitation with carbonates during photosynthesis, uptake by macrophytes, algae, epiphytes, and incorporation by microorganisms (Gopal and Ghosh, 2008).

1.3.11.1 Phosphorus transformation in wetlands

The natural wetlands provide an environment for interconversion of all forms of phosphorus. Soluble reactive phosphorus is taken up by macrophytes and then converted to tissue

phosphorus or may even become absorbed to wetland soil and sediments. If organic matrix is oxidized, organic structural phosphorus may then be released as soluble phosphorus. In some instances, it may be in a form of insoluble precipitates but may re-dissolve under altered conditions. Transformations of phosphorus in wetland systems occur through: peat/soil accretion, adsorption/desorption, precipitation, dissolution, plant/microbial uptake, fragmentation and leaching as well as mineralization and burial (Vymazal, 2006).

1.3.11.2 Peat/soil accretion

According to Lee *et al.* (2009), phosphorus cycling in wetland systems has shown that soil/peat accumulation is a major long-term phosphorus sink, as accretion in tidal marshes ultimately leads to the formation of peat soils (Drexler *et al.,* 2009; Morris *et al.,* 2016). Vertically, peat accretion rates vary both spatially and temporally. Accretion in spatial variation therefore, may be attributed to differences in ecological and or physical factors. In contrast, accretion in temporal variation is greatly by extreme natural events such as fire and climatic fluctuations. Sediment supplying channels and flooding that affect plant productivity are also part of temporal variations (Nyman *et al.,* 2006; Morris *et al.,* 2016).

1.3.11.3 Soil adsorption and precipitation

The movement of soluble inorganic phosphorus from soil pore water to soil mineral surface is referred to as adsorption. Generally, the soil adsorption capacity of phosphorus increases with clay content or the mineral components of that soil. The equilibrium between the solid phase and phosphorus in soil pore water is maintained by the balance between phosphorus adsorption and desorption. This phenomenon is known as the phosphate buffering capacity, and is analogous to the pH buffering capacity of the soil. While the adsorption of inorganic phosphorus is related to either high Al, Fe or Ca levels, phosphorus sorption capacity of wetland soil may be predicted solely from the oxalate extractable (amorphous) aluminium

content of the soil. The concentration of phosphorus in the soil pore water, and the ability of the solid phase to replenish phosphate into the soil control the sorption of phosphorus. The net movement of phosphorus from soil to soil pore water phosphorus concentrations results when soil particles becomes saturated with phosphorus and soil pore water has low concentrations of phosphorus. Sorption can be generally described in a two-step process. The first is the rapid exchange of phosphorus between the soil pore water and soil particles or mineral surfaces (adsorption). The second is the slowly penetration of phosphate into the solid phases, which is referred to as absorption (Vymazal, 2006).

1.3.11.4 Microbial uptake

The microbial uptake of phosphorus by microbiota (bacteria, fungi, algae, micro invertebrates, etc.) is very fast, yet the amount stored is very low. The uptake by these organisms is rapid because of their fast growth and multiplying at high rates. Significant amount of phosphorus may be released in response to seasonal conditions from the microbial biomass. This depends on either carbon availability, soil undergoing the process of wetting and drying or during the process of higher trophic level predation (Richardson and Simpson, 2011). Vymazal (2006) pointed out in his study that the trophic of the wetlands plays an important role in the amount of microbial storage. Soil microorganisms are also known in the solubilisation of the soil phosphorus. Phosphorus cycling can be affected by algae and algal assemblages either directly (uptake, release) or indirectly through photosynthesis, induced as well as through changes in water and soil/water interface parameters (pH, dissolved oxygen).

1.3.11.5 Plant uptake

Plant roots take up most of the phosphorus while absorption through shoot and leaves is restricted to submerged species. However, phosphorus removal through this mechanism is very low. The uptake of phosphorus by macrophytes is usually highest during the beginning

of growing seasons. The translocation of nutrients within the plant serves as an important response to seasons. The majority of important *ions* are translocated from the shoot portions to the roots and rhizomes prior to autumn senescence and these stored nutrients are then used during early spring growth. Storage of phosphorus can range from short to long term in vegetation. This depends on a type of vegetation, litter decomposition rates, leaching of phosphorus from detrital tissues and its translocation from above to below ground biomass. The storage of phosphorus in above ground biomass of emergent macrophytes is usually short term with large amounts of phosphorus released during litter decomposition. Most parts of the aboveground macrophytes grow and decay on a cycle ranging from annual growing season in northern climates to faster cycles in southern climates. After the plant decay, phosphorus is released back to the wetland ecosystem from the biomass (Vymazal, 2006).

1.3.12 Significance of wetland studies

The deterioration of water quality is of high concern since water is an important natural resource in the world, and without it, life cannot exist. Therefore, there is a crucial need to conserve and protect waters and natural wetlands. Studies on wetlands have shown a positive contribution towards water quality improvement. According to Gopal and Ghosh (2008), natural wetlands within the United States have received high protection due to recognition of their ability to improve water quality. The study further reported that during the past two decades, there has been an increasingly great attention paid to natural wetlands potential of improving water quality as well as for the development of an energy efficient technology for wastewater treatment that is inexpensive and based on wetlands. Kotze (2000) also pointed out in his study that wetlands have demonstrated the high effectiveness in removal of nitrogen and phosphorus.

1.3.13 Important of conducting this study

From the literature and previous studies as well as general observation, it has been observed that eutrophication poses dangers and threaten wetland systems. Eutrophication yields negative impacts in aquatic ecosystems, for instance, the loss of wetland component species and ecosystem function (Wu *et al.,* 2011). Anthropogenic activities such as agriculture, land development and other activities destroy the inhabitants of the wetland ecosystem, thus degrading the wetland (Zinhiva *et al*., 2014). Also, weathering and erosion are natural processes that contribute to wetland degradation.

Natural wetland systems are most valued for their function that result in water quality improvement. Therefore, microbial diversity studies are significant in order to understand the soil microbial ecology and other ecosystems (Wu *et al.,* 2011). Most previously conducted studies have demonstrated that nutrients may be removed by wetland systems (Huett *et al.,* 2005; Vymazal, 2006; Lee *et al.,* 2009). Thus, this study investigated the effects of microbial communities and diversity along with concentration of nutrients in improving the quality of water by wetland systems. Moreover, understanding the relationship between microbial composition, structures, diversity and the environmental parameters that affect these ecosystem is of significant in order to understand soil microbial ecology that is associated with concentration of nutrients. These may aid in determining their specific roles and improving the protection of the natural wetland systems, and by extension enhances the management of natural wetlands.

1.3.14 Conclusion

Altogether, the literature has highlighted the importance of natural wetlands systems. Wetland systems are very important for human survival. They improve the quality of water by trapping sediments, filtering out pollutants and absorbing nutrients that would otherwise results in poor quality of water for downstream users. Wetlands are essential for the numerous benefit services that they provide as an ecosystem to humankind. These benefits

range from freshwater supply, food and building materials, and biodiversity to flood control, groundwater recharge, and climate change mitigation. Despite the benefits, excess presence of nutrients in wetlands ecosystem is a problematic phenomenon worldwide since they contaminate the potable water and decreases the biodiversity, and thereby resulting in hypoxia (Roley *et al.,* 2016). Studies have demonstrated that wetlands and their quality continue to decline in most regions of the world. Moreover, the ecosystem services that wetlands provide to people are being compromised (Drenovsky, 2010; Peralta *et al.,* 2010). Looking after wetlands therefore is highly important. Drastically, modification of natural wetlands systems gradient and natural ecological functioning often results from anthropogenic activities. In another view, the activities of diverse microbial communities play a crucial role in transformation of nutrients through different mechanisms (Ligi *et al.,* 2014). Undoubtedly, excessive nutrients decrease water quality and impact negatively on aquatic ecosystem, as well as resulting to loss of component species, and malfunction ecosystem (Wu *et al.,* 2011).

1.3.15 References

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CHAPTER 2

NUTRIENT CONCENTRATIONS AND PHYSICAL AND CHEMICAL PARAMETERS IN THE WETLAND SYSTEMS

2.1 Introduction

The nutrient availability in wetland soils may be influenced by physicochemical properties, especially of the soil (Husseum and Magsood, 2011). A study conducted by Qiuyan *et al.* (2001) concluded that the nature of temperature affected the concentration of nutrients.Also, excess nutrients availability in wetlands induces eutrophication, thus resulting to potential effects on aquatic organisms (Wu *et al.*, 2011; Hu *et al.*, 2017).Importantly, nutrient pollution in wetlands has become an increasing concern in many developing countries and across the world (Gao *et al.*, 2014; Wu *et al.*, 2014; Gao *et al.*, 2017), thereby leading to serious degradation of these ecosystems (Adyel *et al.*, 2017). In addition to the above, excessive nutrient presence in aquatic environments results in negative environmental problems such as depletion of oxygen, loss of biodiversity as well as changes in the hydrological and

morphological characteristics of the wetland (Gao *et al.,* 2017). In a bid to forestall, reduce and / or correct such negative happenings (which centre on: impacts of nutrients pollution), appropriate measures should be put in place and thereby contributing to a good environmental management practices.

Physiochemical factors such as temperature, dissolved oxygen (DO), organic carbon etc. have an influence on denitrification in the natural wetland system (Song *et al.,* 2011). Vegetation, soil texture and hydrologic regime also directly influence denitrification rates by altering the environmental characteristics (Song *et al.,* 2011). Therefore, spatial and temporal changes in environmental conditions may affects denitrification rates.

Various studies conducted on treatment of wastewater has reported that both nitrite and nitrate bacteria are inhibited at low temperatures (Kim *et al.,* 2006; Zhang *et al.,* 2016), leading to poor nitrogen removal efficiency. Moreover, the literature reviewed showed that physiochemical factors such as: temperature, DO and others plays a vital role in wetland systems through influencing degradation of pollutants (We *et al.,* 2015; Liu *et al.,* 2016). Furthermore, DO is amongst the most vital factors influencing the microbial activities and efficiency required for pollutant removal (Liu *et al.,* 2016).

Another noticeable trend is that as pollution from both point and non-point sources are being discharged into wetlands (Cao *et al.,* 2015), suspended solids (SS), microbial pathogens and other pollutants may occur due to these discharges in the natural wetland systems. Reports have it further that numerous life-threatening pollution incidents in wetland systems have been largely attributed to eutrophication (Correll, 1999; Nixon and Fulweiler, 2009). Thus, eutrophication in aquatic ecological research has become a matter of concern (Wu *et al.,* 2014; Cao *et al.,* 2015), and gaining attention on a daily basis worldwide.

In another view, seasonal variation also brings about a huge challenge for the sustainable functioning of natural wetland systems (Fan *et al.,* 2016). Under cold temperatures, decaying of plants is one of the most reasons that account for poor removal efficiency of pollutants. This is apart from the fact that microbial activities are being inhibited (Wu *et al.,* 2015; Fan *et al.,* 2016). In that light, most wetlands plants senescence during cold winter and performs better in warmer climates (Fan *et al.,* 2016; Yan and Xu 2017). Previous studies have shown that the performance of wetlands noticeably declined at low temperatures (Wang and Li *et al.,* 2015; Zhang *et al.,* 2016) since temperature alters water characteristics, and controls aquatic plant growth as well as how aquatic ecosystem functions (Deepmalaand Anil Kumar, 2016).

Based on the above background, the main aim of this chapter was to determine nutrient levels, as well as evaluate the effect of physiochemical parameters on nutrients concentration in both coastal and inland wetland systems in order to improve the wetlands management. The work was conducted seasonally to enable comparisons of nutrients concentrations and physicochemical parameters of two wetland systems. The contribution of physicochemical parameters on nutrients was investigated.

In South Africa, natural wetlands make up a very small portion of 2.4%, and 48% of them are critically endangered by pollution and excess nutrients, erosion, drainage, mining, etc. (DEA, 1996). Moreover, wetlands are the most threatened ecosystems in South Africa as they provide various important benefits such as water purification, flood control, water storage, sediment and nutrient retention, etc. (DEA, 1996; Villiers, 2007; Gordon *et al*., 2011). One of such threats is observed in eutrophication of wetland systems from nutrient enrichment which is impacting negatively on water quality and biodiversity (Villiers, 2007). The nutrient enrichment maybe from natural sources such as weathering and anthropogenic activities such

as agricultural activities, waste water from livestock farming, discharge from domestic waste, runoff from nitrogen saturated sources and grasslands (Villiers and Thiart, 2007).

2.2 Aim and objectives

2.2.1 Aim

To determine nutrient levels and to evaluate the effect of physiochemical parameters on nutrients concentration in both coastal and inland wetland systems

2.2.2 Objectives

- To determine the physicochemical properties (temperature, pH, COD, BOD and suspended solids) in the two wetland systems.
- To determine nutrient concentrations in the natural wetlands.

2.3 Materials and methods

2.3.1 Wetlands site description

Samples were collected monthly in triplicates using the sterile Schott bottles for twelve months from July 2016 to June 2017 from two different natural wetland systems. Temperature and pH were measured on site using an InoLab_IDS multimeter 9310, and then transported to the laboratory for further analysis in a cooler box with ice. This was done in order to determine the influence of physiochemical parameters variation on nutrients concentration in the natural wetlands systems. The two wetlands investigated were Nhlabane mouth Estuary which is located along the Indian Ocean, and Lake Icubhu which is a fresh water wetland located within the homesteads of Esikhaleni and Mandlankala and Empembeni.

,2.3.1.1 Nhlabane Mouth Estuary

Nhlabane is an Estuary located along the east coast of Indian Ocean in Richards Bay (Figure 4). This Estuary is a salt water wetland and receives most of its water from the Indian Ocean. It is documented that this Estuary has been greatly disturbed by anthropogenic activities such as construction of the weir by the nearest mining company (Rainbow, 2002; Jerling, 2010; Vivier, 2010). Its ecological integrity has therefore been largely compromised by human impacts through industrial development (Vivier, 2010), and thus receives excessive nutrients input that compromise the quality of water and ecosystem functioning in this Estuary (Kennish, 2002). This may result in habitat alteration and modification of structure, as well as functioning of the estuarine, leading to declining ecological biodiversity (Rainbow, 2002).

Figure 4: Sampling point at Nhlabane mouth Estuary at coordinates 28.6567°S, 32.2314°E.

2.3.1.2 Icubhu Lake

Lake Cubu (Figure 5) below is a shallow natural fresh water wetland located between areas of Mandlankala and Empembeni at Esikhaleni Township near Richards Bay. The Icubhu lake catchment is about 80 km^2 (Martin and Cyrus, 1994). It supplies Esikhaleni Township with portable water. The lake is to some extent, being affected by industrial and urban developments (Martin and Cyrus, 1994). It also functions as a nursery habitat for fishes (Weerts and Cyrus, 2010).

Figure 5: Sampling site of an inland wetland (Lake Icubhu) at coordinates -28°50'30.2" S and 31°57'56.88" E

2.3.2 Water samplings

Water samples were collected monthly in triplicates from the two different wetland systems using 1000 ml sterile (autoclaved) Schott bottles. Water samples were collected in the mornings at 10 cm depth. The physicochemical assessment of the water was conducted on site and specifically temperature, pH and dissolved oxygen using an InoLab_IDS multimeter 9310 (Merck).

An additional water analysis included the determination of BOD_5 using a 5-day BOD test. Chemical oxygen demand, nitrogen species such as total nitrogen that included ammonium, nitrite, nitrate and total phosphorus including orthophosphate were analysed in the laboratory using spectrophometric methods with Spectroquadrant Pharo 100 cell tests using respective test kits according to manufacturer instructions.

2.3.3 Measurement of temperature and pH

To determine the physicochemical characteristics of the water, the InoLab_IDS 9310 was dipped in the sample immediately after collection on site to read for temperature and pH.

2.3.4 Chemical oxygen demand (COD)

The COD meter which had a digester was used to measure the amount of COD present in water samples. Three millilitres of the sample were pipetted into the reaction cell. The samples were first placed on a digester for 2 hours at 148°C in bottles to be used to measure COD. The cells were removed from the thermoreactor after 2 hours and placed in test tubes rack for cooling. After complete cooling to room temperature, they were placed into the cell compartment of the photometer and the results were recorded.

2.3.5 Measurement of nutrients

The Spectroquadrant Pharo 100 was used to quantify the nutrients. The dissolved nutrients analysed were ammonium, nitrate, nitrite and phosphate. The respective methods are explained below.

2.3.5.1 Ammonia

Ammonia was analysed using the Spectroquant Merck KGaA HC551009 ammonium test kit at pH ranging between 4 and 13. Two hundred microliters of the sample was pipetted into 5 ml of NH_4^{-1}. That was followed by adding one level micro-spoon of NH_4^{-2} and vigorously hand shakingof the test tube until the particles are completely dissolved. After 15 minutes' reaction time, the solution was transferred into a cell and placed on the spectrophotometer for reading. The results were recorded.

2.3.5.2 Nitrate

Nitrate was analysed using Spectroquant KGaA HC549510 nitrate test kit (Merck). Five millilitre of NO_3^{-2} was pipetted into 1 level micro-spoon of NO_3^{-1} in a clean empty round cell. This was followed by vigorously hand shaking of the cell to dissolve the particles, and 1.5 ml of the sample was slowly pipetted into the cell. The cell screw cap was closed and mixed. After 10 minutes of reaction time, the solution was transferred into a corresponding cell and the results were read.

2.3.5.3 Nitrite

Nitrite was analysed using Spectroquant KGaA HC567526 (Merck) nitrite test kit at pH 2 - 10. One levelled micro-spoon of NO_2^{-1} was added into 5 ml of the sample in a clean test tube.

This was followed by vigorously shaking of the test tube to dissolve the particles. At this stage, the pH ranged between 2.0 – 2.5. If needed, Sodium hydroxide solution or sulphuric acid was used to adjust the pH. After 10 minutes of reaction time, the solution was transferred into a cell and the results were recorded.

2.3.5.4 Phosphate

Phosphate was analysed using Spectroquant KGaA HC558742 phosphate test kit (Merck) at pH 0 - 10. One dose of P-1K was added into 1 ml of the sample in a clean reaction cell. The reaction cell was closed and mixed. This was followed by heating the cell in the thermoreactor at 120 ^{o}C for 30 minutes. After completely cooling of the cell to room temperature, 5 drops of P-2K were added and vigorously shaken to dissolve the particles. After another 5 minutes of reaction time, the cell was placed into a cell compartment aligning the cell mark with that of the photometer. The results were recorded.

2.3.6 Total suspended solids (TSS)

The filter paper of 56 pore size was weighed before usage. The total suspended solids of water sample were determined by pouring 1000 millilitre volume of water through a pre-weighed filter of a specialised pore size. The filter was then weighed again after drying to remove water. The gain in weight measure was the dry weight of the particulate matter present in water and expressed in milligrams per litre (mg/l) (Olivia *et al.*, 1980).

2.4 Results and discussion

2.4.1 Physicochemical parameters

The results of the physicochemical parameters measured in the wetland systems are presented below:

2.4.1.1 Temperature

The results of water temperature are presented in Figure 6. The temperature of Lake Icubhu ranged between 17.7^{o}C to 28.6^{o}C, while Nhlabane mouth Estuary ranged between 10.2^{o}C to 28.1^{o}C as presented in Appendix 2.1 and 2.2. The results of this study were similar to the findings of the study by Kaevska *et al.* (2016), where they also find temperature to be highest in the summer (20^{o}C), followed by the spring (12.1^{o}C) then the autumn (7.5^{o}C), and in the winter (1.7^{o}C). In this study, the average temperature in the summer (December, January, and February) was found to be 26.4^{o}C in Lake Icubhu and 26.6^{o}C in Nhlabane mouth Estuary. This was followed by 24.9^{o}C in Lake Icubhu and 25^{o}C in Nhlabane month Estuary in spring (September, October, and November). In the autumn (March, April, and May), Lake Icubhu had 23.1^{o}C and 21.6^{o}C, and in the winter (June, July, and August), Lake Icubhu had 20.6^{o}C and 16.3^{o}C. The freshwater wetland temperature was mostly high as compared to the estuarine temperature except in the spring (September, October, and November). Temperatures were highest in the summer (December, January, and February) in both wetlands and lowest in the winter (June, July, and August).

In relation to the findings above, Fink (2005), and EPA (2012) noted in their studies that metabolic rates, physiological function and biological activity of the aquatic organisms may be affected by temperature. Furthermore, McNally and Mehta (2004) also stated that enzymes denature at temperature above 35^{0}C leading to reduction of metabolic function. Temperature also affect aquatic plant growth and photosynthesis (Fink, 2005). It was also reported that dissolve oxygen decreases as the temperature increases (EPA, 2012). The results of

temperature found in this study are similar to those reported by Brett (2011), where temperature was between 10 ⁻35^{o}C in his study of natural wetlands. According to Robert *et al.*,(2011), the wetland optimal temperature is 20 – 30^{o}C for wetlands processes to occur and Robert *et al.*, (2011) also noted that wetland processes were affected at temperature lower than 15^{0}C. The stipulated recommended temperature is 15-35^{o}C(S.A. Water Quality Guidelines for Coastal Marine Waters, 2012). Nhlabane Estuary had 10^{0}C in July 2016, however this was a cold dry season. Low temperatures were expected in winter since it is a cold season and high temperatures were also expected in the summer since it is a warm season.The results of temperature in this study were also similar to those of the study conducted by Bezuidenhout *et al.*, (2002), at uMhlathuze river in KwaZulu-Natal.

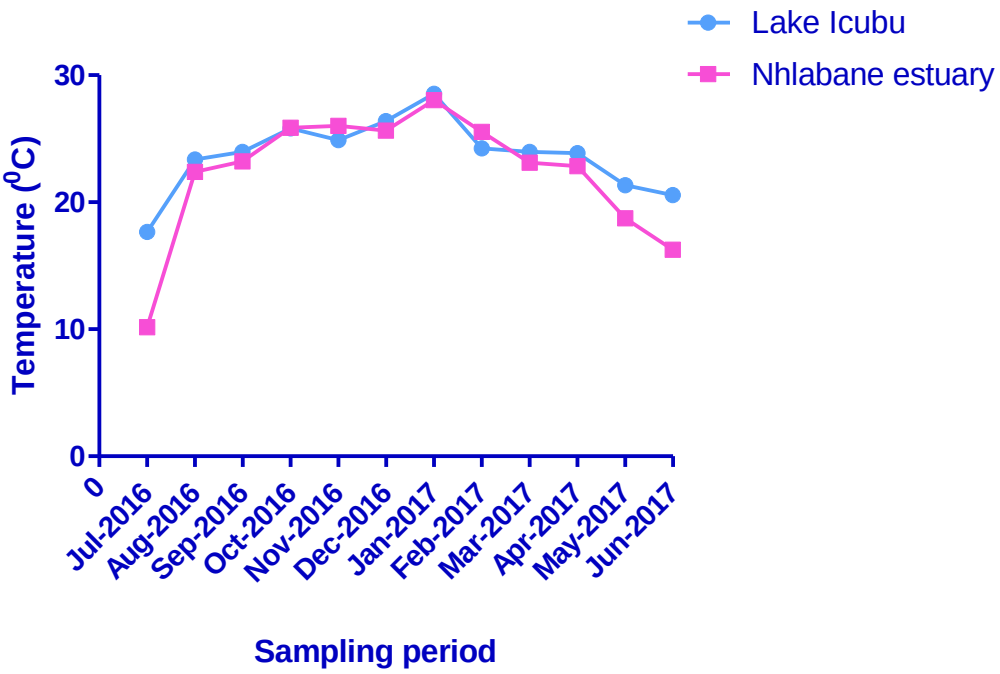

Figure 6: Temperature variation in Lake Icubhu and Nhlabane mouth Estuary between July 2016 and June 2017

2.4.1.2 pH

The pH is another physicochemical property that affects nutrient concentrations in wetland systems (Brandy and Ray, 2010) through altering microbial diversity (Fierer and Jackson

2006). The results of pH for both wetlands systems are presented in Figure 7. The pH of Lake Icubhu ranged between 6.3 and 7.3 as presented in Appendix 2.3, while for the saline influenced Nhlabane wetland system, the range was between 6.6 and 7.4 as presented in Appendix 2.4. In Lake Icubhu, the pH was highest in the autumn (March, April, and May), and was more or less the same throughout other seasons. The pH in Nhlabane mouth Estuary was highest in the spring (September, October, and November), followed by the autumn (March, April, and May), then in the winter (June, July, and August), and the summer (December, January, and February) had the least. For Lake Icubhu, its pH ranged between 6.4 – 7.5 in the autumn (March, April and May), 6.2 – 6.9 was recorded in the spring (September, October, and November), 6.2 – 6.8 in the summer (December, January, and February), and 6.2 – 6.7 in the winter (June, July and August). Nhlabane mouth Estuary's pH ranged between 6.8 – 7.5 in the spring (September, October, and November), 6.9 – 7.5 in autumn (March, April and May), 6.7 – 7.2 in winter (June, July and August), and 6.7 – 7 in the summer (December, January, and February). Overall, it could be said that the pH was around neutral throughout the duration of the study period. The pH values of Nhlabane were found to be higher throughout the study than those of Lake Icubhu with an exception of January 2017 where Nhlabane mouth Estuary values were slightly lower than Lake Icubhu. The pH of aquatic ecosystem should range between 4 and 11 as per South African Water Guidelines (S.A. Water Guidelines, 1996). This means that the pH values for both wetlands were within South African standards stipulated in South African water guidelines. However, the findings of this study were not in line with the findings of the study conducted by (Barman *et al.*, 2015) where pH was put at highest concentrations of 7.3 – 9.2 in the summer (December, January, and February), followed by concentrations of 7.0 – 7.6 observed in the autumn (March, April, and May), and the lowest concentrations ranging between 6.7 – 7.3 were observed in the winter (June, July, and August). The results of this study were also not in line

with results of studies conducted by (Garg and Soni, 2016; Khan and Predesh 2016 and Ansari, 2017) where their findings indicated that the pH was mostly high in the summer, followed by autumn and winter. This may be due to anthropogenic activities and grazing livestock. The animal waste especial their urine and fertilizers from human activities could have contributed to high concentration of nutrients in these wetlands. Nhlabane generally had higher pH throughout the study than Lake Icubhu, and this could have been due to geological location of the wetlands. It should also be remembered that optimum pH is crucial in wetlands to maintain good chemical and biological processes that are occurring in water (Wang *et al.,* 2008). The pH findings of this study were also in line with the findings of the study that was conducted by Lin *et al.* (2004) at uMhlathuze river in KwaZulu-Natal when studying its water quality.

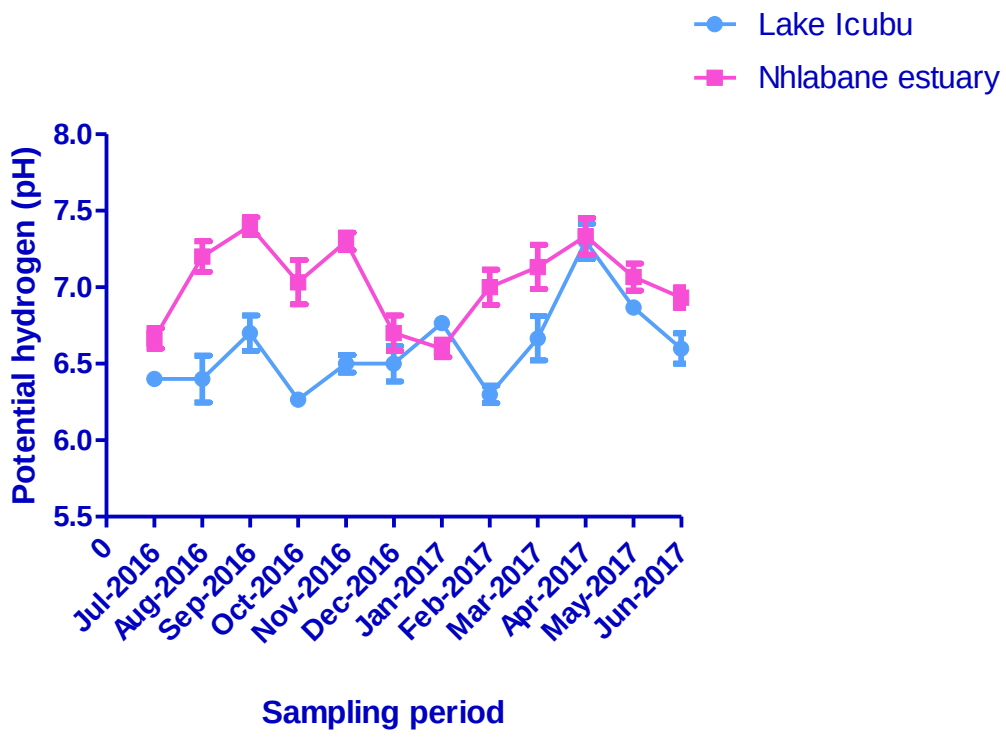

Figure 7: Potential hydrogen variation in Lake Icubhu and Nhlabane mouth Estuarybetween July 2016 and June 2017

2.4.1.3 Chemical oxygen demand

The results of COD for both wetlands are presented in Figure 8. Chemical oxygen demand was highest in the autumn (March, April, and May) for both wetlands, and was least in the spring (September, October, and November) as presented in Appendix 2.5 and Appendix 2.6 respectively. In the autumn (March, April, and May), Lake Icubhu ranged between 65 and 77 mg/l, while ranges of 59 and 84 mg/l were observed in Nhlabane. This was followed by summer (December, January, and February) where 24 to 88 mg/l was observed in Icubhu Lake and 22 to 67 mg/l observed in Nhlabane. Winter (June, July and August.) had ranged between 27 and 68 mg/l in Lake Icubhu and 20 to 28 mg/l in Nhlabane month Estuary. The spring (September, October and November) was also observed with the least COD concentrations recorded, as Lake Icubhu ranged between 24 to 44 mg/l while Nhlabane month Estuary ranged between 20 and 27 mg/l. It can be seen fromFigure 2.5 that COD concentrations for Lake Icubhu was always higher than that of Nhlabane Estuary during the study period, as it ranged between 25 (observed in the spring) to 92 mg/l (observed in the autumn) in Lake Icubhu, while in Nhlabane mouth Estuary, it ranged between 20 (observed in the spring) to 84 mg/l (observed in the autumn).

Generally, chemical oxygen demand concentrations were low in both wetlands in spring (September, October and November) than in autumn (March, April and May). As lakes may carry along numerous amounts of organic matter, this organic matter may comprise of organic, inorganic and dissolved matter. Chemical oxygen demand has a vital bearing on the quality of water in the natural wetland systems (Khurana and Singh, 2012). These pollutants may reduce oxygen levels in the lakes (Ntengwe, 2006). The natural organic detritus and organic waste presence in the wetland systems from agricultural, anthropogenic, industrial, etc., act as food source for water-borne bacteria. Bacteria decomposes the organic material

present in the wetland systems using dissolved oxygen. This has negative impact on aquatic life, since the presence of dissolved oxygen for aquatic animals like fish and others is reduced (Rixen *et al.,* 2010). This could be due to the fact that it was the beginning of cold season when plants were dying off, recycled back to water and then contributing to suspended solids. However, the findings of COD in this study slightly differ seasonally from those of the study by Barman *et al.,* (2015), where they find COD concentrations to be high in summer followed by autumn and low in winter. This may be due to numerous factors. Apart from the anthropogenic activities and livestock grazing around these wetland, March 2017 – May 2017 was very windy and dry. Aquatic plants then were dying, decaying and recycling back to wetland water contributing to an increased concentrations of COD (Yuan *et al.,* 2017). Yuan *et al.* (2017) noted higher COD concentrations when there was high storm water that resulted to introduction of high pollution level in water. The results of COD concentrations obtained in this study were higher than those obtained in previous studies within the same region (Lin *et al.,* 2004; Mthembu *et al.,* 2011). This could have been due to the developments that have been recently occurring, increasing number of human population, industrialization or urbanisation and the constant rise of the sea level.

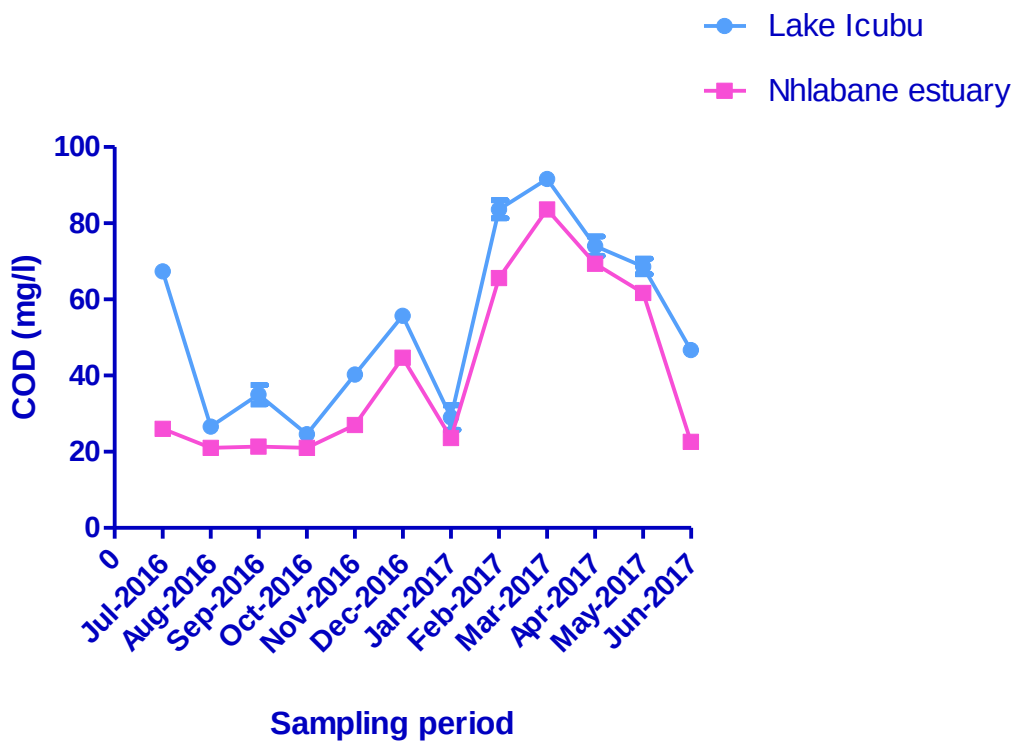

Figure 8: Chemical oxygen demand in Lake Icubhu and Nhlabane mouth Estuary between July 2016 and June 2017

2.4.1.4 Total suspended solids

The results of TSS are presented in Figure 9. There were higher suspended solids in the autumn (March, April, and May) and in the winter (June, July, and August), this could be however attributed to wind actions during winter months (Park, 2007). The wind actions blow small and big particles or pollutants to wetland water, thuscontributing to higher concentrations of suspended solids. It could also be observed from the results that Lake Icubhu generally had higher total suspended solids than Nhlabane estuarine. This was associated with anthropogenic activities since Cubhu is located near the homesteads of Esikhaleni, Mpembeni and Mandlankala. Total suspended solids were observed to range between 2.0 and 16.0 mg/l in Lake Icubhu as presented in Appendix 2.7, and 2.0 and 7.0 mg/l in Nhlabane estuarine as presented in Appendix 2.8. In Lake Icubhu,TSS was in high

concentration ranging between 10.6 – 12.8 mg/l in the autumn (March, April, and May), followed by concentrations of (3.2 - 16.9 mg/l) observed in the winter (June, July, and August), then another concentration of 1.8 – 8.7 mg/l observed in summer (December, January, and February), and a final concentrations between 1.8 – 4.2 mg/l observed in spring (September, October, and November).

In Nhlabane month Estuary, highest concentrations between 2.3 – 7.5 mg/l were observed in the summer (December, January, and February), followed by 2.1 – 3.6 mg/l observed in the winter (June, July, and August), then another concentration between 2.1 – 3.5 mg/l observed in the spring (September, October, November), and concentrations ranging between 1.8 – 33 mg/l were also observed in the autumn (March, April, and May). The suspended solids in wetlands may consist of organic and inorganic mater and often includes high levels of microorganisms (Diaz, 1993). They may be naturally present or introduced (Phyllis *et al.*, 2007).

This means that the TSS values between both wetlands were within the South African standards. The findings of Lake Icubhu are similar to the findings by Khan and Pradesh (2016) where they also observed highest concentrations in autumn followed by summer (December, January and February) and winter (June, July and august). Nhlabane month results were not similar to previous studies conducted by Barman (2015) and Khan and Pradesh (2016), Nhlabane had the highest in summer and the lowest in autumn. This could due to addition of solids particles from high constant rain runoffs to the wetlands during those seasons as well as from decaying plant matter.The total suspended solids values obtained in this study were similar to those obtained by Mthembu et al. (2011) at uMhlathuze area.

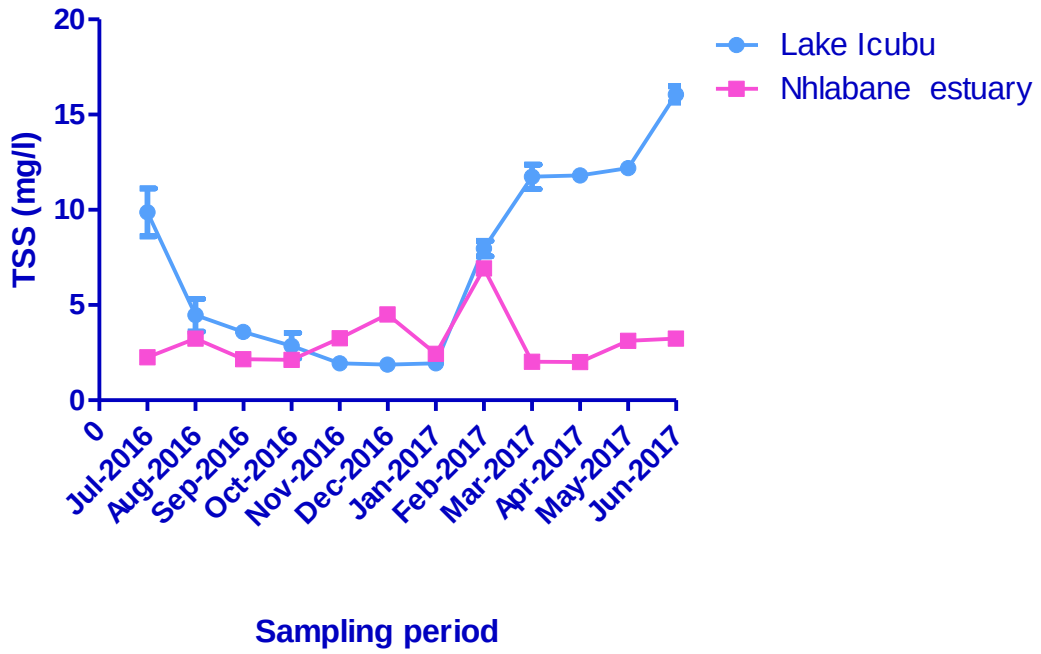

Figure 9: Total suspended solids in Lake Icubhu and Nhlabane mouth Estuary between July 2016 and June 2017

2.4.2 Nutrients

Nitrogen and phosphorus are considered dissolve substances since they do not directly contribute to wetland water pollution (Mannios *et al.*, 2002). Nutrients are rather an indirect contributor of suspended solids since they fuel algal biomass which contributes to TSS and turbidity of the wetland system. Dissolved nutrients impact the wetland system, resulting in eutrophication, which in turn, results to low dissolved oxygen (Mannios *et al.*, 2003). The concentration of nutrients was monitored seasonally in each wetland, and it was also determined how physiochemical properties variations affected the nutrient concentrations. Phosphorus and nitrogen are the most common nutrients found in water (John *et al.*, 2017; Raffensperger *et al.*, 2017). The results of nutrients concentrations are presented below.

2.4.2.1 Phosphorus

The results of phosphorus concentrations ranged between 1.6 to 2.5 mg/l in Lake Icubhu and 1.7 to 1.9 mg/l in Nhlabane Estuary (Figure 2.7). In the winter season (June, July, and August), a highest concentration of 2.1 mg/l was observed at Lake Icubhu. In summer (December, January, and February), and spring seasons (September, October, and November), an average concentration of 2 mg/l was observed each season in Lake Icubhu. For summer (December, January, and February), it ranged between 1.5 - 2.5 mg/l, while the spring (September, October, and November) was between (1.5 – 2.2 mg/l). Lowest concentrations were observed in the autumn (March, April, and May), ranging between 1.5 – 1.8 mg/l. Highest concentrations were also observed in the winter (June, July, and August) in Nhlabane and were ranging between 1.7 -1.9 mg/l. The spring (September, October, and November).), the summer (December, January, and February) and the autumn (March, April, and May) had a mean value of 2 mg/l, which was slightly below that of the winter (June, July, and August). In the spring (September, October, and November), and in the autumn (March, April, and May), phosphorus ranged between 1.6 – 1.8 mg/l and ranged between 1.6 – 1.9 mg/l in the summer. All these concentrations are presented in Appendix 3.1 and 3.2 respectively.

Lake Icubhu generally had higher concentrations of phosphorus throughout the study when compared to Nhlabane Estuary. This was probably due to the fact that Lake Icubhu is located within human settlement areas. It was also observed that domestic waste, runoffs from the human plantations and livestock grazing around the wetland could have been the contributing factors to higher concentrations of phosphate. It was recorded also that phosphorus was higher in cold season than in warm season. Phosphorus is one of the nutrients found in water, and is essential for plant growth (Kozlosski and Pallardy, 1997; Domisch *et al.,* 2002). However, eutrophication may result when this nutrient is present in excess amount (Chislock,

2003; Liu *et al.,* 2010). In literature and previous studies, most phosphorus was taken up by plant roots during the growth season (warm season). It was then released back when plants died and decayed. That followed by phosphorus being released into the water by the decaying plant matter above ground and recreate into the soil by decaying roots (Vymazal, 2006; Chao *et al.,* 2014). The phosphorus uptake by microorganisms is very fast but are unable to store large amounts (Vymazal, 2006). The phosphorus concentrations obtained in both wetlands was less than 5 mg/l throughout the study period. This means that both wetlands had low accumulation of dissolved nutrients. Thus, the condition of these wetlands were oligotrophic (S.A. Water Guidelines, 1996). These conditions usually support moderate levels of species diversity. They also have limited nuisance growth of aquatic plants or blue-green algae (Hardiman, 2010). The results of this study are similar to those obtained in a study by Barakat *et al.* (2016) and (Ansari, 2017), who also observed higher phosphorus concentrations in winter and less in summer (December, January, and February). Barman (2015) and Ansari (2017) however, reported high concentrations in summer and low concentrations in winter. This may be due to agricultural run-offs and livestock grazing around the area. The results of phosphate obtained in this study were higher than those of previous studies by Lin *et al.* (2004), and Mthembu *et al.* (2011). This could be the results of agricultural activities and grazing livestock.

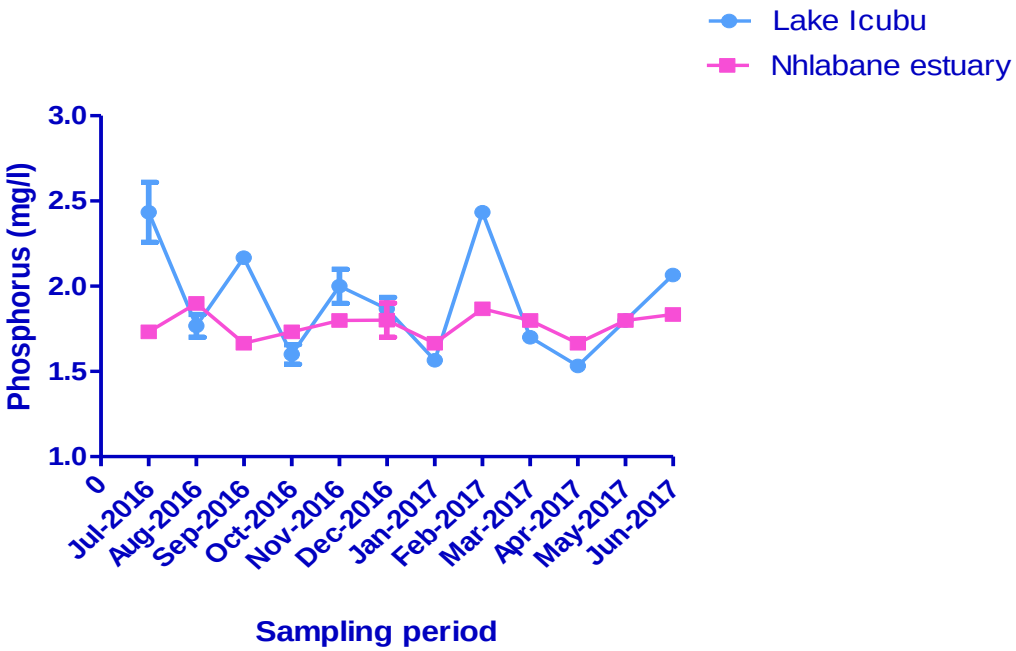

Figure 10: Phosphorus concentration in Lake Icubhu and Nhlabane mouth Estuary between July 2016 and June 2017.

2.4.2.2 Ammonia

Figure 11 presents the results of ammonia concentration in both wetland systems. Ammonia concentrations in Lake Icubhu ranged between 6 mg/l in the winter (June, July, and August) to 11 mg/l obtained in the autumn (March, April, and May) as presented in Appendix 3.3 and 3.4 respectively. Ammonia in Nhlabane Estuary ranged between less than 0.5 mg/l in the spring (September, October, and November) to 14 mg/l observed in the autumn (March, April, and may). Both wetlands had high concentrations of ammonium in the autumn (March, April, and May) with Lake Icubhu ranged between 10 – 13 mg/l, and Nhlabane mouth Estuary was between 11 – 15 mg/l. In both wetlands, the results in the summer were similar to that of autumn (March, April, and May). While Lake Icubhu was between 7 – 12mg/l, Nhlabane month Estuary was between 8 – 13mg/l. Furthermore, Lake Icubhu in the spring was in ranges between 4 – 9mg/l and ranges between 5 – 7 mg/l were observed in the winter (June, July, and August). In NhlabaneEstuary, the spring (September, October, and

November) had the lowest concentrations (0.3 – 2 m/l), while the winter (June, July, and August) had concentrations ranging between 5 – 9 mg/l. It was noted in this study that both wetlands had high concentrations of ammonia in autumn. The results of this study were similar to those of Nair *et al.* (2016), where low concentrations of ammonia were observed in the winter, and higher concentration were observed in the summer and the autumn. In Lake Icubhu, both temperatures and pH values were favourable for nitrification process. Norton (2008) observed that at pH lower than 8.0, volatilization does not occur. He also observed that at pH 9.3, ammonia and ammonium *ions* exist as a 1:1 ratio. This concluded to significant losses of ammonia through volatilization. Vymazal (2006) reported that ammonification rates depend on various factors such as temperature, soil conditions, available nutrients, etc. The values obtained in the winter and in the spring for both wetlands were within the target water quality range of 7 mg/l for aquatic ecosystem (S.A. Water Guidelines, 1996). Values obtained in the winter and in the spring were above the target water quality for aquatic ecosystems. Therefore, they had chronic effect values which ranged between 7.1 mg/l – 15 mg/l (S.A. Water Guidelines, 1996). The chronic effects include: reduction in morphological development, growth rates as well as pathological changes in tissues such as gills, kidneys liver of the aquatic animals. Ammonia concentrations in this study were higher than those of the study conducted by Barakat *et al.* (2016), Lin *et al.* (2004), and Mthembu *et al.* (2011) and this may be due to anthropogenic pollutants, agricultural and domestic activities on my study sites.

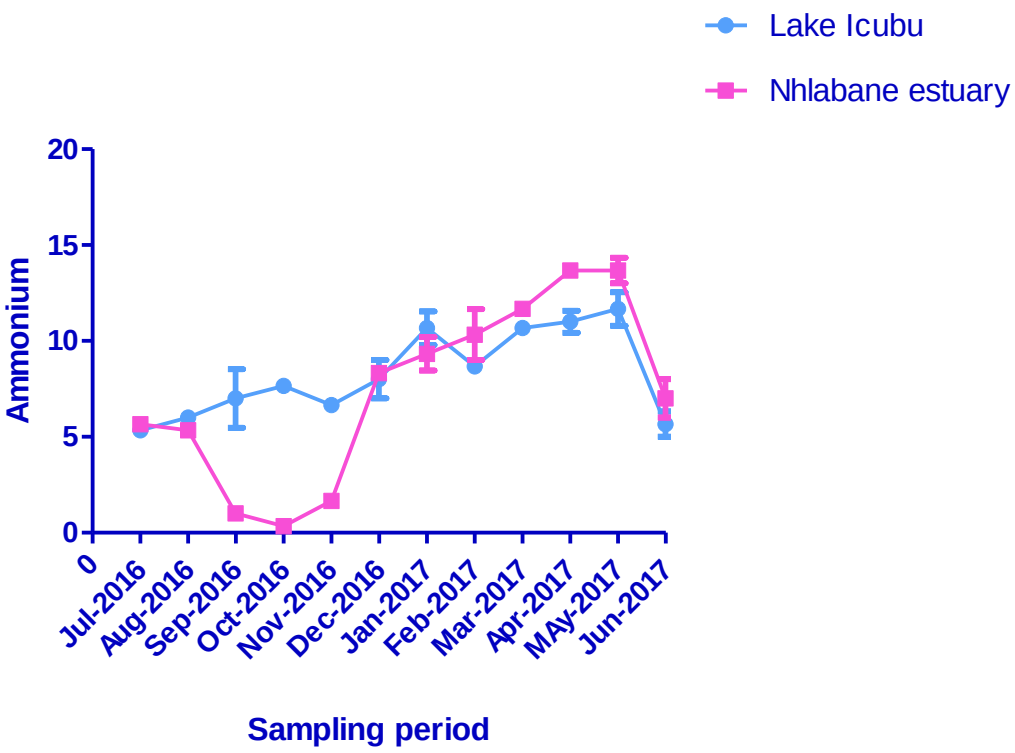

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Figure 11: Ammonia concentration in Lake Icubhu and Nhlabane mouth Estuary from July 2016 to June 2017.

2.5.2.3 Nitrite

The concentrations of nitrite ranged between 0.001 and 0.6 mg/l in both wetlands as presented in Appendix 3.5 and 3.6 respectively. Figure 12 presents the results of nitrite concentrations during the study period. Lake Icubhu had concentrations as low as 0.001 mg/l in the spring (September, October, and November), and in the summer (December, January, and February). Higher concentrations were also observed in the autumn (March, April, and May) in both wetlands. At Nhlabane, the lowest concentrations were observed in the summer (December, January, and February). In both wetlands, nitrite was high in the autumn (March, April, and May), both had a mean value of 0.5 mg/l. Lake Icubhu ranged between 1.3 – 0.7 mg/l, and Nhlabane mouthEstuary ranged between 0.4 – 0.8 mg/l. Nitrite at Lake Icubhu

ranged between 0.01mg/l – 0.06 mg/l in the winter (June, July, and August). It ranged between 0.0 – 0.02 mg/l in the spring (September, October, and November), and between 0.01 – 0.02 mg/l in the summer (December, January, and February). Nitrite at Nhlabane mouthEstuary ranged between 0.1 – 0.2 mg/l in the spring (September, October, and November), ranges of 0.01 – 0.07 mg/l were observed in the winter (June, July, and August) and ranges between 0.01 – 0.02mg/l were observed in the summer (December, January, and February). Nitrite concentrations were within South African standards for aquatic ecosystem guidelines use throughout the study (S.A. Guidelines, 1996). Similar observations were made by Nair *et al.,* (2016), where they observed low concentration of nitrite in the summer and higher concentrations were observed in in the winter and in the autumn. Various factors such as temperature, pH, etc. also influence nitrification (Song *et al.,* 2011).

The results of nitrite obtained in the winter, in the spring and in the summer indicated that water conditions were oligotrophic for both wetlands, which is in line with S.A. Water guidelines (S.A. Guidelines, 1996). This is characterised by rapid nutrient cycling with no presence of blue-green algal blooms and moderate levels of species diversity (S.A. Guidelines, 1996). The conditions were mesotrophic in the autumn, when the concentration was slightly above 0.5 mg/l. This was characterised by seldom toxic algal blooms which is not good for aquatic ecosystem (S.A. Guidelines, 1996). These findings of this study were in line with those by Nwankwoala *et al.* (2009), where they observed high nitrite concentrations in dry seasons (Cold seasons), and lower concentrations in wet seasons (Warm temperatures). The results were also in line with the findings of the study conducted by Lin *et al.* (2004) at uMhlathuze river.

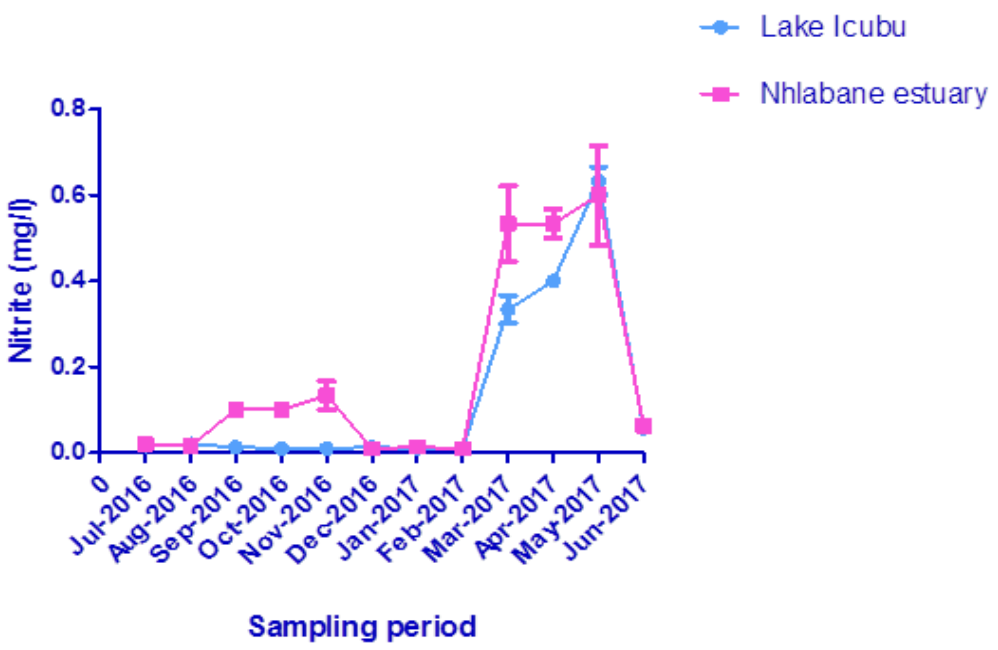

Figure 12: Nitrite concentration in Lake Icubhu and Nhlabane mouth Estuary from July 2016 to June 2017

2.4.2.4 Nitrate

Nitrate was high in both wetlands in the autumn (March, April, and May). Lake Icubhu ranged between 3.1 – 3.6 mg/l as presented in Appendix 3.7, while Nhlabane Estuary was between 1.9 – 2.3 mg/l as presented in Appendix 3.8. In both wetlands, the concentrations obtained in the winter (June, July, and August) were similar to those obtained in the autumn (March, April, and May). In the winter, Lake Icubhu was between 2 – 2.8 mg/l while NhlabaneEstuary was between 1.7 – 1.9 mg/l. In the spring (September, October, and November), and in the summer (December, January, and February), seasons in both wetlands had average values of 0.1 mg/l, and they all ranged between 0.1 – 0.2 mg/l. However, eutrophication results from excessive presence of nitrogen compounds in wetland systems (Gopal and Gosh, 2008; Liu *et al.*, 2010). Norton (2008) reinforced in his study that the optimum temperature for nitrification is 30^{o}C to 40^{o}C at a pH between 6.6 and 8.8. Nitrate concentration results are presented in Figure 13, and it can be seen from the figure that both

wetlands had lowest concentration of nitrate during spring and summer. The highest was obtained in the autumn (March, April, and May) for both wetlands where both the temperature and pH values were optimum for nitrification. It can also be confirmed from the figures that highest concentrations were obtained in autumn for the nitrate, this may be due to decaying plants and animal matter. Nitrate concentrations were within South African ranges for aquatic ecosystem guideline throughout the study period (DWAF, 1996).

In the spring (September, October, and November), as well in the summer (December, January, and February), the wetland water conditions for both wetlands were oligotrophic. This water condition is characterised by moderate diversity of species, blue – green algal blooms are not present and there is rapid cycling of nutrients. The water conditions were mesotrophic in the autumn (March, April, and May), and in the spring (September, October, and November) at Nhlabane. These water conditions are characterised by high levels of species diversity, nuisance growth of aquatic plants, blue – green algal blooms which are seldom toxic to wetland species living in it. Eutrophic conditions were also observed at Lake Icubhu in the autumn (March, April, and May), and in the winter (June, July, and August). They are characterised by low species diversity, nuisance growth of aquatic plants and as well as blue green algae, which is toxic to human beings, wildlife and livestock. The results of this study are similar to those reported by Viswanathan *et al.* (2016), who found low nitrate concentrations during the summer and higher concentrations during cold season. These results are also in line with those of the study by Rusjan (2009), who noted that higher concentrations are in (cold season, while thesummer (warm season) had less concentrations.

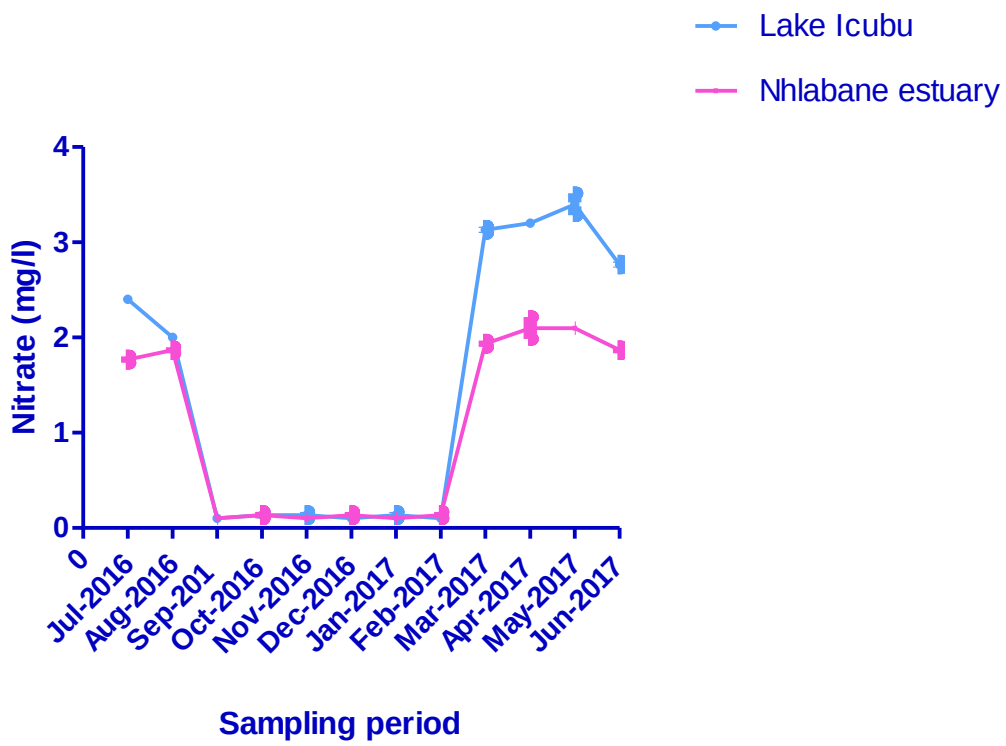

Figure 13: Nitrate concentration obtained in Lake Icubhu and Nhlabane mouth Estuary from July 2016 to June 2017

2.6 Conclusion

The natural wetland systems are a very complex ecosystem. Understanding of the wetland system variables is crucial in order to understand their function. Natural wetland systems are an important ecosystem that provides numerous benefits that include economic, social and environmental. In some areas of South Africa, people largely depend on wetlands for living, production of food, portable water, livestock grazing and many more. As a result of those activities eutrophication sets in and results in death of sensitive aquatic animals and their rotting carcasses may further contribute bacterial bloom. Furthermore, seasonal variation of temperature affected photosynthetic activities. The nutrient concentration varied with season depending on source of water and their location. Therefore, it can be concluded that seasonal

variation together with the physiochemical parameters influenced nutrients concentrations in the natural wetlands systems. Natural wetlands systems are available in small percentages in South Africa, and yet are suffering a great threats and loss by high level of nutrients that get introduced to them. They are also threatened by factors such as population growth, urban development, poor management and many more factors. The development of educational programmes about the wetland systems is essential to raise the awareness of wetlands values and uniqueness in South Africa. The results of phosphorus obtained in this study showed no significant threat to both wetlands since it was within S.A. standards stipulated in S.A. guidelines (DWAF, 1996). However, the results of ammonia, nitrite and nitrate levels obtained in this study showed significant threats in these two wetlands studied. Therefore, appropriate protections should be put in place in order not to degrade and lose them in the future. Since both these wetlands are easily accessible by both humans and livestock, more research need to be conducted in order to increase the understanding of natural wetland systems, their development, the way they function as well as to design effective restoration efforts.

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CHAPTER 3

EXAMINATION OF THE RELATIONSHIP BETWEEN PHYSICOCHEMICAL PARAMETERS AND NUTRIENTS IN LAKE ICUBHU AND NHLABANE ESTUARY.

3.1 Introduction

Seasonal physicochemical parameters variations in wetland system affect the quality of water, and this occurs differently between the seasons (Saravanakumar *et al.*, 2007; Mustapha *et al.*, 2012; Vajravelu *et al.*, 2017). Firstly, physicochemical parameters are regarded as important factors that influence biotic assemblage composition in the natural wetland ecosystems (Bird &Day, 2014). Secondly, they are known to exert significant structural effect on natural wetland habitats (De Roeck, 2008). Thus, good quality of wetland water depends highly on physiochemical properties and pollution load (Rahaman *et al.,* 2014). Therefore, regularly monitoring of the wetland water quality is required for an efficient management of these important resources, as wetlands play multiple roles in maintenance of water quality.

Jagadeshappa *et al.*, (2011), and Bertoli *et al.*, (2016) reported that natural wetland systems play an important role in maintenance of water quality, nutrients cycling, regulation of biological cycles as well as supporting the food chain. Similarly, natural wetland systems also maintain the aquatic biodiversity, filter nutrients, buffer coastal region zones, protect from floods and act as breeding grounds as well as water reservoirs (Sasa *et al.*, 2014). However, natural and human activities affects water quality and quantity, and specifically, a wide range of natural and human activities affect the quality as well as the quantity of natural wetland

water present (Bartram and Balance, 1996). In another words, natural processes such as weathering and anthropogenic activities such as discharge of agricultural and domestic wastewater results in wetland deterioration (Shrestha and Kazama, 2007; Kumar *et al.,* 2009, Rahaman *et al.,* 2014; Barakat *et al.,* 2016). Based on previous researches and the present one, wetlands are considered so sensitive such that its ecosystem is easily disrupted by any significant change in the water quality (Bartram and Balance, 1996).

In terms of their advantages, the wetlands are an important source of water for human consumption, industrial purposes and irrigation of agriculture crops. Efficient management of wetland system is crucial to maintain the good health of these systems. Moreover, this requires information about the wetland water quality and its variability (Vajravelu *et al.,* 2017). Therefore, the main objective of this chapter was to determine the influence of physiochemical parameters on nutrients concentration in both coastal and inland wetland systems in order to improve wetland management. For this study, statistical methods like correlation matrices, analysis of variance (ANOVA) and exploratory data analysis (EDA) were carried out to assess the variations in both wetlands water quality and to estimate the seasonal differences caused by natural and anthropogenic factors. Therefore, the influence of physicochemical parameters on nutrients concentration was investigated.

The chemical, physical and biological contents of natural wetland determine its quality of water. Temperature, pH, TSS along with TSS are the important parameters that determines the wetland aquatic environment. These parameters limit the survival of aquatic organisms and proper functioning of the wetland system when are available at high concentrations (Kane *et al.,* 2015). The changes in physicochemical parameters may negatively or positively affect the wetland biota (Usuman *et al.,* 2017). Therefore, physicochemical parameters govern the wetland aquatic life and their stability (Olele andEkelemu, 2008). It is thus crucial

to monitor the wetland physicochemical as well as chemical parameters and nutrients to keep the wetland aquatic habitat favorable.

3.2 Aim and Objectives

3.2.1 Aim

The main aim of this chapter was to determine the influence of physiochemical parameters on nutrients concentration in a coastal and an inland freshwater wetland system in order to improve wetland management.

3.2.2 Objectives

To establish the relationship between physicochemical parameters and nutrient concentrations in the wetland system.

3.3 Materials and methods.

3.3.1 Samples collection

Samples were collected per month in triplicate using sterile Schott bottles over a period of twelve months. Physiochemical properties such as temperature and pH were measured on site using the hand held InoLab IDS multimeter 9310. Samples were then transported in a cooler box with ice for further analysis of TSS, COD and nutrients concentrations in the laboratory using spectrophotometric methods. The data was recorded,and statistical analysis was then conducted.

3.3.1 The influence of physicochemical parameters on nutrient concentrations

Correlation was used to assess the relationship between the physicochemical parameters such as temperature, pH, COD and TSS with nutrient concentrations (phosphate, ammonium, nitrate and nitrite). A linear and non-linear models were used to express the strength of the relationship based on physical observation of the coordinates.

3.3.3 Statistical analysis

The data was analyzed using two-way analysis of variance (ANOVA) test, correlation analysis of water parameters in both wetlands for the seasons under review and some exploratory data analysis (EDA) were carried out as well. The model falls under the univariate general linear model. SPSS 23.0 was used for the analysis. Statistical software Prism5 was also used to correlate changes in physiochemical parameters and nutrient concentrations. The relationship was considered significant when the *p* value was less than 0.05.

3.4 Results and discussion

3.4.1.1 Temperature

A significant difference (P = 0.003) was observed in the temperatures in both wetlands and for the seasons at 5% level of significant. The post hoc test was carried out using the Duncan multiple range test. The result shows that all the seasons have different temperatures and the temperatures in Lake Icubhu are significantly higher than that of Nhlabane Estuary for all the seasons, except for summer (December, January,and February) where they have the same temperature. This is depicted in Figure 6 in chapter 2. Sukumaran *et al., (*2013), Rahaman *et al.,* (2014), Barakat *et al.,* (2016), and Vajravelu *et al.,* (2017) also made similar observations in in their studies. The water temperature outside the normal ranges may result in negative impact on aquatic organisms, which may result in an impact on growth, metabolism and reproduction (Dallas, 2008). Water temperature in Lake Icubhu showed a significant negative

high correlation with Nitrate (r = -0.648), a negative moderate with phosphorus (r = -0.476), a negative weak with nitrite (r = -0.227), a positive weak correlation with ammonia (r = 0.539) as presented in Appendix 1.1. In Nhlabane Estuary, temperature showed a significant negative high correlation when compared with nitrate (r = -0.669), a negative weak correlation with phosphorus (r = -0.367), a very week negative nitrite (r = -0.086) and a very weak positive correlation with ammonium (r = -0.158) as presented in Appendix 1.2.

It could be seen from Figure 14 that as the temperature increases, the concentrations of phosphate, nitrite and ammonium at Nhlabane eEstuary were decreasing. Ammonium showed a lower likelihood that a concentration of ammonium is influenced by the temperature in lake Icubhu. As temperature increased, a significant decrease was observed in nitrate concentrations in both wetlands. Similar observations on temperature with phosphate, ammonium and nitrite were also made in the study conducted by Sunetra *et al.* (2016). Similar observations were also made in the study conducted by Onkar and Sunil (2008), and Abir (2014). Temperature with nitrate correlation was not similar to previously conducted studies by Sunetra *et al.* (2016), Onkar and Sunil (2008), and Abir (2014). This was due to high concentrations on nitrate by anthropogenic and grazing of livestock in these wetlands. It was then observed that temperature variations in the wetland systems play a huge role in influencing the concentration of nutrients present. Dallas (2008) also reported that temperature exerts strong influence on biological, physical and chemical characteristics of water, including the chemical reaction rates. Therefore, the change in temperature yielded in a noticeably change or effect in all nutrients (phosphate, ammonium, nitrite and nitrate) correlated with it throughout this study period as shown in Figure 14.

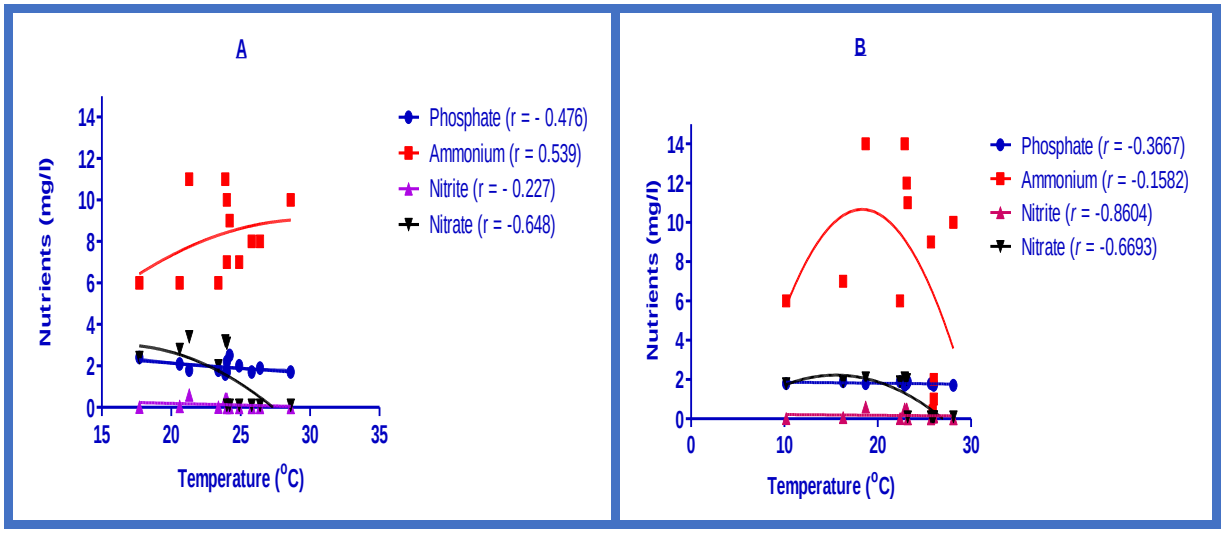

Figure 14: Effect of temperature on nutrient concentrations at Lake Icubhu (A) and Nhlabane mouth Estuary (B) from July 2016 to June 2017.

3.4.1.2 pH

The result presented in appendix 2.3 and 2.4 (Figure 7) showed that all the seasons have different pH values, and the pH in Lake Icubhu are significantly less than that of Nhlabane Estuary for all the seasons. This is indicated in Figure 15. Potential hydrogen in Lake Icubhu showed a significant positive high correlation when compare with ammonium (r = 0.667) and nitrite (r = 0.693). It showed a positive moderate correlation when compared with nitrate (r = 0.505), a negative moderate correlation with phosphorus (r = -0.525). In Nhlabane Estuary, pH showed a negative weak correlation with ammonium (r = -0.200), positive weak correlation with nitrite (r = 0.437), a positive weak correlation with nitrate (r = 0.106) and COD perfect correlation with phosphorus (r = 1.00). A significant increase in these nutrients were observed with increase in temperature (Figure 15). A linear relationship between pH and all the nutrients was observed in both wetlands. Phosphate and ammonium showed a decrease in their concentrations when pH values were increasing. A slight increase in nitrate was observed with increase in pH and moderate increase observed with increase in

temperature. Similar observations on the relationship between pH and nutrients were also made by Vajravelu *et al.*, (2017). Kumar *et al.*, (2009); and Rahaman *et al.*, (2014) made different observation because of tidal influence (Qasim, 2003; Rahaman *et al.*, 2014). According to Kshirsagar *et al.* (2016), the concentration of pH may be influenced by the air temperature and available nutrients. Therefore, the change in pH values resulted in either a constant positive or negative change in all nutrients (phosphate, ammonium, nitrite and nitrate) depending on a location of the wetland system.

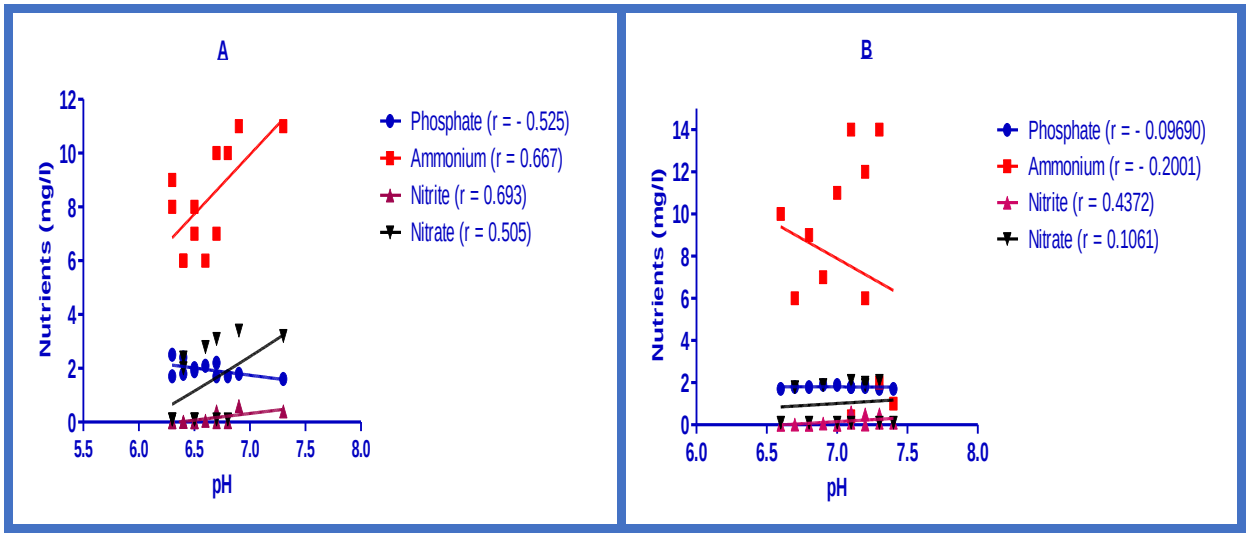

Figure 15: Effect of pH on nutrient concentrations at Lake Icubhu (A) and Nhlabane mouth Estuary (B) from July 2016 to June 2017

3.4.1.3 Chemical oxygen demand

Chemical oxygen demand in Lake Icubhu showed significant positive moderate correlation when compared with nitrite (r = 0.582). It also showed positive moderate correlation with Nitrate (r = 0.507), and ammonium (r = 0.478). It showed positive correlation with phosphorus (r = 0.622) as indicated in Figure 16. In Nhlabane mouth Estuary, a significant positive high correlation was observed when compared with nitrite (r = 0.723), a significant positive moderate correlation with ammonium (r = 0.797), a positive moderate with nitrate (r

= 0.357). A negative very weak correlation was observed when compare with phosphorus (r = -0.016), as shown in Figure 16.

The result shows that all the seasons have different COD values, and the COD in Lake Icubhu are significantly (P = 0.001) larger than that of Nhlabane Estuary for all the seasons. COD showed significant variation between the seasons in the study conducted by Mustapha *et al.* (2012). Importantly, the findings of this study were similar to those by Sunetra *et al.* (2016). Nutrients showed increase with increase in COD concentrations. Chemical oxygen demand also contributes on the concentration of nutrients in the wetland systems. Therefore, the change in COD concentrations yielded in a noticeably change or effect in all nutrients (phosphate, ammonium, nitrite and nitrate) correlated with it throughout this study period.

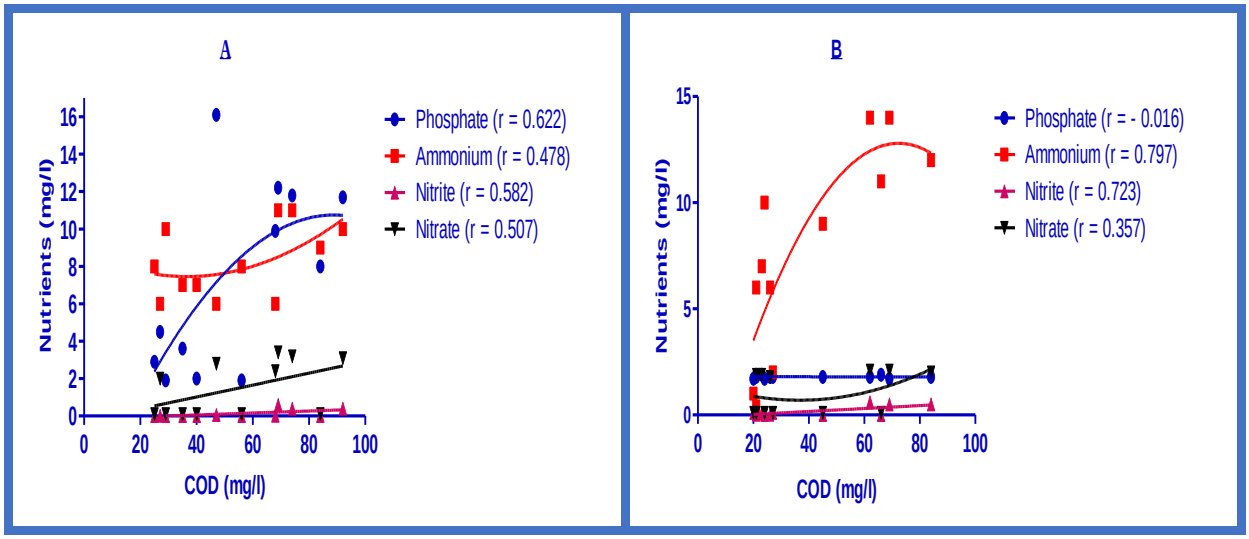

Figure 16: Effect of COD on nutrient concentrations at Lake Icubhu (A) and Nhlabane mouth Estuary (B) from July 2016 to June 2017

3.4.1.4 Total suspended solids

The result shows that the TSS values in Lake Icubhu are significantly (p = 0.001) greater than that of Nhlabane Estuary. In Lake Icubhu,TSS significant positive very high correlation when

compare with Nitrate (r = 0.860), significant positive moderate correlation nitrite (r = 0.601). It showed positive very weak correlation with ammonium (r = 0.180), and phosphorus (r = 0.180) as depicted in figure 17. In Nhlabane Estuary, a negative moderate correlation was observed with nitrate (r = -0.335), and nitrite (r = -0.338). Positive correlation was observed when compared with ammonium (r = 0.199),and phosphorus (r = 0.592). This is shown in Figure 17.

A perfect positive correlation was observed between phosphate and TSS at lake Icubhu, as showed in Figure 17. A significant high increase of nitrate concentrations as TSS concentrations increase was also observed. Nitrite also showed a significant moderate increase with an increase in TSS concentrations at Lake Icubhu. At Nhlabane Estuary, a significant increase between phosphate with increase of TSS concentrations was observed. A non-significant increase in ammonium concentrations with increase in TSS was observed. Both nitrite and nitrate concentrations were decreasing as concentrations of TSS increases.

Similar observations were also made by Vajravelu *et al.* (2017) where with a positive increase in TSS was an increase in nutrients concentrations as well but with an exception of nitrite and nitrate which showed a weak inverse relationship with TSS. Total suspended solids in wetland systems also influences the nutrient concentrations depending on the nature and amount dissolved solids. Therefore, the variation in TSS concentrations resulted in a noticeably change or effect in all nutrients (phosphate, ammonium, nitrite and nitrate) correlated with it throughout this study period. A positive correlation was observed with all the correlated nutrients in both wetlands studied with an exception of nitrate at Nhlabane Estuary which had a negative correlation.

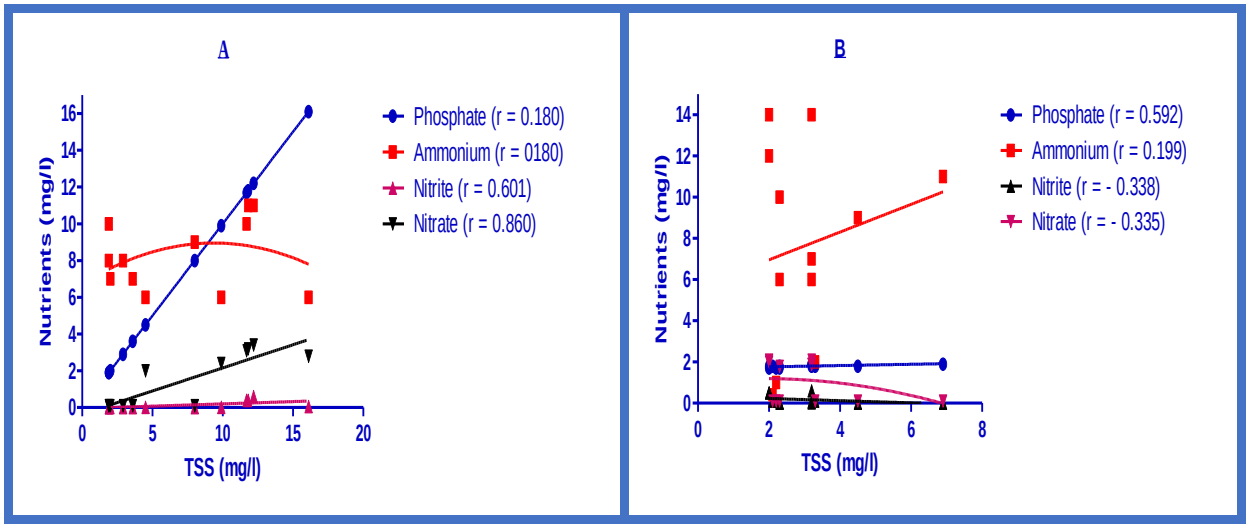

Figure 17: Effect of TSS on nutrient concentrations at Lake Icubhu (A) and Nhlabane mouth Estuary (B) from July 2016 to June 2017

3.6 Conclusion

Correlation coefficient of each wetland system was analyzed in order to understand the influence of physicochemical parameters to the nutrients on the two wetland systems. It was observed that the physiochemical parameters do have an influence on the concentration of nutrients in the wetland system. Nutrient concentration was also observed to change with variation of physicochemical parameters. Hence, they are interrelated, and should be considered together. Therefore, understanding the biological, chemical and physical aspects of wetland water is crucial for proper management of the wetland system. The fluctuations in these parameters may be possible contributed by anthropogenic activities through agricultural practices, including the application of fertilizer and grazing livestock in these wetlands.

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CHAPTER 4

MIROBIAL DIVERSITY AND SEASONAL VARIATION IN NATURAL WETLANDS

4.1 Introduction

The activity of microbial communities in natural wetlands is vital for the decomposition of the possible present organic matter. Wetland sediment microorganisms may alter both chemical as well as physical features of the wetland soil. Moreover, they are also important in biochemical cycling in a wetland system (Angeloni *et al.,* 2006; Wang *et al.,* 2010; Gemma *et al.,* 2014). Microbial diversity is vital for wetland ecosystem processes and nutrient cycling (Loreau, 2001; Wang *et al.,* 2010). Anthropogenic activities and other natural disturbances such as weathering may results in wetland loss or degradation (Peng *et al.,* 2003; Riis *et al.,* 2003; Moreno-Mateos *et al.,* 2008). Furthermore, microorganisms are sensitive to environmental changes (Wang *et al.,* 2010). The changes in environment result in alteration of microbial communities and negative effect on the health of the soil ecosystem (Riis *et al.,* 2003; Lin *et al.,* 2008; Herbert *et al.,* 2015). Different species and richness may have different manner of utilizing nutrients or pollutants in water systems. This may affect the wetland system ecological responses (Wang *et al.,* 2015).

Microorganisms in the natural wetlands ecosystems play an important role in removing pollutants in the system. These pollutants may include: organic matter (Zhang *et al.,* 2015), nitrogen, industrial organic pollutants (Lin *et al.,* 2012), and emerging organic contaminants (Jaatina *et al.,* 2008). Natural wetlands systems remove pollutants and nutrients at a low cost when comparing to other recognized available methods (Adrados *et al.,* 2014).

In coastal wetlands systems, salinity drives major functions and structures of the ecosystem since it impacts both biotic and abiotic processes (Brunet *et al.,* 2012). Chambers *et al.* (2011) also confirmed in his study that microbial community structures and associated biogeochemistry of wetlands systems are impacted by salinity.

Studying the natural wetlands is vital in order to understand their unique ecological roles in pollution filtration, erosion control and nutrients cycling. Understanding of bacterial community structure composition and diversity between the natural fresh water and coastal wetland is also vital since bacteria are known to be the key factors in many of the environment processes (Gemma *et al.,* 2014)

The aim of this study was to determine effect of seasonal variation on microbial communities and diversity in Lake Icubhu and Nhlabane mounth Estuary sediments through amplification of 16S rDNA. They were then compared to their seasonal shift in response to change in environmental parameters such as temperature and pH since microorganisms have different optimum ranges. Polymerase chain reaction (PCR) was used to determine and monitor the presence of microbial communities associated with transforming nutrients. Next generation – Illumina MiSeq sequencing was conducted to observe, compare and contrast changes in microbial community composition, structure as well as diversity.

4.2 Aim

To determine microbial communities in the natural wetlands systems in Lake Icubhu and Nhlabane as well determine the effect of seasonal variations of the microbial communities.

4.3 Objectives

To determine microbial community structures, present in Lake Icubhu and Nhlabane mouth Estuary in four different seasons.

4.4 Materials and methods

4.4.1 Collection of samples

Sediment samples were collected seasonally or quarterly in triplicates in the mornings from both Nhlabane mouth Estuary (coastal wetland) and at Icubhu Lake (Inland fresh water wetland) from July 2016 to June 2017 using polystyrene bags. This was done in order to study the microbial communities that were present in each wetland per season. The samples were then transported in cooler box with ice to the lab for the analysis of genomic DNA. The Xpedition Soil/Fecal DNA mini prep kit from Zymo Research (Inqaba Biotech) was used in the lab for DNA extraction from the samples collected in the morning, following the manufactures protocol describe in section 4.4.2.1 and samples were sent to Inqaba Biotech in cooler box with ice for further analysis of microbial communities using the NGS method describe in section 3.4.7.

4.4.2 Isolation of microorganisms from the soil samples

4.4.2.1 Soil DNA extraction

Sediment DNA was extracted using the Xpedition^{TM} Soil/fecal DNA MiniPrep kit following the manufacturer's (distributor's) protocols. One hundred milligrams of wet sediment sample were transferred to ZR bashing bead^{TM} Lysis tube, and centrifuged in a microcentrifuge at 10000 xg for 1 minute. This was followed by transferring of 400 *u*l supernatant to a Zymo-Spin^{TM} IV spin filter in a collection tube and centrifuged again at 7,000 x *g* for 1 minute. One thousand and two hundred microliters of Soil/Fecal DNA binding buffer were added to the

filtrate in a collection tube, and 800 *u*l of the mixture was then transferred to a Zymo-SpinTM IIC Column in a collection tube and centrifuged at 10,000 x *g* for 1 minute. The flow through was then discarded from the collection tube and the previous step was repeated. Two hundred microliter of DNA pre-wash buffer was added to a Zymo-SpinTM IIC column in a new collection tube and centrifuged at 10,000 x *g* for 1 minute, then followed by addition of 500 *u*l Soil/Fecal DNA wash buffer to the Zymo-Spin IIC column and centrifuged at 10,000 x *g* for 1 minute. The Zymo-SpinTM IIC column was then transferred into a clean 1.5 ml microcentrifuge tube and 100 *u*l of DNA elution Buffer was added directly to the column matrix. The DNA was then eluted by centrifuging the solution at 10,000rpm for 30 seconds. The eluted DNA from the previous step was then transferred to a Zymo-SpinTM IV-HRC spin filter in a clean microcentrifuge tube and centrifuged at exactly 8000rpm for 1 minute. The filtered DNA was kept at -80^{0}C before further processing.

4.4.3 DNA analysis.

The Extracted DNA was visualised in 0.8% agarose gel. Ethidium bromide was added for clear visualisation of DNA.

4.4.4 The principle of PCR

The PCR was used to amplify copies of a specific segment of DNA or strand of nucleic acid. This amplification was accomplished by polymerases which aided in stringing together individual DNA building blocks to form long molecular strands. For efficient PCR, polymerases required a supply of nucleotides, primers and template for construction of new strands. When these ingredients were supplied, the enzymes constructed exact copies of the templates. Three major steps that were involved in PCR technique and included; denaturation, annealing and extension. In the first step, DNA was being denatured at high temperatures (90^{0}C – 97^{0}C). This was followed by the second step where by the primers are

being annealed to the DNA template strands to prime extension. And the last step where by the complimentary copy strand of DNA is being created by extension at the end of the annealed primers. Therefore, in order to amplify a segment of DNA using PCR, the sample were first heated in order to separate into two pieces of single-stranded DNA. Then the synthesis of a Taq polymerase enzyme will build two new strands of DNA, utilising an original strand as template. This whole process resulted in a duplication of the original DNA. And new molecules containing both the old and new strands of DNA (Mohini and Deshpande, 2010).

4.4.5 PCR amplification

After the DNA was extracted, samples were sent to Inqaba Biotech in cooler box with ice for Polymerase chain reaction (PCR), and determination of microbial community structures as well as their diversity in both wetlands systems using the NGS method describe in section 4.4.6.

4.4.6 Next Generation – Illumina MiSeq sequencing

The DNA samples were further analyzed for microbial communities using Next Generation – Illumina MiSeq sequencing. Basically, this method was followed: starting with the genomic DNA (gDNA), the V3 and V4 region of the 16S rRNA were amplified using Next Generation Sequencing modified primers 341F, and 785R. PCR products were thereafter gel purified, and quantified with a Nanodrop 3300 Flourospectrometer. The amplicons obtained were indexed, size selected and quality controlled using Agilent's Bioanalyzer. Approximately 20 mb of (2 X 300 bp paired end reads) on Illumina MiSeq platform using MiSeq V3 (600 cycle) kit. The generated sequences were analyzed using the CLC Genomic Workbench version 10.1.1.

4.5 Results and discussion

Figure 4.1 presents the results of PCR products of soil samples that were collected from Lake Icubhu and Nhlabane mouth Estuary. The fragments had DNA size ≥0.50Kb and were separated using polyacrimide gel. The results for bacteria isolated in each season are presented on Table 4.1 (Bacteria identified at Lake Icubhu) and Table 4.2 (Bacteria identified at Nhlabane Estuary) and recorded based on the maximum identity from the Gene bank pool. The dominant kingdom was bacteria in both wetland systems throughout the study period of June 2016 to July 2017 as presented in Appendix 4.1 and 4.2 respectively. Other kingdoms were present in significantly low proportions, including Plantae, Archea, Viruses, Animalia, Chromista and Chromalveolata. The highest percentage of kingdom bacteria was observed in spring at Lake Icubhu and in winter at Nhlabane Estuary. The lowest percentages of kingdom bacteria were observed in summer in both wetlands and this could be due high temperatures.

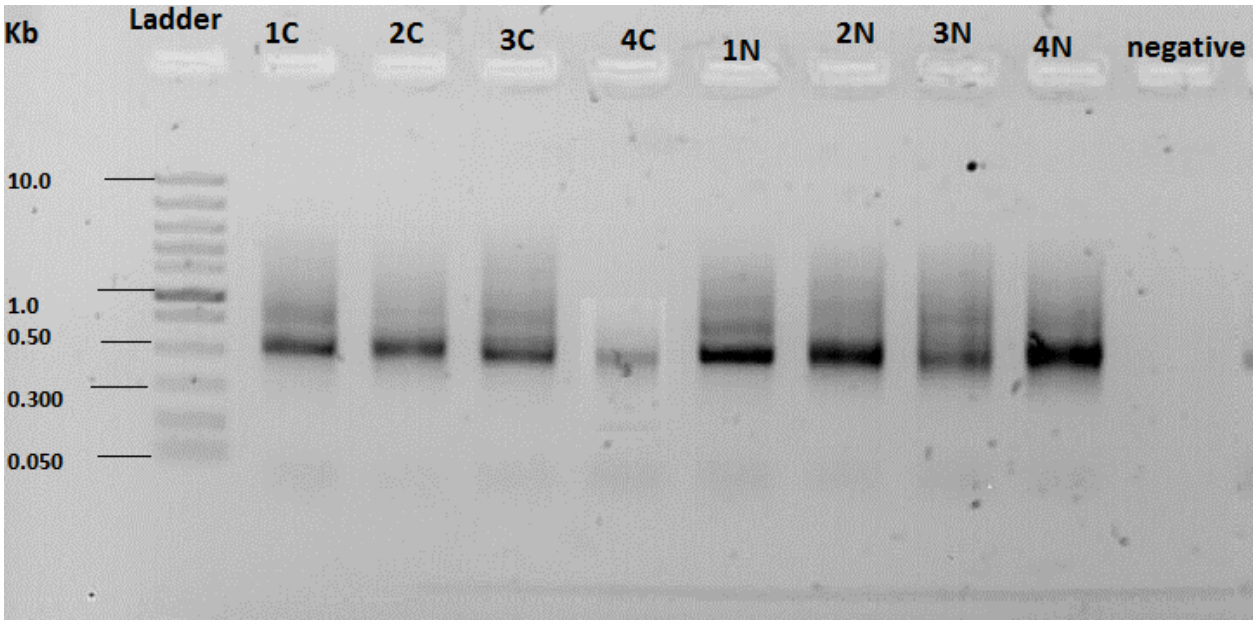

Figure 18: Agarose gel electrophoresis analysis of 16S rDNA genes amplified from 8 bacterial isolates. The PCR products were run on 1% agarose gel. Lane ladder represents the DNA ladder used, Markers with high intensity were indicated by their sizes. Lane 1C – 4C represents the PCR amplicon of Lake Icubhu while 1N – 4N represents the PCR amplicon of Nhlabane mouth Estuary.

4.5.1 Microorganisms identified at Lake Icubhu.

Different microorganisms were isolated in both wetlands at different seasons. Some microorganisms such as *E. coli* were present in all the samples collected. *E. coli* are facultative anaerobes of fecal origin (Tenaillon *et al.,* 2010). During the winter (June, July, and August) the phylum Proteobacteria was most predominant (30.85%) as presented in Appendix 4.3. It was represented by microorganisms such as *E. coil* (13.40%), *Aeromonas sp* (6.31%), etc. Others such as *Shewanella xiamenensis* (*Betaproteobacteria*) were also present in lower percentages (0.23%) others as low as 0.01% in the case of *Xanthobacter autotrophicus* (*Alphaproteobacteria*). The Proteobacteria abundance in winter was followed by the percentage present in the spring (September, October, and November) which was 21.56% and included microorganisms such as *Methylibium sp.* (11.29%), *Dokdonella sp* (0.66%) among others. In theautumn (March, April, and May) followed after the spring at 18.17%. These were represented by microorganisms such as *Burkholderia sp (Betaproteobacteria)* at 4.22%, *Bradyrhizobium sp* (Alphaproteobacteria) at 1.65%, *Geobacter sp* (*Deltaproteobacteria*) at 0.79%. The summer (December, January, and February) had the lowest at 7.51% represented by microorganisms such as *E. coil* (1.88%), *Rhodobacter sp* (0.56%), etc. which are denitrification bacteria (Adrados *et al.,* 2014). These microorganisms are also present in activated sludge and plays an important role in the removal of phosphate (Ivanov *et al.,* 2005).

Winter (June, July, and August) had the highest percentage of bacteroidetes (3.48%) represented by microorganisms such as *Flavobacterium sp* (0.79%), followed by spring at 1.98% including *Flavobacterium sp* (0.04%), *Alistipesshahii* (0.02%), etc. Then summer (December, January, and February) followed by the spring (September, October, and November) at 1.72%. *Flavobacterium sp (0.34*%) represented bacteroidetes in this season. And lastly, was the autumn (March, April, and May) with 0.38% bacteroidet*es.*

Flavobacterium sp known as potential denitrifying bacteria, and may also be found in activated sludge (Park *et al.,* 2007; Adrados *et al.,* 2014).

Actinobacteriamicroorganisms such as *Blastococcus saxobsidens* were highest in the autumn (March, April, and May) with a total average of 3.89% as presented in Appendix 4.6, while thesummer (December, January, and February) was second in Actinobacteria abundance with 1.84%, and included microorganisms such as *Corynebacterium xerosis* (0.90%). This was followed by the winter period (June, July, and August) with a total percentage of 1.55% and included microorganisms such as *Propionibacterium sp*. (0.64%), *Streptomyces hygroscopicus (0.01%),* etc. Spring (September, October and November) was last with 0.60% including *Actinobacterium sp* (0.04%), *Mycobacterium sp.* (0.04), *Conexibacter woesei* (0.03%). These group of microorganisms are known to be involved in nitrogen cycle, especially nitrogen fixation (adrados *et al.,* 2014).

Bacillus sp. belonging to a phylum Firmicuteswere also present in all the seasons. The highest percentage was observed during the winter (June, July, and August) at 1.87%, followed by the spring (September, October, and November) at 0.37%, then the summer (December, January, and February) at 0.36%, and the autumn (March, April, and May) was last at 0.185%. And they are believed to be potential denitrification bacteria (Adrados *et al.,* 2014). Others such as *Acaryochloris sp*. belonging to phylum Cyanobacteria, *Spirochaeta sp* belonging to phylum Spirochaetes were also observed at relatively low percentages. The microorganism's activities are involved in major roles in the natural wetland systems sediments that involves biochemical processing of nitrogen as well as other organics that may be deposited (Sezanna, 2011). The change in microbial community structures diversity may indicate nutrient input, degradation of the wetland system and environmental contamination (Judd *et al.,* 2006)

The results of this study were similar to the findings by Adrados et al. (2014). Who reported that the most represented taxonomic group in all their samples belonged to Proteobacteria.

Table 1: Microorganisms identified at Lake Icubhu between July 2016 and June 2017

	Spring (Sep, Oct and Nov.)	Summer (Dec, Jan and Feb)	Autumn (Mar, Apr and May)	Winter (Jun, Jul and Aug.)
Lake Icubhu	*Methylibium sp.* *Spirochaeta sp.* *Gemmatimonas* *Dokdonella sp.* *Escherichia coli*	*E coli* *Acidobacterium sp.* *Corynebacterium xerosis* *Bacteroides* *Cyanobacterium* *Rhodobacter*	*Burkholderia sp.* *Frankia sp.* *Bradyrhizobium* *Geobacter sp.* *Ramlibacter* *Epsilon sp.* *Hydrogenophaga* *Comamonas*	*E coli* *Aeromonas sp* *Aeromonas hydrophila* *Cryomorpha* *Pseudomonas sp* *Flavobacterium* *Kurthia gibsonii* *Propionibacterium*

4.5.2 Microorganisms identified at Nhlabane Estuary

Nhlabane mouth Estuaryin the winter (June, July, and August) had the highest percentage ofProteobacteria *(68.11%).* Among the identified microorganisms were: *Shewanella sp.* (7.49%), *P. migulae* (2.17%), *P. aeruginosa* (0.92%) etc. The percentage in the winter was followed by that of summer (December, January, and February) at 27.41%. Among the microorganisms identified at Nhlabane Estuary in summer (December, January, and February) were:*Pseudomonas sp. (0.52%), Enterobacter cloacae* (2.83%), *Sphingomonas sp (1.70%), Bradyrhizobium japonicum* (0.15%) all known to be under the phylum *Proteobacteria.* In the autumn (March, April, and May)Proteobacteriaaccounted for 11.23% were identified as: *Aeromonas hydrophila* (1.98%), *Pseudomonas sp.* (0.12%), *E. coli* (0.96%). For the spring (September, Octobe,r and November), the lowest percentage of

microorganisms belonging to phylum Proteobacteria (10.15%), among others identified were *Massilia oculi* (0.39), *Desulfuromonas sp.* (1.38%) and *E. coli* (0.86%).

Bacteroidetes percentages were in highest in the spring (September, October, and November) with 2.56% as presented in Appendix 4.4. Microorganisms such as *Alistipes shahii* (0.06%), and *Flavobacterium sp.* (0.09%) were also. identified. This was followed by the autumn (March, April, and May) with 1.10%, which was then followed by the summer (December, January, and February) at 0.65%, and the winter (June, July, and August) had the lowest with 0.44%, while some of the microorganisms isolated included *Dysgonomonas sp* (0.02%).

Actinobacteria were highest in the summer (December, January, and February) as presented in Appendix 4.5 at 4.43% including microorganisms such as *Microbacterium* (0.40%). That was followed by the winter (June, July, and August) at 0.83% including microorganisms such as *Microbacterium oxydans* (0.17). Spring then followed after winter at 0.57% and microorganisms such as *Streptomyces bingchenggensis* (0.04) were identified. Autumn (March, April, and May) had the lowest at 0.19% and microorganisms such as *Cellilomonas flavigena* (0.02) were identified.

Firmicutes in the summer (December, January, and February) were the highest at 1.60% including *Bacillus sp.* (0.04%), followed by the autumn (March, April, and May) at 1.38%, and among the identified microorganism was *Bacillus cereus* (0.01%). Spring followed after the autumn at 0.77% with 0.52% identified *Bacillus species.* Winter had the lowest percentage of isolated Firmicutes at 0.75%, but the microorganisms identified included:*Kurthia gibsonni* (0.41%), and *Veillonella parvula* (0.04%). Microorganisms isolated at Nhlabane Estuary were related to nutrient concentrations as they play a major role in nutrient cycling in the wetland system.

The findings of this study were similar to those by Cotterell and Kirchman (2000), and Heike *et al.* (2005), who also observed dominance by Proteobacteria followed by Bacteroidetes whereas Planctomycetes, Actinobacteria, Firmicus, acidobacteria and verrucomicrobia were less dominant. The land – sea interface is documented to be the most productive coastal marine ecosystem (Alongi, 1998). However, in this study, Bacteroidetes were only second highest in the spring (2.56%). In the autumn, it was the fourth highest with (1.10%) coming after Chloroflexi and Firmicutes. In the winter, it was also fourth coming after Actinobacteria and Firmicus. In the summer, it was the fifth dominant, coming after Planctomycetes, Actinobacteria and Firmicutes. This could have been due to their locations, and partly that these wetlands are highly exposed to livestock and human activities. In literature, it is documented that Bacteroidetes and Proteobacteria (α, β, gamma, delta and sometimes epsilonproteobacteria) are often the most abundant group of bacteria in coastal habitats (Hagstrom *et al.,* 2000; Eilers *et al.,* 2001 and Alonso *et al.,* 2007). Various lines of evidence state that Bacteroidetes play an important role in degrading complex as well as polymeric organic matter (Kirchman, 2002, Alonso *et al.,* 2007).

Table 2: Microorganisms identified at Nhlabane Estuary between July 2016 and June 2017.

	Spring (Sep, Oct and Nov.)	Summer (Dec, Jan and Feb)	Autumn (Mar, Apr and May)	Winter (Jun, Jul and Aug.)
Nhlabane Estuary	*Chloroflexus sp.* *Desulfuromonas sp.* *Xanthomonas* *Bacteroides* *E. coli* *Saprospira* *Bacillus sp.* *Rubrobacter*	*E. coli* *Enterobacter cloacae* *Aeromonas hydrophila* *Sphingomonas sp.* *Nocardioides marinisabuli* *Clostridium sp.* *Burkholderis sp.* *Pseudomonas sp.* *Acidovorax temperans*	*Chloroflexus sp.* *Aeromonas hydrophila* *Bacteroides sp.* *E. coli* *Cyanobacterium sp.* *Desulfobulbus sp.* *Bacillus sp.* *Anaerolinea sp.*	*Aeromonas hydrophila* *Shewanella sp.* *Stenotrophomonas sp.* *P. migulae* *Comamonas sp.* *E. coli* *Clostridium sp.* *P. aeruginosa* *Aeromonas veronii*

4.6 Conclusion

The seasonal variation appeared to have an influence on microbial communities in both wetland systems. *Proteobacteria sp* were predominant across all the seasons from the dataset generated from the GenBank which supports the previous studies (Bucci *et al.,* 2014; Crump and Hobbie, 2015). The sequences from the bacterial different phyla include Proteobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Actinobacteria and others were well presented by the available sequences and the microorganisms identified were probably the important participants in the wetland system. Physicochemical parameters such as temperature, and pH also have a huge impact in regulating the optimum environments for microbial communities. Hence, microbial populations vary with season, location and the environmental nature of the wetland system. Precisely, Lake Icubhu had more diverse microbial communities compared to Nhlabane Estuary. The highest dominant communities were noted in spring from Lake Icubhu. Hence, it is concluded from this study that both season and location of the wetland do have an effect on microbial community population. Therefore, more research needs to be conducted with regards to maintenance as well as improvement of microbial activity during different seasons.

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CHAPTER 5

GENERAL DISCUSSION

5.1.1 Physiochemical effect on nutrients

Temperature is a parameter known to play a key role in biogeochemical processes of the wetland systems. The temperature was high in summer and low in winter as expected, and it was in line with literature (Dallas, 2008). Nutrients like ammonium and nitrate showed a positive increase with increase in temperature. Whereas phosphate and nitrite showed a decrease with increase in temperature in both wetlands.

The increase in pH resulted in high concentrations of ammonium, nitrate and nitrite at Lake Icubhu. Phosphate decrease with increase in pH concentration in both wetlands. A significant decrease of ammonium with increase in pH was also observed in Nhlabane Estuary. The pH concentrations were generally higher at Nhlabane Estuarywhen compared to Lake Icubhu.

Nutrients such as ammonium, nitrate, nitrite and phosphate at Lake Icubhu showed a positive increase with the increase in COD. The phosphate concentrations at Nhlabane Estuary were decreasing as COD concentrations increases. This could have been due to Indian Ocean tidal influences.

Lake Icubhu had higher TSS concentrations than Nhlabane Estuary, with an exception in summer. The TSS concentrations also do have an effect on nutrients concentrations. All nutrients showed a positive increase with an increase in TSS concentrations at Lake Icubhu. At Nhlabane, a decrease in ammonium, nitrate and nitrite was observed with when TSS concentrations were increasing. Phosphate positively increase with increase in TSS concentrations.

5.1.2 Seasonal shift of microbial communities

Microbial communities in the natural wetland systems play a key role in cycling of nutrients that involves nitrogen fixation, ammonification, nitrification and denitrification. The activities of nutrient cycling in the wetland systems is carried out by different microorganisms. The physicochemical parameters such as temperature and pH also plays an important role in enabling proper activities of different microorganisms. Moreover, the change in these parameters affected the activity of microorganisms, since they functioned within their optimum ranges. The growth rate, behavior and microbial structures was affected with parameters outside optimum ranges. The alteration in pH concentrations also affected microbial diversity, since many of the microbial species could not tolerate extreme pH levels.

Seasonal microbial communities were isolated from both wetlands. Lake Icubhu had most microbial communities throughout the study period. This could have been due to the fact that Nhlabane is an Estuarine, and it had higher salt content compared to Lake Icubhu. Salinity is known to negatively affect microbial communities negatively. It is also documented in literature that increased salinity levels result in less active and smaller microbial communities (Wang *et al.*, 2010).

Most of the analyzed sequences corresponded to uncultured microorganisms, while others matched with certain number of percentage of similarity to culture bacteria. Typical microorganisms were found in all seasonal sample analyzed. The core composition of bacterial communities consisted of: Proteobacteria, Bacteroides, Actinobacteria, Firmicutes and others. *Acinetobacter sp.* is known to enhance biological phosphate removal and usually present in river sediments and activated sludge (Ivanov *et al.,* 2005). Other microbe such as *Arthrobacter sp*. participates in nitrogen cycle, especially nitrogen fixation (Adrados *et al.,* 2014). While members of Actinobacteria, bacteroidetes and Acidobacteria were observed in Lake Icubhu, members of Firmicutes, Chloroflexi and Bacteroidetes were also dominant at Nhlabane Estuary. Finally, some representatives of Thermomicrobia, Fusobacteria, Verrucomicrobia and Fusobacteria were also obtained from some samples. Therefore, it was concluded that seasonal variation and nutrients pollution loads were related to microbial community structures.

5.2 Conclusion

The natural wetland systems are a very complex ecosystem. It is thus crucial to understand its variables in order to understand how they function, and for proper management of the wetland system. The results revealed that the wetland parameters were interrelated with each other. Therefore, it can be concluded that seasonal variation together with the physiochemical parameters influenced nutrients concentrations in the natural wetlands systems. Nutrients concentrations such as phosphorus, ammonium and nitrate were found to be significantly higher in Lake Icubhu than Nhlabane mounth Estuary, and this was assumed to be as a result of agricultural activities and livestock grazing in close proximity of the lake. Alos, nitrite was found significantly less in Lake Icubhu than Nhlabane month Estuary.

The seasonal variation appeared to have an influence on microbial communities in both wetland systems. *Proteobacteria sp* were predominant across all the seasons from the dataset

generated from the GenBank. The sequences from the bacterial different phyla that include Proteobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Actinobacteria and others were well presented by the available sequences and the microorganisms identified were probably the important participants in the wetland system. Physicochemical parameters such as temperature and pH also have a huge role in regulating the optimum environments for microbial microorganisms. Hence, microbial populations vary with season, location, and the environmental nature of the wetland system. Furthermore, Lake Icubhu had more diverse microbial communities compared to Nhlabane Estuary. Nutrients including phosphate, ammonium and nitrate were generally higher in Lake Icubhu. Therefore, it is concluded that high concentration of nutrients supports high microbial diversity.

5.3 Future studies / Recommendations

Natural wetland systems have demonstrated positive contribution towards water quality improvement at low cost and in an environmental friendly manner.Further studies on effective reduction of nutrients pollution in wetlands throughout the year (all seasons) is essential, because some seasons hinder the full potential of wetlands functioning.

Some groups of analyzed microorganisms had no close cultured representative. Therefore, more multiple approaches should be made available to characterize microbial communities and their function in the wetland systems.

5.4 References

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Appendix 1.

Correlation matrix between nutrients and physicochemical parameters.

1.1: Correlation of Water Parameters at Lake Icubhu throughout the study period July 2016 – June 2017

	Temp	**pH**	**COD**	**TSS**	**Phosphorus**	**Ammonium**	**Nitrite**	**Nitrate**
Temperature	1							
pH	0.060	1						
COD	-0.357	0.256	1					
TSS	-0.695*	0.368	0.622*	1				
Phosphorus	-0.476	-0.525	0.199	0.087	1			
Ammonium	0.359	0.667*	0.478	0.180	-0.509	1		
Nitrite	-0.227	0.693*	0.582*	0.601*	-0.461	0.731*	1	
Nitrate	-0.648*	0.505	0.507	0.860*	-0.248	0.238	0.756*	1
*. Correlation is significant at the 0.05 level (2-tailed).								

1.2: Correlation matrix of water parameters at Nhlabane Estuary throughout the study period July 2016 – June 2017

	Temp	**pH**	**COD**	**TSS**	**Phosphorus**	**Ammonium**	**Nitrite**	**Nitrate**
Temperature	1							
pH	-0.041	1						
COD	0.162	0.056	1					
TSS	0.443	-	-0.050	1				

		.0763*						
Phosphorus	-0.381	0.000	-0.001	0.130	1			
Ammonium	0.190	-0.293	0.599*	0.477	0.268	1		
Nitrite	-0.048	0.384	0.839*	-0.518	-0.085	0.226	1	
Nitrate	-0.815*	0.174	0.426	-0.536	0.320	0.119	0.586*	1
*. Correlation is significant at the 0.05 level (2-tailed).								

Appendix 2.

Physicochemical parameters measured in both wetlands between July 2016 – June 2017

2.1: Lake Icubhu Temperature (^{0}C)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	17.7	17.7	17.6	17.7	winter
Aug-2016	23.4	23.3	23.5	23.3	winter
Sep-2016	24	23.9	24	24	spring
Oct-2016	25.8	25.6	25.9	25.9	spring
Nov-2016	24.9	24.7	25	25	spring
Dec-2016	26.4	26.6	26.3	26.3	summer
Jan-2017	28.6	28.6	28.5	28.5	summer
Feb-2017	24.2	24.2	24.2	24.3	summer
Mar-2017	24	24	23.9	24	autumn
Apr-2017	23.9	23.9	23.8	23.9	autumn
May-2017	21.3	21.3	21.3	21.4	autumn
Jun-2017	20.6	20.6	20.5	20.6	winter
Spring	24.9	24.7	25	25	
Summer	26.4	26.5	26.3	26.4	
Autumn(Fall)	23.1	23.1	23	23.1	
Winter	20.6	20.6	20.5	20.5	

2.2: Nhlabane Estuary Temperature (^{0}C)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	10.2	10.1	10.1	10.3	winter
Aug-2016	22.4	22.9	22.1	22.2	winter
Sep-2016	23.2	23.4	23	23.2	spring
Oct-2016	25.9	25.8	25.9	25.9	spring
Nov-2016	26	26	26	26	spring
Dec-2016	25.7	25.5	25.8	25.6	summer
Jan-2017	28.1	28	28	28.1	summer
Feb-2017	26	25	26	25.6	summer
Mar-2017	23.1	23.1	23.1	23.1	autumn
Apr-2017	22.9	22.7	23.1	22.7	autumn
May-2017	18.7	18.5	19.1	18.6	autumn
Jun-2017	16.3	16.3	16.2	16.3	winter
Spring	25	25.1	25	25	
Summer	26.6	26.2	26.6	26.4	
Autumn(Fall)	21.6	21.4	21.8	21.5	
Winter	16.3	16.4	16.1	16.3	

2.3: Lake Icubhu pH

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	6.4	6.4	6.4	6.4	winter
Aug-2016	6.4	6.3	6.2	6.7	
Sep-2016	6.7	6.9	6.7	6.5	Spring
Oct-2016	6.3	6.3	6.2	6.3	
Nov-2016	6.5	6.5	6.6	6.4	
Dec-2016	6.5	6.3	6.7	6.5	Summer

Jan-2017	6.8	6.8	6.8	6.7	
Feb-2017	6.3	6.3	6.2	6.4	
Mar-2017	6.7	6.4	6.7	6.9	Autumn
Apr-2017	7.3	7.1	7.5	7.3	
May-2017	6.9	6.9	6.8	6.9	
Jun-2017	6.6	6.4	6.7	6.7	Winter
Spring	6.5	6.6	6.5	6.4	
Summer	6.5	6.5	6.6	6.5	
Autumn(Fall)	7	6.8	7	7	
Winter	6.5	6.4	6.4	6.6	

2.4: Nhlabane Estuary pH

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	6.7	6.6	6.8	6.6	Winter
Aug-2016	7.2	7.3	7	7.3	
Sep-2016	7.4	7.3	7.5	7.4	Spring
Oct-2016	7.1	7	7.3	6.8	
Nov-2016	7.3	7.2	7.3	7.4	
Dec-2016	6.8	6.5	6.7	6.9	Summer
Jan-2017	6.6	6.5	6.7	6.6	
Feb-2017	7	6.8	7.2	7	
Mar-2017	7.2	6.9	7.1	7.4	Autumn
Apr-2017	7.3	7.1	7.4	7.5	
May-2017	7.1	6.9	7.1	7.2	
Jun-2017	6.9	7	6.8	7	Winter
Spring	7.3	7.2	7.4	7.2	
Summer	6.8	6.6	6.9	6.8	
Autumn(Fall)	7.2	7	7.2	7.4	
Winter	6.9	7	6.9	7	

2.5: Lake Icubhu COD (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	68	68	67	67	winter
Aug-2016	27	27	26	27	
Sep-2016	35	37	38	30	spring
Oct-2016	25	25	24	25	
Nov-2016	40	44	38	39	
Dec-2016	56	56	56	55	summer
Jan-2017	29	24	28	35	

Feb-2017	84	80	88	83	
Mar-2017	92	91	92	92	autumn
Apr-2017	74	69	77	76	
May-2017	69	69	72	65	
Jun-2017	47	45	46	49	winter
Spring	33.3	35.3	33.3	31.3	
Summer	56.3	53.3	57.3	57.7	
Autumn(Fall)	78.3	76.3	80.3	77.7	
Winter	47.3	46.7	46	47.7	

2.6: Nhlabane Estuary COD (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	26	24	28	26	winter
Aug-2016	21	21	20	22	
Sep-2016	20	21	20	23	spring
Oct-2016	21	21	20	22	
Nov-2016	27	27	27	27	
Dec-2016	45	44	46	44	Summer
Jan-2017	24	22	26	23	
Feb-2017	66	67	64	66	
Mar-2017	84	84	84	83	Autumn
Apr-2017	69	70	67	71	
May-2017	62	65	59	61	
Jun-2017	23	20	25	23	Winter
Spring	22.7	23	22.3	24	
Summer	45	44.3	45.3	44.3	
Autumn(Fall)	71.7	73	70	71.7	
Winter	23.3	21.7	24.3	23.7	

2.7: Lake Icubhu TSS (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	9.9	8.1	12.3	9.2	winter
Aug-2016	4.5	6.1	3.2	4.1	
Sep-2016	3.6	4.2	3.5	3.1	spring
Oct-2016	2.9	2.1	4.2	2.3	
Nov-2016	2	2.1	1.9	1.8	
Dec-16	1.9	1.9	1.8	1.9	summer
Jan-2017	1.9	1.9	2	1.9	

Feb-2017	8	7.3	7.9	8.7	
Mar-2017	11.7	10.6	11.8	12.8	autumn
Apr-2017	11.8	11.2	11.8	12.4	
May-2017	12.2	12.2	12.6	11.8	
Jun-2017	16.1	15.5	15.8	16.9	Winter
Spring	2.8	2.8	3.2	2.4	
Summer	3.9	3.7	3.9	4.2	
Autumn(Fall)	11.9	11.3	12.1	12.3	
Winter	10.2	9.9	10.4	10.1	

2.8: Nhlabane Estuary TSS (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	2.3	2.1	2.1	2.6	winter
Aug-2016	3.2	3.3	3.2	3.2	
Sep-2016	2.2	2.3	2.1	2.1	spring
Oct-2016	2.1	2.1	2.1	2.2	
Nov-2016	3.3	2.9	3.5	3.4	
Dec-16	4.5	4.1	4.8	4.6	summer
Jan-2017	2.3	2.6	2.4	2.3	
Feb-2017	6.9	6.4	6.9	7.5	
Mar-2017	2	1.8	1.8	2.5	autumn
Apr-2017	2	2.4	1.8	1.8	
May-2017	3.2	3.1	3.3	3	
Jun-2017	3.2	3.2	3.6	2.9	winter
Spring	2.5	2.4	2.6	2.6	
Summer	4.6	4.4	4.7	4.8	
Autumn(Fall)	2.4	2.4	2.3	2.4	
Winter	2.9	2.9	3	209	

Appendix 3.

Nutrients concentrations measured in both wetlands between July 2016 – June 2017

3.1: Lake Icubhu Phosphate (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	2.4	2.7	2.1	2.5	winter
aug-2016	1.8	1.7	1.9	1.7	
Sep-2016	2.2	2.2	2.2	2.1	spring
Oct-2016	1.7	1.6	1.7	1.5	
Nov-2016	2	2.1	2.1	1.8	
Dec-2016	1.9	1.8	1.8	2	Summer
Jan-2017	1.7	1.5	1.6	1.6	
Feb-2017	2.5	2.4	2.5	2.4	
Mar-2017	1.7	1.7	1.7	1.7	Autumn
Apr-2017	1.6	1.5	1.6	1.5	
May-2017	1.8	1.8	1.8	1.8	
Jun-2017	2.1	2.1	2	2.1	Winter
Spring	2	2	2	1.8	
Summer	2	1.9	2	2	
Autumn(Fall)	1.7	1.7	1.7	1.7	
Winter	2.1	2.2	2	2.1	

3.2: Nhlabane Estuary Phosphate (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	1.8	1.7	1.7	1.8	Winter
aug-2016	1.9	1.9	1.9	1.9	
Sep-2016	1.7	1.7	1.6	1.7	spring
Oct-2016	1.8	1.7	1.7	1.8	
Nov-2016	1.8	1.8	1.8	1.8	
Dec-2016	1.8	1.6	1.9	1.9	summer
Jan-2017	1.7	1.7	1.7	1.6	
Feb-2017	1.9	1.9	1.9	1.8	
Mar-2017	1.8	1.8	1.8	1.8	autumn
Apr-2017	1.7	1.6	1.7	1.7	
May-2017	1.8	1.8	1.8	1.8	
Jun-2017	1.9	1.8	1.9	1.8	winter
Spring	1.8	1.7	1.7	1.8	
Summer	1.8	1.7	1.8	1.8	
Autumn(Fall)	1.8	1.7	1.8	1.8	
Winter	1.9	1.8	1.8	1.8	

3.3: Lake Icubhu Ammonium (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	6	5	5	6	Winter
Aug-2016	6	6	6	6	
Sep-2016	7	4	9	8	Spring
Oct-2016	8	8	7	8	
Nov-2016	7	7	6	7	

Dec-2016	8	10	7	7	Summer
Jan-2017	10	12	11	9	
Feb-2017	9	8	9	9	
Mar-2017	10	11	10	11	Autumn
Apr-2017	11	12	11	10	
May-2017	11	13	12	10	
Jun-2017	6	7	5	5	Winter
Spring	7.3	6.3	7.3	7.7	
Summer	9	10	9	8.3	
Autumn(Fall)	10.7	12	11	10.3	
Winter	6	6	5.3	5.7	

3.4: Nhlabane EstuaryEstuary Ammonium (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	6	6	5	6	Winter
Aug-2016	6	5	5	6	
Sep-2016	1	1	1	1	spring
Oct-2016	0.4	0.3	0.3	0.4	
Nov-2016	2	2	1	2	
Dec-2016	9	8	8	9	summer
Jan-2017	10	8	11	9	
Feb-2017	11	13	9	9	
Mar-2017	12	12	11	12	autumn
Apr-2017	14	14	14	13	
May-2017	14	13	15	13	
Jun-2017	7	9	6	6	winter
Spring	1.1	1.1	0.8	1.1	
Summer	10	9.7	28	9	
Autumn(Fall)	13.3	13	13.3	12.7	
Winter	6.3	6.7	5.3	6	

3.5: Lake Icubhu Nitrite (mg/l)

Sampling period	Averages	Sample #1	Sample #2	Sample #3	
Jul-2016	0.02	0.02	0.02	0.01	winter
Aug-2016	0.02	0.02	0.02	0.02	
Sep-2016	0.01	0.01	0.02	0.01	spring
Oct-2016	0.01	0.01	0.01	0.01	
Nov-2016	0.01	0.01	0.01	0.01	

Dec-2016	0.01	0.02	0.01	0.01	Summer
Jan-2017	0.01	0.01	0.01	0.01	
Feb-2017	0.01	0.01	0.01	0.01	
Mar-2017	0.4	0.3	0.4	0.3	Autumn
Apr-2017	0.4	0.4	0.4	0.4	
May-2017	0.6	0.7	0.6	0.6	
Jun-2017	0.06	0.05	0.06	0.06	Winter
Spring	0.01	0.01	0.01	0.01	
Summer	0.01	0.01	0.01	0.01	
Autumn(Fall)	0.5	0.5	0.5	0.4	
Winter	0.03	0.03	0.03	0.03	

3.6: Nhlabane Estuary Nitrite (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	0.02	0.02	0.02	0.02	Winter
Aug-2016	0.02	0.02	0.02	0.01	
Sep-2016	0.1	0.1	0.1	0.1	Spring
Oct-2016	0.1	0.1	0.1	0.1	
Nov-2016	0.1	0.1	0.2	0.1	
Dec-2016	0.01	0.01	0.01	0.01	Summer
Jan-2017	0.01	0.01	0.01	0.02	
Feb-2017	0.01	0.01	0.01	0.01	
Mar-2017	0.5	0.7	0.5	0.4	Autumn
Apr-2017	0.5	0.5	0.6	0.5	
May-2017	0.6	0.8	0.6	0.4	
Jun-2017	0.07	0.07	0.05	0.07	Winter
Spring	0.1	0.1	0.1	0.1	
Summer	0.01	0.01	0.01	0.1	
Autumn(Fall)	0.5	0.6	0.6	0.4	
Winter	0.04	0.04	0.03	0.03	

3.7: Lake Icubhu Nitrate (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	2.4	2.4	2.4	2.4	winter
Aug-2016	2	2	2	2	
Sep-2016	0.1	0.1	0.1	0.1	Spring
Oct-2016	0.1	0.1	0.1	0.2	
Nov-2016	0.1	0.2	0.1	0.1	

Dec-2016	0.1	0.1	0.1	0.1	Summer
Jan-2017	0.1	0.1	0.2	0.1	
Feb-2017	0.1	0.1	0.1	0.1	
Mar-2017	3.1	3.2	3.1	3.1	Autumn
Apr-2017	3.2	3.2	3.2	3.2	
May-2017	3.4	3.6	3.4	3.2	
Jun-2017	2.8	2.8	2.7	2.8	Winter
Spring	0.1	0.1	0.1	0.1	
Summer	0.1	0.1	0.1	0.1	
Autumn(Fall)	3.2	3.3	3.2	3.2	
Winter	2.4	2.4	2.4	2.4	

3.8: Nhlabane Estuary Nitrate (mg/l)

Sampling period	**Averages**	**Sample #1**	**Sample #2**	**Sample #3**	
Jul-2016	1.8	1.7	1.8	1.8	Winter
Aug-2016	1.9	1.9	1.9	1.8	
Sep-2016	0.1	0.1	0.1	0.1	Spring
Oct-2016	0.1	0.1	0.1	0.2	
Nov-2016	0.1	0.1	0.1	0.1	
Dec-2016	0.1	0.1	0.2	0.1	Summer
Jan-2017	0.1	0.1	0.1	0.1	
Feb-2017	0.1	0.2	0.1	0.1	
Mar-2017	2	1.9	2	1.9	Autumn
Apr-2017	2.1	2.3	2.1	1.9	
May-2017	2.1	2.1	2.1	2.1	
Jun-2017	1.9	1.9	1.8	1.9	Winter
Spring	0.1	0.1	0.1	0.1	
Summer	0.1	0.1	0.1	0.1	
Autumn(Fall)	2.1	2.1	2.1	2	
Winter	1.9	1.8	1.8	1.8	

Appendix 4.

Kingdoms of microorganisms isolated in both wetlands

4.1: Kingdoms of microorganisms isolated at Lake Icubhu

Kingdom	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
Bacteria	99.81	98.86	98.93	99.04
Unkown	0.11	0.40	0.51	0.39
Protozoa	0.01	0.29	0.17	0.20
Fungi	0.03	0.24	0.10	0.05
Plantae	0.02	0.10	0.09	0.03
Archaea	0.01	0.01	0.03	0.03
Viruses	0.00	0.07	0.01	0.00
Virus	0.00	0.01	0.14	
Animalia	0.00		0.01	0.00
Chromista	0.01			
Chromalveolata		0.03	0.00	0.26

4.2: Kingdom of microorganisms isolated at Nhlabane Estuary

Kingdom	Spring (%)	Summer (%)	Autumn (%)	Winter (%)
Bacteria	99.27	98.38	99.54	99.88
Plantae	0.42	0.18	0.07	0.01
Unkown	0.09	0.28	0.11	0.08
Fungi	0.09	0.10	0.15	0.01
Archaea	0.04	0.09	0.07	0.00
Protozoa	0.03	0.37	0.03	0.00
Viruses	0.01	0.33	0.01	
Virus	0.03		0.00	0.00
Chromalveolata		0.22	0.02	
Animalia		0.04		0.00
Chromista			0.00	

Appendix 4.

Phyla classification results for both wetlands.

4.3: Phyla classification isolated in winter 2016.

Phyla classification of Lake Icubhu in winter 2016.

Phyla Classification	%
Unknown	60.51
Proteobacteria	30.85
Bacteroidetes	3.48
Firmicutes	1.87
Actinobacteria	1.55
Planctomycetes	0.44
Cyanobacteria	0.35
Heterokontophyta	0.26
Verrucomicrobia	0.21
Ciliophora	0.20
Acidobacteria	0.11
Gemmatimonadetes	0.09
Basidiomycota	0.04
Tracheophyta	0.03
Ascomycota	0.01
Chloroflexi	0.00
Chordata	0.00
Fusobacteria	0.00
Crenarchaeota	0.00
Chlorobi	0.00
Not assigned	0.00
Bryophyta	0.00

Phyla classification of Nhlabane Estuary in winter 2016.

Phyla Classification	%
Proteobacteria	68.11
Unknown	29.82
Actinobacteria	0.83
Firmicutes	0.75
Bacteroidetes	0.44
Tracheophyta	0.01
Basidiomycota	0.01
Verrucomicrobia	0.01
Chordata	0.00
Fusobacteria	0.00
Ciliophora	0.00
Planctomycetes	0.00
Cyanobacteria	0.00
Euryarchaeota	0.00
Chlorobi	0.00

4.4: Phyla classification isolated in spring 2016.

Phyla classification of Lake Icubhu in spring 2016.

Phyla Classification	%
Unknown	70.56
Proteobacteria	21.56
Bacteroidetes	1.98
Gemmatimonadetes	1.03
Planctomycetes	1.02
Acidobacteria	0.81
Fibrobacteres	0.75
Cyanobacteria	0.71
Actinobacteria	0.60
Spirochaetes	0.38
Firmicutes	0.37
Chloroflexi	0.05
Verrucomicrobia	0.04
Nitrospira	0.04
Basidiomycota	0.03
Tracheophyta	0.02
Thermomicrobia	0.01
Deinococcus-thermus	0.01
Ciliophora	0.01
Chordata	0.00
Crenarchaeota	0.00
Radiozoa	0.00
Ascomycota	0.00
Euryarchaeota	0.00
Oomycota	0.00
Thermodesulfobacteria	0.00
Chlorobi	0.00
Lentisphaerae	0.00
Aquificae	0.00

Phyla classification of Nhlabane in spring 2016.

Phyla Classification	%
Unknown	80.86
Proteobacteria	10.15
Bacteroidetes	2.56
Chloroflexi	2.35
Planctomycetes	1.46
Firmicutes	0.77
Actinobacteria	0.57
Acidobacteria	0.47
Tracheophyta	0.42
Basidiomycota	0.08
Nitrospira	0.06
Gemmatimonadetes	0.04
Verrucomicrobia	0.04
Cyanobacteria	0.04
Euryarchaeota	0.04
Ciliophora	0.03
Fusobacteria	0.03
Ascomycota	0.01

4.5: Phyla classification isolated in summer 2016.

Phyla classification of Lake Icubhu in summer 2016.

Phyla Classification	%
Unknown	84.43
Proteobacteria	7.51
Actinobacteria	1.84
Acidobacteria	1.82
Bacteroidetes	1.72
Cyanobacteria	0.61
Chloroflexi	0.46
Planctomycetes	0.44
Firmicutes	0.36
Ciliophora	0.29
Basidiomycota	0.13
Chlamydiae	0.11
Glomeromycota	0.11
Tracheophyta	0.09
Heterokontophyta	0.03
Spirochaetes	0.02
Verrucomicrobia	0.01
Deinococcus-thermus	0.01
Euryarchaeota	0.01
Bryophyta	0.01
Ascomycota	0.00
Gemmatimonadetes	0.00
Thermomicrobia	0.00
Not assigned	0.00

Phyla classification of Nhlabane Estuary in summer 2016.

Phyla Classification	%
Unknown	58.87
Proteobacteria	27.41
Planctomycetes	4.52
Actinobacteria	4.43
Firmicutes	1.60
Bacteroidetes	0.65
Chloroflexi	0.64
Cyanobacteria	0.38
Ciliophora	0.35
Spirochaetes	0.28
Heterokontophyta	0.22
Tracheophyta	0.18
Crenarchaeota	0.07
Gemmatimonadetes	0.07
Verrucomicrobia	0.06
Basidiomycota	0.06
Acidobacteria	0.05
Ascomycota	0.04
Deinococcus-thermus	0.04
Chordata	0.04
Not assigned	0.02
Euryarchaeota	0.01

4.6: Phyla classification isolated in autumn 2017.

Phyla classification of Lake Icubhu in autumn 2017.

Phyla Classification	%
Unknown	75.51
Proteobacteria	18.17
Actinobacteria	3.89
Acidobacteria	0.52
Bacteroidetes	0.38
Chloroflexi	0.25
Spirochaetes	0.19
Firmicutes	0.18
Cyanobacteria	0.17
Ciliophora	0.17
Planctomycetes	0.11
Gemmatimonadetes	0.09
Basidiomycota	0.08
Tracheophyta	0.06
Verrucomicrobia	0.05
Nitrospira	0.03
Deinococcus-thermus	0.03
Bryophyta	0.03
Crenarchaeota	0.02
Fibrobacteres	0.02
Ascomycota	0.02
Euryarchaeota	0.01
Chordata	0.01
Not assigned	0.01
Thermodesulfobacteria	0.01
Heterokontophyta	0.00

Phyla classification of Nhlabane Estuary in autumn 2017.

Phyla Classification	%
Unknown	75.10
Proteobacteria	110.23
Chloroflexi	8.14
Firmicutes	1.38
Bacteroidetes	1.10
Cyanobacteria	0.97
Planctomycetes	0.86
Actinobacteria	0.43
Acidobacteria	0.19
Verrucomicrobia	0.18
Basidiomycota	0.14
Tracheophyta	0.07
Euryarchaeota	0.06
Spirochaetes	0.03
Ciliophora	0.03
Deinococcus-thermus	0.02
Thermomicrobia	0.02
Heterokontophyta	0.02
Fibrobacteres	0.01
Ascomycota	0.01
Nitrospira	0.01
Gemmatimonadetes	0.00
Chrysiogenetes	0.00
Crenarchaeota	0.00
Radiozoa	0.00