

**AN ECOLOGICAL STUDY OF A CONSTRUCTED TREATMENT  
WETLAND ON A COMMERCIAL CROCODILE FARM NEXT TO THE  
OKAVANGO DELTA, BOTSWANA**

By

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10 February 2020

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# ABSTRACT

Constructed treatment wetlands (CTWs) are hidden weapons for improving water quality of which we have not yet discovered the full potential. The study area was based on a CTW treating the wastewater of Krovovango – a commercial crocodile farm in the village of Samochima next to the Panhandle of the Okavango River, Botswana. The Krovovango CTW cannot specifically be classified as a true CTW, since it does not fit into any of the existing criteria used. This wetland can rather be referred to as a simplified vertical surface flow wetland with no outflow, which is a unique scenario. The aim of the study was to contribute to our general understanding of constructed wetland functioning and, more importantly, the role that planktonic organisms play within these wetlands to improve water quality. Secondly, the study attempted to highlight the potential of simple wastewater treatment systems to show that more expensive or complex systems are not necessarily the only option to be considered for water quality improvement, especially in developing and arid countries such as Botswana. The study took place during July-August 2017, with a follow-up study during June-July 2018. Results from the Krovovango wetland showed that planktonic community comprised of five taxa with a total of 50 species sampled and identified. These organisms depend on each other for survival by maintaining balanced community structures and ultimately ensuring ecosystems remain as natural as possible. Interactions within the trophic structure of wetlands improve water quality and degrade pollutants. Phytoplankton, for example *Anabaena* sp. (cyanobacteria) and *Nitzschia* sp. (diatom), form the base of aquatic food webs as the primary producers. Protozoans, for example *Paramecium* sp., occupy a wide range of trophic levels. Rotifers, such as *Brachionus* spp. and *Platyias patulus*, are primarily omnivorous and commonly feed on dead or decomposing organic material, making wheel animalcules critical role players in organically rich water bodies, like the Krovovango CTW. Cladocerans (e.g. *Alona affinis*) and copepods (e.g. *Thermocyclops neglectus*) create a trophic link between primary producers and bigger predators. Examples of species mentioned above were also the most abundant within each taxon collected from the Krovovango wetland. Microorganisms in association with wetland vegetation contributed substantially to nutrient cycling and energy flow. Physical water quality parameters were measured, and results indicated that total

dissolved oxygen, conductivity, temperature, and pH levels of the Krovovango CTW falls in the range of the Okavango River. The Krovovango CTW has been in operation since 2012 and has become an additional habitat for a variety of bird species. The diversity of microinvertebrates, as well as other invertebrates and bird species recorded and identified, is a valuable indication of the wetland's success as a constructed treatment facility.

**Keywords:** Krovovango constructed treatment wetland, biological indicator species, phytoplankton, Protozoa, Rotifera, Cladocera, Copepoda, water quality.

# OPSOMMING

Mensgemaakte waterbehandelings vleilande (MWBVe) is versteke wapens waarvan ons nog nie die volle potensiaal ontdek het nie. Die studie area was gebasseer op 'n MWBV wat die afvalwater van Krokovango behandel. Krokovango is 'n kommersiële krokodilplaas in die dorpie van Samochima langs die Pypsteel van die Okavangorivier, Botswana. Die Krokovango MWBV kan nie spesifiek as 'n ware MWBV geklassifiseer word nie, aangesien dit nie inpas by enige van die kriteria wat gebruik word nie. Hierdie vleiland kan eerder na verwys word as 'n vereenvoudigde vertikale oppervlak vloei vleiland met geen uitvloei, wat 'n unieke geval is. Die doel van die studie was om by te dra tot ons algemene kennis van mensgemaakte vleiland funksionering, en mees belangrik, die rol wat planktoniese organismes binne hierdie vleilande vervul om watergehalte te verbeter. Tweedens het die studie gepoog om die potensiaal van eenvoudige afvalwaterbehandelingstelsels uit te lig, om aan te toon dat duurder of ingewikkelde stelsels nie noodwendig die enigste opsie is wat oorweeg kan word vir waterbehandeling nie, veral in ontwikkelende en droë lande soos Botswana. Die studie het gedurende Julie-Augustus 2017 plaasgevind, met 'n opvolg studie gedurende Junie-July 2018. Resultate van die Krokovango vleiland het aangedui dat die planktoniese gemeenskapstruktuur bestaan het uit vyf taksa met 'n totaal van 50 spesies wat versamel en geïdentifiseer is. Hierdie organismes is van mekaar vir oorlewing afhanklik deur gebalanseerde gemeenskapstrukture te handhaaf en uiteindelik te verseker dat ekosistels so natuurlik as moontlik bly. Interaksies in die trofiese struktuur van vleilande verbeter watergehalte en degradeer besoedelingstowwe. Verteenwoordigers van fitoplankton soos *Anabaena* sp. (sianobakterieë) en *Nitzschia* sp. (diatoom) vorm die basis van akwatiese voedselwebbe as die primêre produseerders. Protozoa verteenwoordigers soos *Paramecium* sp. kom op verskillende trofiese vlakke voor. Rotifera verteenwoordigers bv. *Brachionus* spp. en *Platyias patulus* is hoofsaaklik omnivories en voed op dooie- of ontbindende organiese materiaal. Hierdie feit maak wieldiere kritiese rolspelers in organiesryke watermassas soos die Krokovango MWBV. Verteenwoordigers van die Cladocera (bv. *Alona affinis*) en Copepoda (bv. *Thermocyclops neglectus*) skep 'n trofiese skakel tussen primêre produseerders en groter roofdiere. Voorbeelde van bogenoemde spesies was ook die vollopste in elke takson wat in die Krokovango

vleiland versamel was. Mikroörganismes in samewerking met vleilandplantegroei dra aansienlik by tot die sirkulering van voedingstowwe en vloei van energie. Die fisiese parameters van waterkwaliteit was gemeet, en die resultate het aangedui dat totale opgeloste suurstof-, elektriese geleiding-, temperatuur- en pH vlakke van die Krokovango MWBV ooreenstem met die van die Okavangorivier. Die Krokovango MWBV is sedert 2012 in werking en het ook tot 'n habitat vir talle voëlspesies ontwikkel. Die verskeidenheid mikroörganismes-, sowel as ander ongewerweldes en voëlspesies wat waargeneem en geïdentifiseer is, is 'n waardevolle aanduiding van die sukses van hierdie vleiland as 'n waterbehandelingstelsel.

**Sleutelwoorde:** Krokovango mensgemaakte waterbehandelings vleiland, biologiese indikator spesies, fitoplankton, Protozoa, Rotifera, Cladocera, watergehalte.

## CHAPTER 1: INTRODUCTION

Wetlands are considered as the earth's natural kidneys, and if we could build more kidneys for our earth, then - why not do so? Constructed treatment wetlands (CTWs) are manmade treatment systems that functions in the same way as natural wetlands. The world is changing rapidly, natural environments are deteriorating on a global scale and humans/establishments are consistently striving towards approaching more environmentally friendly practices or being recognised as environmentally considerate. The study of natural systems is, therefore, crucial in understanding how we as humans influence lower trophic forms, and vice versa (top-down and bottom-up processes) in order to make informed decisions for the future.

The world's water contains 2.5% that is fresh, of which only 1% is in liquid form on the earth's surface. Ultimately, a mere 0.01% is readily available for anthropogenic purposes (Dudgeon et al. 2006; Balian et al. 2008; Van As et al. 2012). There are approximately 126 000 freshwater species described, making up 9.5% of the total number of species on earth (Balian et al. 2008; Strayer and Dudgeon 2010). Considering that fresh water covers such a small percentage of the earth's surface, it becomes evident that the biodiversity residing in these freshwater ecosystems comprises a disproportionately large fraction of the world's total biodiversity.

According to Zaman and Sizemore (2017), freshwater ecosystems are under enormous threat on a global scale and human activities are to blame for it. The effective management of these systems are crucial, since not only freshwater organisms depend on it, but life on earth (including humans) also depends on it (Dudgeon et al. 2006; Zaman and Sizemore 2017). The idea of water quality being improved by small living organisms in aquatic systems is a concept not fully understood/recognised by some. In the general public, microorganisms are often associated with dirty conditions and/or disease (Nai et al. 2016). People should be made more aware of the value that wetland microorganisms might have.

Constructed treatment wetlands are water treatment systems that are more often and -successfully implemented in developed countries, but the potential that uncomplicated constructed wetland systems might have in developing countries can

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be substantial for various reasons, including low costs and simplicity of operation and -maintenance (Gorgoglione and Torretta 2018).

Globally the production of solid wastes and contaminants are increasing rapidly, and it is becoming more and more challenging to protect the environment and human health (Abdel-Shafy and Mansour 2018). The list of chemical compounds that we release into the environment continues to expand. Despite this ongoing issue, the effects caused by these chemicals remain poorly understood (Elosegi et al. 2019). More extensive research is needed on these topics, because water authorities and policy makers rely on it to implement long-term strategies to mitigate future environmental challenges (Sabater et al. 2019).

Microorganisms in association with wetland vegetation within these aquatic ecosystems contribute substantially to nutrient cycling and energy flow. Ultimately interactions within the trophic structure of wetlands improve water quality and degrade environmental pollutants (Cotner and Biddanda 2002; Battin et al. 2003; Hahn 2006; Barnett et al. 2007). These interactions can be very complex to understand and there is much room for research in this area. Understanding these interactions more in depth assists us in altering community compositions within constructed treatment wetlands to improve its efficiency.

It was noted that most research regarding constructed treatment wetlands focusses on performance in relation to the combination of effective vegetation types, overall structure and hydraulics. Examples of related studies include Klomjek and Nitorisavut (2005), Knight RL et al. (2000) and Sundaravadivel and Vigneswaran (2001). All these aspects directly influence microorganism community structure and it might be very beneficial for us to know more about the role of these microscopic organisms within these treatment systems. It is therefore important to expand our knowledge on this topic, since it can substantially improve our existential quality of life.

With this in mind, the **first** aim was to contribute to our general knowledge of constructed wetland functioning and the role that planktonic organisms play within these wetlands to improve water quality. The objectives here were to observe, identify and quantify phyto- and zooplankton species collected from the Krokovango CTW and to determine their potential as biological indicator species. Focus was placed on the

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microinvertebrates, while other vegetation, insects and birds were also noted, observed and identified. **Secondly**, the present study also attempted to illustrate the potential of the use of simple wastewater treatment systems to show that more expensive or complex systems are not necessarily the only option to be considered, especially in a developing and arid country like Botswana.

Following this brief introduction (**Chapter 1**), **Chapter 2** provides a general background on constructed treatment wetlands referring to the different types found and the different physical- and biological components and how these components influence one another to improve water quality (among other benefits). In **Chapter 3**, the material and methods used in this dissertation are described. **Chapter 4** includes all results gathered for the five groups collected during the study, including species lists, -descriptions, -ecology and statistical analysis on zooplankton abundances. In **Chapter 5**, the Krokovango CTW is compared to the textbook definition of the “perfect” CTW, in order to make recommendations on improving its efficiency. The trophic structure- and role of vegetation and microinvertebrates within freshwater aquatic systems are discussed. Alongside this, biological indicator phytoplankton- and zooplankton species of eutrophication are also examined. The thesis is concluded with concluding remarks followed by references used in **Chapter 6**. This dissertation ends with **Appendix 1** that contains counts for species of each day with abundances for 2017 and **Appendix 2** that contains counts for species of each day with abundances for 2018.

## **CHAPTER 2: WETLANDS AND CONSTRUCTED TREATMENT WETLANDS (CTWs)**

A wetland is an area of land consisting of marshes or swamps. It can be any piece of land saturated with water (Finlayson et al. 2018). A wetland that is constructed for the primary purpose of water quality improvement is called a Constructed Treatment Wetland (CTW). There are several terms used e. g. reed beds, engineered wetlands, man-made- or artificial wetlands, but for this dissertation the term constructed treatment wetland (CTW) will be used. As defined by Interstate Technology and Regulatory Council (ITRC 2003): "Constructed treatment wetlands are engineered systems, designed and constructed to utilise the natural functions of wetland vegetation, soils and their microbial populations to treat contaminants in surface water, groundwater or waste streams".

Wetlands are unique ecosystems compared to other natural ecosystems found on earth. Not only do wetlands provide a habitat for animals and plants, but they are important to humans for many reasons as well, as will be discussed in "The functions and values of CTWs" section of this chapter. Many CTWs are constructed to closely resemble natural wetlands (Mitsch and Gosselink 2007). Wetlands normally have an abundance of water and this promotes most forms of biological productivity. These high rates of biological activity enable wetlands to transform many of the more common pollutants into harmless by-products or essential nutrients. These nutrients can additionally be used for other biological activities occurring in wetlands (Kadlec and Wallace 2008).

According to Birch and Wachter (2011), countries such as Australia, United States and New Zealand are increasingly constructing these manmade wetlands. In most cases these systems also offer tertiary treatment to towns and cities. Being larger in size, they operate through a surface-flow system to remove low concentrations of nitrogen (N) and phosphorus (P) as well as suspended solids (Vymazal 2010).

### 2.1: The history of CTWs

Disposal of wastewater has been a challenge for humanity since the industrial revolution. Over the past century, natural wetlands have been one of the major go-to methods utilised by communities to dispose of wastewater (Murphy and Cooper 2010). Up until today, wetland technology has been improved substantially and people are starting to create wetlands themselves for wastewater treatment. The Max Planck Institute in Germany was the first institute to conduct studies on the use of constructed wetlands for wastewater treatment (Seidel 1976). Some CTW systems were installed in the 1970s and this number increased notably towards the 1980s. The first CTW that was designed for the main purpose of treating wastewater, was constructed in 1901 in the United States of America (Kadlec and Wallace 2009). It was only during the 1990s where people really started to realise the potential of CTWs and that these systems can be used to treat different types of wastewater. Initially, CTWs were mostly used to treat municipal wastewater, until it was discovered to be just as effective at treating other wastewater types such as stormwater and agricultural wastewater (Murphy and Cooper 2010).

During the last two centuries, people have increasingly started using cities and urban areas as their primary living areas. It is estimated that human populations living in urban areas globally increased from 10% to more than 50% since the 1900's (Birch and Wachter 2011). By 2050, it is possible that the percentage of people living in urban areas might increase up to 80% (Grimm et al. 2008). Along with this, the human population has grown well over 7.7 billion people. Due to the constantly growing human population and people moving to metropolitan areas, it is becoming increasingly important for cities to provide resources and ecosystem services (Everard 2017). Costanza et al. (1997) mentioned that the need for sustainability and utilising environmentally friendly practices as far as possible, is also becoming increasingly more important. Constructed treatment wetlands are being implemented as ecosystem services to benefit rural and urban infrastructure (Mitsch and Gosselink 2007).

### **2.2: Components of CTWs**

According to Gelt (1997), wetlands may be comprised of a complex mass of organic and inorganic materials and these ecosystems allow for water and gas to interexchange creating diverse communities of microorganisms. These microorganisms can break down or transform various substances, which also form part of the purification process occurring in wetlands (Davis 1995).

Sunlight, soil, wind, plants and animals also assist in the transformation processes. Wetland vegetation is specially adapted to water-saturated conditions. According to Kadlec and Wallace (2009), wetland vegetation has adapted to overcome the periodic shortage of other chemical elements, such as oxygen, needed by most plants to survive in saturated conditions. For this reason, wetlands are very productive biological systems. Wetland fauna include mammals, birds, reptiles, amphibians, fish and invertebrates (Kadlec and Wallace 2008).

### **2.3: Wetland hydrology**

According to Cherry (2011), all wetlands have one common characteristic, which is the presence of surface- or near-surface water, whether it is permanent or periodically saturated. This includes natural-, constructed-, freshwater- and saltwater wetlands. These saturated conditions are a perfect habitat for the dense growth of vascular plants that prefer these conditions. Microenvironments, in turn, are created by wetland vegetation for the attachment of microbial communities. Microbial processes are enhanced as plants die back during winter times, because the litter provides a source of nitrogen, phosphorus and carbon (Davis 1995).

Davis (1995) mentioned that CTWs normally receive water from two sources namely, surface water from precipitation and the source of wastewater it was initially built to treat. Hydrology is a very important aspect in CTWs, since it alone can determine a wetland's success or failure. Wastewater need enough contact time with plants and substrates to be treated properly. This should be managed without overloading a treatment wetland with wastewater, since it might cause clogging (Kadlec and Wallace 2009).

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There are several important things to keep in mind concerning the hydrology of wetlands. Wetlands usually have a larger surface area and shallower depths (Tiner 1999). For this reason, Davis (1995) argued that the functionality of wetlands is easily altered by precipitation, either through rainfall or snowfall and evapotranspiration, combined water loss through evaporation from the water surface and transpiration from plants.

### **2.4: Wetland substrates**

Certain soils, sand, gravel and rocks with different sizes and textures are used to construct artificial wetlands. Organic material such as compost is also regularly used to construct CTWs. As a CTW matures, sediments and litter accumulate because of all the input of waste (Chen et al. 2018).

Chen et al. (2018) also mentioned that sediments, substrates and litter of wetlands are very important in the sense that it provides a habitat for numerous living organisms. The substrate in wetlands also restricts water flow and influences water flow paths. It all depends on the permeability of the substrates found in wetlands (Cherry 2011). The substrates of wetlands also allow chemical and biological transformation to take place within it. Many contaminants are trapped and stored in substrates. Important biological reactions rely on carbon to take place. The volume of organic matter (which is a source of carbon) in wetlands are increased by the accumulation of litter (Kadlec and Knight 1996). Microbial attachment and material exchange also rely on enough volumes of organic matter within wetland compositions. Flooding of wetlands cause soils and other substrates to become physically and chemically altered (Cherry 2011). According to Davis (1995), atmospheric gasses in pore spaces are replaced by water in saturated substrates and the available oxygen is consumed by microbial metabolism. Substrates then become anoxic, because oxygen consumption occurs faster than the replacement of oxygen by diffusion from the atmosphere. This process is important for the removal of pollutants such as nitrogen and metals (Davis 1995).

Chen et al. (2018) mentioned that it is important that these components must have the perfect balance between enough restrictions of wastewater flow, since this might affect the treatment efficiency.

### 2.5: Types of CTWs

Kadlec and Wallace (2009) mentioned that CTW systems in modern times, have been designed to emphasise specific characteristics of specific wetland ecosystems to improve treatment efficiency. The flow of CTWs are divided into two main types known as surface flow- and subsurface flow wetlands. The latter can be divided into horizontal subsurface flow- and vertical subsurface flow wetlands. Hybrid systems also exist that incorporate these two main types. CTWs can also be combined with other manmade filter systems (Davis 1995).

#### 2.5.1: Surface flow (SF) wetland

Surface flow wetlands (SF) have a shallow basin and usually water flow occurs horizontally (Fig. 2.1). The water level and waterflow are mainly above the substrate surface. Macrophytes in these systems can be rooted (Davis 1995; Fonder and Headley 2013) and grow higher than the water surface. Floating vegetation and emergent plants can also be found within SF wetlands. These wetlands nearly resemble natural marshes (Kadlec and Wallace 2008).

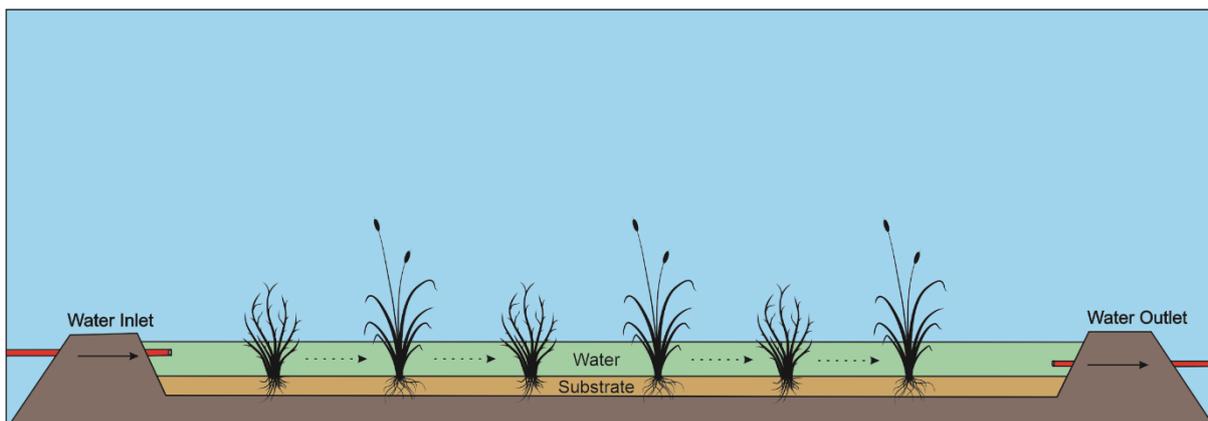
Along with water treatment, Kadlec and Knight (1996) mentioned that SF wetlands additionally provide a habitat for a variety of wildlife species, such as mammals, birds, reptiles, fish, amphibians, as well as insects and molluscs. Usually these systems are aerobic near the surface and anaerobic within the deeper water and substrate. Surface flow wetlands are primarily used to treat storm water, mine drainage and agricultural runoff. Surface flow wetlands that treat mine drainage can also be called aerobic wetlands. In most scenarios, SF wetlands are used for treatment of effluent coming from secondary or tertiary wastewater treatment processes (Kadlec and Wallace 2008). Primary treatment processes refer to sedimentation of solid waste within water. Secondary wastewater treatment processes involve the removal of nutrients and remaining solids through bacterial composition. Tertiary wastewater treatment processes are designed to achieve higher effluent quality than secondary treatment processes. These processes are described by Ramalho (2012), and include organic removal, suspended solid removal, reverse osmosis, ion exchange, chemical

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oxidation, electro dialysis, inorganic compound- and nutrient removal and sonozone wastewater purification processes.

Surface flow wetlands can deal with pulse flow and changing water levels, which make these wetlands very effective for the treatment of agricultural-, urban-, and industrial stormwater. Other types of wastewater treatable by SF wetlands include leachate, groundwater and mine water (Kadlec and Wallace 2008).

Surface flow wetlands can operate and be utilised in all climate types (Davis 1995). These are simple systems and cost-effective to operate. Surface flow wetlands are simplistic to construct, operate and maintain (Wang et al. 2017). According to Kadlec and Wallace (2009), as well as Fonder and Headley (2013), the only negative setback of these systems is that they need a large area to be effective.



**Figure 2.1:** Diagram of a typical surface flow (SF) wetland adapted from Kadlec and Wallace (2008).

### 2.5.2: Horizontal subsurface flow (HSSF) wetland

Kadlec and Wallace (2008), mentioned that HSSF wetlands can also be referred to as plant-rock filter-, root zone method-, vegetated submerged bed- and microbial rock reed filter systems. Horizontal subsurface flow wetlands have a sealed basin. The water level in such a system is below the substrate surface. Water flow mainly occurs through a sand or gravel bed. In these wetlands the roots of the vegetation usually penetrate the bottom of the sand- or gravel bed (Weerakoon et al. 2018). A typical HSSF wetland (Fig. 2.2) will be comprised of an inlet pipe system, filter media, a clay

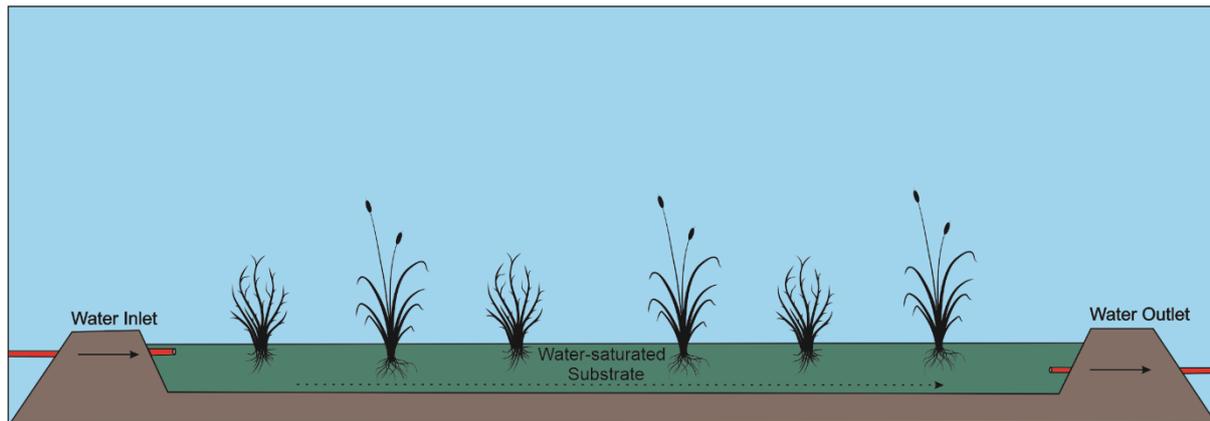
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or synthetic liner, emergent vegetation, berms and an outlet pipe system, which also acts as a mechanism for water control (Kadlec and Wallace 2008).

The risk of humans or animals being exposed to pathogenic organisms are minimalised by the fact that the water is not exposed during the treatment process (Almuktar et al. 2018). When HSSF wetlands are operated in a proper manner, mosquitoes for example, should not be able to breed in these wetlands. The insulation caused by the vegetation enables HSSF to be more effective in colder weather conditions than SF wetlands (Kadlec and Wallace 2008).

Horizontal subsurface flow wetlands are usually utilised as primary water treatment systems preceding either surface water discharge or soil dispersal. Horizontal subsurface flow wetlands are best suited to treat wastewater with moderately uniform flow conditions and low solid concentrations, as the substrate usually constrains hydraulic flow (Kadlec and Wallace 2008).

Kadlec and Wallace (2008) also mentioned that HSSF wetlands are more effective in reducing pest problems and are also effective at reducing the odour of foul-smelling wastewaters. The water surfaces of HSSF wetlands are usually not fully exposed to air (Bentley 2003). The porous medium seen in HSSF wetlands has a greater surface area for the attachment of waste particles. For this reason, HSSF wetlands are designed to be smaller to treat the same volume of wastewater as a larger SF wetland (Austin and Yu 2016). According to Kadlec and Wallace (2008), this is also why HSSF wetlands are more expensive to construct compared to SF wetlands. Horizontal subsurface flow wetlands are also more difficult to maintain and repair. Therefore, they are also more prone to having clogging problems and are mostly used to treat wastewater flowing in at a slow pace. Considering this, the operation costs of HSSF wetlands are still far less expensive than many other treatment options (Davis 1995; Kadlec and Wallace 2008).



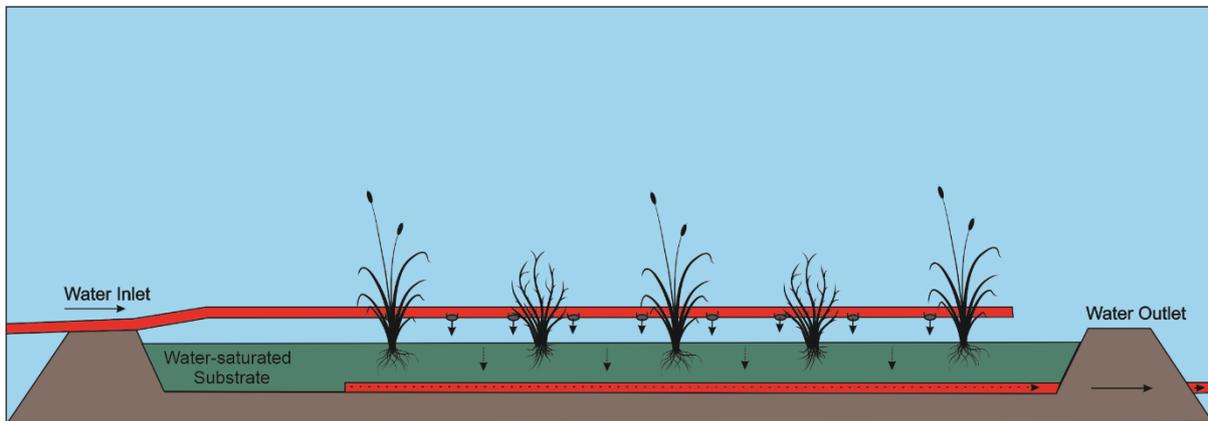
**Figure 2.2:** Diagram of a typical horizontal subsurface flow (HSSF) wetland adapted from Kadlec and Wallace (2008).

### 2.5.3: Vertical subsurface flow (VSSF) wetland

Vertical subsurface flow wetlands usually consist of a sand or gravel bed with wetland vegetation. Water is distributed across the sand or gravel bed (Fig. 2.3). As the water percolates through the plant root zone, it is treated (Kadlec and Wallace 2008).

In most cases, surface flooding or pulse loading are implemented in VSSF wetlands. This technique simply entails the wetland being fed large volumes of wastewater once, at certain times. Vertical subsurface flow wetlands were first constructed in Europe for the main reason of enhancing oxygen transfer, which in turn leads to the production of a nitrified effluent (Kadlec and Wallace 2008). Mander (2016) suggested that VSSF wetlands can be combined with SF or HSSF wetlands to create nitrification-denitrification systems.

Vertical subsurface flow wetlands are effective at oxidising ammonia and this has resulted in these systems being implemented for wastewater treatment with higher ammonia levels than domestic- or municipal wastewater (Vymazal 2006).



**Figure 2.3:** Diagram of a typical vertical subsurface flow (VSSF) wetland adapted from Kadlec and Wallace (2008).

### 2.5.4: Hybrid systems

Hybrid systems exist where certain wastewater requires more complex treatment. In hybrid systems both surface flow (SF) - and subsurface flow (SSF) wetlands can be built in one treatment area. Different CTWs are built in what is called “cells”. Hybrid systems are used to treat wastewater such as mine drainage and ammonia concentrations from agricultural establishments. These types of wastewater require both aerobic- and anaerobic reactions to take place (Davis 1995).

The complexity and design choices of CTWs depend on the region where it will be constructed. Factors that might influence the type of CTW built in a specific area, includes available capital, legislation on wastewater treatment of the area, and the nature of the site, as well as climatic changes (Nivala et al. 2013; Sanchez et al. 2016).

## 2.6: Functions and values of CTWs

CTWs have the potential to provide significant benefits to human communities. The benefits of these wetlands are not restricted to developed countries, but can be adapted to simpler, more affordable systems in developing countries that are just as effective. The idea of constructing a wetland today, entails finding the most effective way to treat waste- and storm water, while also doing so in the most cost-effective way possible. Worldwide CTWs are used successfully to improve water quality (Kadlec and Knight 1996).

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According to Austin and Yu (2016), CTWs differ from natural wetlands in the sense that it is usually designed to enhance natural processes that take place in natural wetlands. This involves modification of the vegetation, soil and microbial and aquatic communities in CTWs.

Inherent processes occurring in wetlands are called wetland functions. Wetland values are perceived as the attributes that wetlands can provide to benefit humanity. An ideal wetland would be able to provide most or all the wetland functions and -values. Davis (1995) compiled a list of all the functions and values that wetlands can provide, which are:

- Education and research
- Passive recreation
- Water quality improvement
- Cycling of nutrients and other materials
- Active recreation
- Flood storage and desynchronisation of storm rainfall and surface runoff
- Habitat for wildlife and plants
- Aesthetic and landscape enrichment

Constructed treatment wetlands utilise natural energy, i.e. solar energy, kinetic energy, microorganisms and wetland plants, as far as possible, depending on the complexity of the system and the level of contamination of the water it is treating. This makes CTWs extremely environmentally friendly, compared to other complex manmade systems. Ultimately CTWs enable and simplify water reuse and -recycling (Huang et al. 2000).

Constructed treatment wetlands are a very cost-effective way to treat wastewater. Minimal to zero fossil fuel energy is usually needed depending on the treatment objectives, because of the ongoing processes in a CTW system (Kadlec and Wallace 2008). It can be very simple to construct and easy to maintain. CTWs only require maintenance periodically, compared to more continuous maintenance of other treatment options. CTWs can also adjust to changes in flow of water into the system (Hammer and Bastian 1989).

Eutrophication is a troubling issue in many parts of the world. CTWs can effectively remove macronutrients such as nitrogen and phosphorus from the water to prevent

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the process of eutrophication from occurring when water reaches natural systems (Huang et al. 2000).

### 2.7: CTWs as a habitat

CTWs can provide habitats to numerous species of large animals. Invertebrates are known to fragment detritus and consume organic matter, which contributes to the water treatment process (Anderson and Sedell 1979). Insect larvae occurring in wetlands usually consume large volumes of organic material during the larval developmental stages. Other zooplankton in wetlands such as crustaceans, rotifers and ciliates, also contribute to breaking down excess organic material (Wolters 2000).

### 2.8: Microbial populations in CTWs

Wetland plant roots often create oxic-anoxic conditions, which facilitates simultaneous activity of aerobic and anaerobic microbial communities (Bodelier and Dedysch 2013). Some bacteria are facultative anaerobes, which means that they can function with- or without the presence of oxygen (Davis 1995). Wetland systems are highly productive due to input of nutrients and fast recycling caused by active aerobes and anaerobes (Bodelier and Dedysch 2013).

Some microbial populations can easily adjust to new environmental conditions to survive. They are all, however restricted by extreme changes. When microorganisms are provided with enough energy-containing materials, the populations can expand very rapidly (Rajan et al. 2019). In the case of environmental conditions that change to such an extent that microorganisms find it unfavourable, they can become dormant for several years. These microorganisms stay dormant, until conditions become favourable, which is when they will emerge and reproduce again (Hilton 1993). Pesticides and heavy metals are among the toxic substances that can affect the microbial communities in CTWs. The volumes of these substances must be controlled to prevent any long-term detrimental effects (Davis 1995).

Weller et al. (2015) mentioned that excess nutrients that are deposited from storm water runoff like nitrogen and phosphorous are also taken up by macrophytes, as well as microorganisms and absorbed by soils. Wetland microbes can process organic nitrogen into inorganic forms i.e. nitrogen ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4$ ). These

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inorganic forms are used by plants to grow, while the rest of the organic nitrogen is converted into gasses that escape into the atmosphere (Ghaly and Ramakrishnan 2015).

### **2.9: Zooplankton in CTWs**

Zooplankton plays an essential role in any healthy aquatic ecosystem, including wetlands. According to Eivers et al. (2017), zooplankton communities in agricultural CTWs remain unstudied. Large zooplankton communities can also limit the production rates of algae, which can cause a decline in efficiency for CTWs that rely on algae to function properly e.g. wastewater treatment algal ponds (Schlüter et al. 1987; Montemezzani et al. 2015). These pond systems are usually intensively managed and controlled and differ from agricultural CTWs. The habitat preferences, feeding guilds and community composition of zooplankton could contribute to improve the effectiveness of agricultural CTWs. This knowledge could assist in wetland design, reduction of pathogens and controlling high nutrient levels (Eivers et al. 2017).

### **2.10: Wetland vegetation**

Vegetation in wetlands, which are primarily macrophytes, play an important role since it effects the system in several ways. According to Bentley (2003), the three vegetation types normally used in CTWs include submerged plants (grow below the water surface), emergent plants (rooted in the soil with stems and leaves growing above the water level) and floating plants (float on water surface with roots in the water column). The services that macrophytes can provide to CTWs are very beneficial and a very crucial component of these systems (Thullen et al. 2005). Wetland vegetation alters hydrology by slowing the flow paths of water as it flows through the wetland (Brix 1997). Wetland vegetation also restricts sunlight and wind from the system.

Plants in general are important for the success of CTWs. This includes vascular plants and algae. Davis (1995) mentioned that the dissolved oxygen content of wetland water is increased by algal photosynthesis. Vascular plants play a huge role in treating wastewater and can also reduce the flow speed of water, which allows for suspended metals to settle in a wetland. Along with this, the reduction in water velocity also provides time for nitrogen removal to take place (Brix 1997).

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Vascular plants die-back creating litter and restricting channelised flow of water. Vascular plants stabilise substrates and their root- and stem systems are used by microorganisms for attachment (Davis 1995). Oxygenated microsites are created by oxygen coming from subsurface plant structures into the substrate (Stefanakis 2018). Trace elements, i.e. carbon and nutrients are taken up by vascular plants, to be used as building blocks for plant tissues. Gasses are also transferred between sediments and the atmosphere through the presence of the vascular plants (Finlayson and Woodroffe 1996). The most effective plants to be used for CTWs are emergent plants, of which the roots grow in the substrate. The stems and leaves of these plants usually emerge from the water surface. Cattails, reeds, bulrushes and some broad-leaved species are emergent plants that are usually used as treatment vegetation in CTWs (Davis 1995). Emergent macrophytes are very effective in removing nitrogen from wetlands. Brisson and Chazarenc (2009) noted that wetlands without, or with minimal macrophyte populations are less effective at nitrogen removal.

According to Davis (1995), vegetation traps suspended solids because of the low waterflow, causing the suspended solids to settle out. Other pollutants become inactive and are taken up by plants or transformed to forms that are less soluble. Microorganisms also flourish in the habitats that wetland plants provide. Microorganisms play a role in recycling nutrients in wetlands (Denny 1985). The processing capacity of wetlands can be affected by the presence of microorganisms in the substrate, since these organisms change the redox (reduction/oxidation) conditions of the substrate. Organic- and inorganic substances are also transformed into harmless- or insoluble substances by microbial activities (Davis 1995).

### **2.11: Processes at work in CTWs**

The tempo of water flow is lowered by vegetation as soon as it enters the wetland. This is where the cleaning process starts. Pollutants in wetlands can be removed via physical-, chemical- and biological processes.

CTWs remove pathogens from water through three main processes: sedimentation, filtration and absorption (Sundaravadivel and Vigneswaran 2001; Ibekwe et al. 2016). Sedimentation is the process of gravitational settling of solids and constituent contaminants. Filtration occurs when particles get stuck in the substrate as the water

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passes through it. Absorption occurs because of inter-particle attractive forces. Sorption is important in wetlands for various reasons and contributes to pollutant removal (Kadlec and Wallace 2008).

Gasses created by various processes in wetlands, are released into the atmosphere, these include methane ( $\text{CH}_4$ ), nitrous oxide ( $\text{N}_2\text{O}$ ), dinitrogen, hydrogen sulphide ( $\text{H}_2\text{S}$ ) and ammonia ( $\text{NH}_3$ ). Atmospheric carbon dioxide is taken in by wetlands to be used by vegetation for photosynthesis (Kadlec and Wallace 2008).

According to Bavor and Schulz (1993), nitrogen will be taken up by macrophytes in a mineralised state and incorporated into plant biomass. Accumulated nitrogen is released into the system during a die-back period. Nutrients are taken up by plants to aid in metabolism. Trace chemicals are also taken up in the root zone. These trace chemicals can be stored or may even be released into the atmosphere as gasses (Reddy et al. 2010). Volatile organic contaminants can also be taken up by plants in CTWs and removed through volatilisation. Daily transpiration is positively related to mineral adsorption and could be used as an index of the water purification capability of plants (Kadlec and Wallace 2008).

Metals such as zinc (Zn) and copper (Cu) occur in soluble or particulate associated forms and the distribution in these forms are determined by physio-chemical processes (Jackson et al. 2014). Metals accumulate in a bed matrix through adsorption and complexation with organic material. Metals are also reduced through direct uptake by wetland plants. However, over-accumulation may kill the plants (Kadlec and Wallace 2008).

According to Celenza (2000), substrates may remove wastewater constituents by ion exchange/non-specific adsorption, specific adsorption/precipitation and complexation, making the system more complex. During ion exchange an ion from a solution is exchanged for a similarly charged ion attached to an immobile solid particle. This is a reversible chemical process (Gupta et al. 2009). Non-specific adsorption occurs when ions are held together by electrostatic forces (Yong 2001). Sposito (1984) defined specific adsorption as: "The effects of inner-sphere surface complexation of the ions in solution by the surface functional groups associated with the soil fractions".

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Microbial pollutant removal occurs during the activities of bacteria or other microorganisms. These organisms are mainly attached to solid surfaces and only a few of them are free-floating (Kadlec and Wallace 2008). Kadlec and Wallace (2008) also mentioned that photo-degradation occurs when sunlight degrades or converts substances in water. Ultraviolet radiation can also kill many microorganisms such as viruses and pathogenic bacteria.

Biodegradable organic matter is removed by decomposing microorganisms in the water. Biodegradation occurs when dissolved organic matter is carried into biofilms that are attached on submerged plant stems, root systems and surrounding soil, or media by the diffusion process. Decomposers such as bacteria, fungi, and actinomycetes are active in any wetland, breaking down dissolved and particulate organic material to carbon dioxide and water (Cecen and Aktas 2011).

### **2.12: How CTWs improve water quality**

Various mechanisms are responsible for the water treatment processes occurring in wetlands. To begin with, suspended particulate matter can easily settle in wetlands due to the restricted water flow. Wetlands create good conditions for pathogens to be preyed upon by certain microinvertebrates, such as bacterivorous zooplankton, i.e. bdelloid rotifers (Davis 1995; Schallenberg et al. 2005). CTWs also create a space for pathogens to naturally die off. Wetlands create conditions where water has contact with substrates for long periods at a time and this allows chemical precipitation and filtration to take place effectively. Plants, sediment, substrate and litter provide surfaces for ion exchange and absorption to take place (Davis 1995).

### **2.13: Seasonal operation of CTWs**

Providing that the water does not freeze, physical processes such as deposition are not dependent on temperature to take place (Davis 1995). The substrate of a wetland facilitates many reactions to take place within it. Microbial activity and decomposition within the substrate prevent subsurface layers from freezing by building up enough heat (Celenza 2000). When the top surface layer of the water does freeze, the treatment process is able to continue. When this happens, the water level can be raised to create space for water to flow under the ice (Kadlec and Wallace 2008). The

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water level can later be dropped again. This is just to prevent the wetland from freezing throughout the whole profile. When temperatures drop to a point where water can freeze, CTWs might need to be enlarged slightly, since microbial decomposition rates decrease as water temperature decreases (Wallace et al. 2000). This can especially be important in the case of agricultural wetlands, since the organic wastes of these establishments are broken down by microbial activities. Some CTW systems have pre-treatment units for such occasions. An example would be the Sunrise Potato Storage LTD farm in Alliston, Ontario, Canada. A study was conducted by Bosak et al. (2016), where they studied the performance of a CTW (treating potato wash water) and the pre-treatment system on the farm. Results indicated optimal treatment during spring months, for both pre-treatment and the wetland itself. Enlarging the pre-treatment system improved performance during spring and summer months, as a result of seasonal loading of the wetland during these seasons.

During colder periods, wastewater is stored in these units and is treated during warmer periods (Vymazal 2010). Even though microbial activity rates are faster during the warmer months, the volume of water flowing through some wetlands can also be higher during these times because of spring rains, snow melting and higher groundwater tables. This can cause inadequate treatment due to reduced retention time. During summer months wetlands can lose large volumes of water due to evapotranspiration (Davis 1995).

According to Weller et al. (2015), hot, arid climates may affect the functionality of CTWs in several ways. Extreme temperatures during the warmer summer months can potentially constrain microbial- and plant activities, while microbial- and plant activities may increase during warm winters. Hot and arid climates might affect the functionality of certain macrophyte species. Thullen et al. (2008), argued that decomposition rates of senesced plant material might be increased by high temperatures, which might reduce nutrient accumulation in dead plant material. Transpiration and evaporation are increased by high temperatures and shortages in low vapor pressure, which may also affect the hydrology of wetland systems (Ong et al. 1995; Sanchez et al. 2016). In general, these wetlands become oxygen poor systems due to the long periods of saturation during the growing season (Davis 1995).

### **2.14: CTW limitations**

According to Campbell and Ogden (1999), CTWs usually require a larger area of land to be equally effective, compared to other wastewater treatment options. This means that the use of CTWs depends on land availability and -affordability to be economically viable. The effectiveness of CTWs might be influenced by seasons of the year and arising environmental conditions. This makes CTWs less consistent in their performance compared to other treatment options. Toxic chemicals can negatively influence the performance of CTWs, since these systems are made up of biological components (Kadlec and Wallace 2008). CTWs always need a certain volume of water to stay effective, because complete drought could destroy the entire system. Since the world is still quite new to CTWs as a wastewater treatment option, environmentalists and scientists are still in the process of perfecting the designs of CTWs for different areas and -purposes. Little is also known on the performance or environmental impact of CTWs over a longer period (Davis 1995).

## CHAPTER 3: MATERIAL AND METHODS

### 3.1: Study area and field laboratory

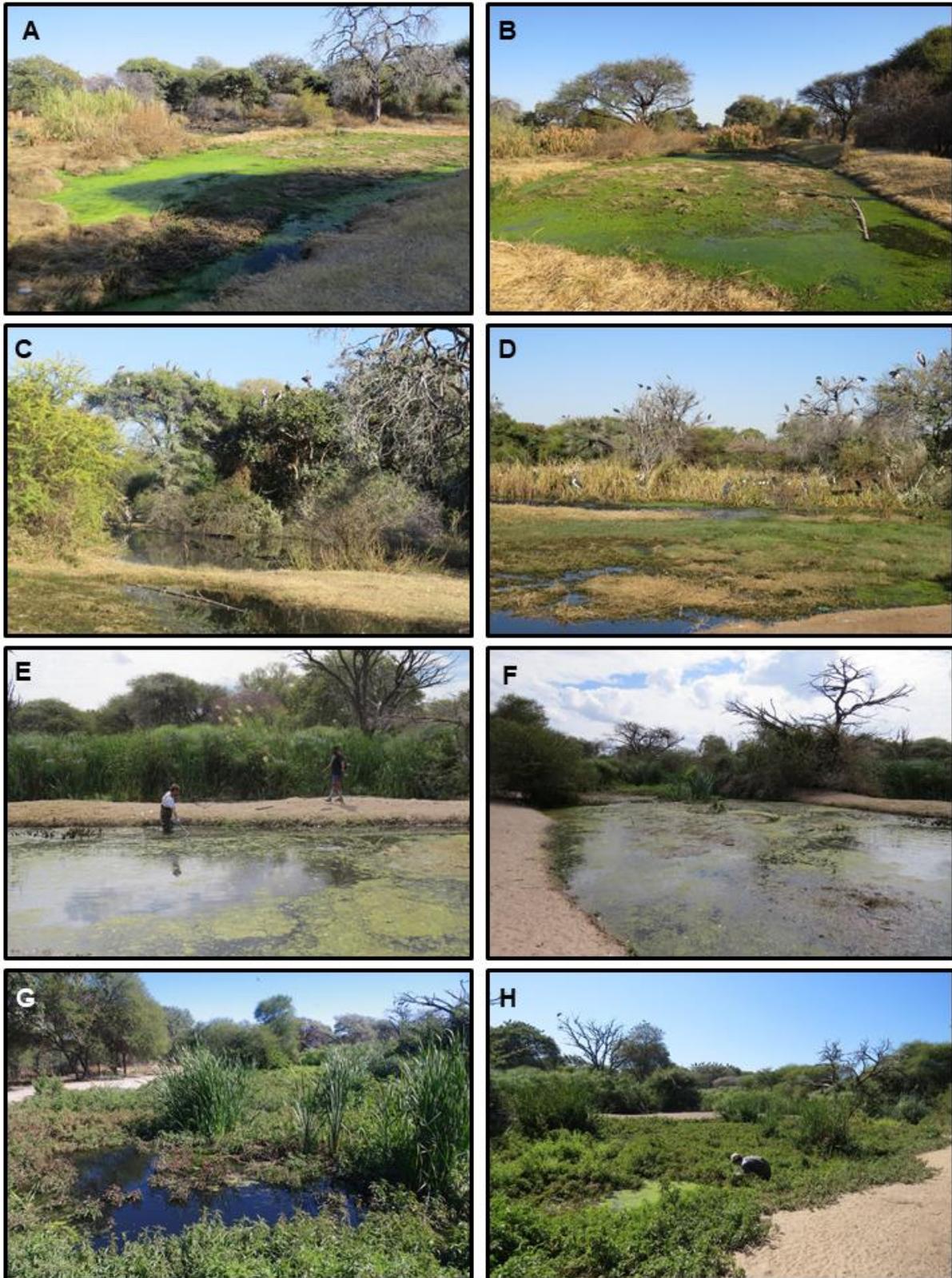
The study was conducted in the Krovovango CTW (18°26'00.0" S; 21°53'38.2" E), which is a CTW treating the wastewater of Krovovango – a commercial crocodile farm next to the Okavango River, in Samochima village, northern Botswana.

Krovovango was opened by the then Minister of Environmental Affairs, Mr. Kitso Mokaila, in April 2005. According to McMillan and McCraig (2019), the unemployment rate of Botswana is around 20%, which is very high. This establishment provides both permanent and temporary employment for several people from the Samochima village, which is a good initiative for the community, as work is not easy to find in the northern parts of Ngamiland.

The Krovovango wetland has been operating since 2012 (period of effectiveness) and has in the recent years become an additional habitat- for a variety of mostly birdlife. Figure 3.1 illustrates how the Krovovango CTW matured from 2014 to 2018. The Krovovango CTW can be described as a simplified vertical surface flow wetland with no outflow, which is a unique scenario. The wetland is approximately 50x50m in size and it is covered by *Cyperus capensis*, *Phragmites australis*, *Typha capensis* and *Wolffia arrhiza*.

The current study took place during July-August 2017 and June-July 2018 at the Leseding Research Camp, located on the premises of the Krovovango crocodile farm. The Leseding Research Camp was constructed by members of the Aquatic Ecology Research Group from the Department of Zoology and Entomology, University of the Free State. The camp is sufficiently equipped to conduct research, comprising tented accommodation, a kitchen, ablution facilities and a field laboratory. Laboratory equipment such as chemicals for specimen preservation and microscopes were transported from Bloemfontein.

### CHAPTER 3: MATERIAL AND METHODS



**Figure 3.1:** Images indicating the maturation of the Krovovango CTW from 2014 to 2018. **A-**  
**B:** 2014; **C-D:** 2015; **E-F:** 2017; **G-H:** 2018.

### CHAPTER 3: MATERIAL AND METHODS

The crocodile farm contains approximately 11 500 crocodiles (11 453 counted in 2017). Hatchlings are moved to a hot house after emerging from their eggs. From here crocodiles are sorted into different dams according to size. Crocodile size on the farm is directly related to feeding circumstances. Smaller and weaker hatchlings are at a disadvantage from the start and grow slower than their relatives due to competition for food. Larger and stronger juveniles outcompete weaker individuals through bullying and by eating more in the same time span. The largest nonbreeding crocodile, Sam (Fig. 3.2A) is nearly 5m in length and is named after the village Samochima. Amos is another huge crocodile, not used for breeding purposes and was named after the foreman of Krovovango. Sam and Amos form part of the educational and tourist section of the farm. The crocodiles on the farm are bred for their skins to be sold, mainly to clients overseas.

Larger crocodiles (Figs. 3.2B, C) do not get fed during the winter months. The success of the crocodile farm largely relies on the continuous growth of the crocodiles. This means that the Krovovango staff members need to feed the juvenile crocodiles (Fig. 3.2D) throughout the year. For this to occur, the water of the dams is heated in the winter since reptiles tend to feed much less during the winter months.

The basic layout of the crocodile dams is presented in Figure 3.3. The Krovovango Crocodile Farm consists of 2 separate dams for Sam and Amos, 3 larger dams containing the breeding crocodiles, 1 dam with crocodiles almost ready for breeding (breeding stock), 28 dams containing younger crocodiles and 2 hot houses containing the juveniles.

### CHAPTER 3: MATERIAL AND METHODS



**Figure 3.2:** Photos of **A:** The largest nonbreeding crocodile, Sam; **B:** The breeding crocodiles; **C:** The breeding stock and **D:** The younger crocodiles from the Krokovango Crocodile Farm.

CHAPTER 3: MATERIAL AND METHODS

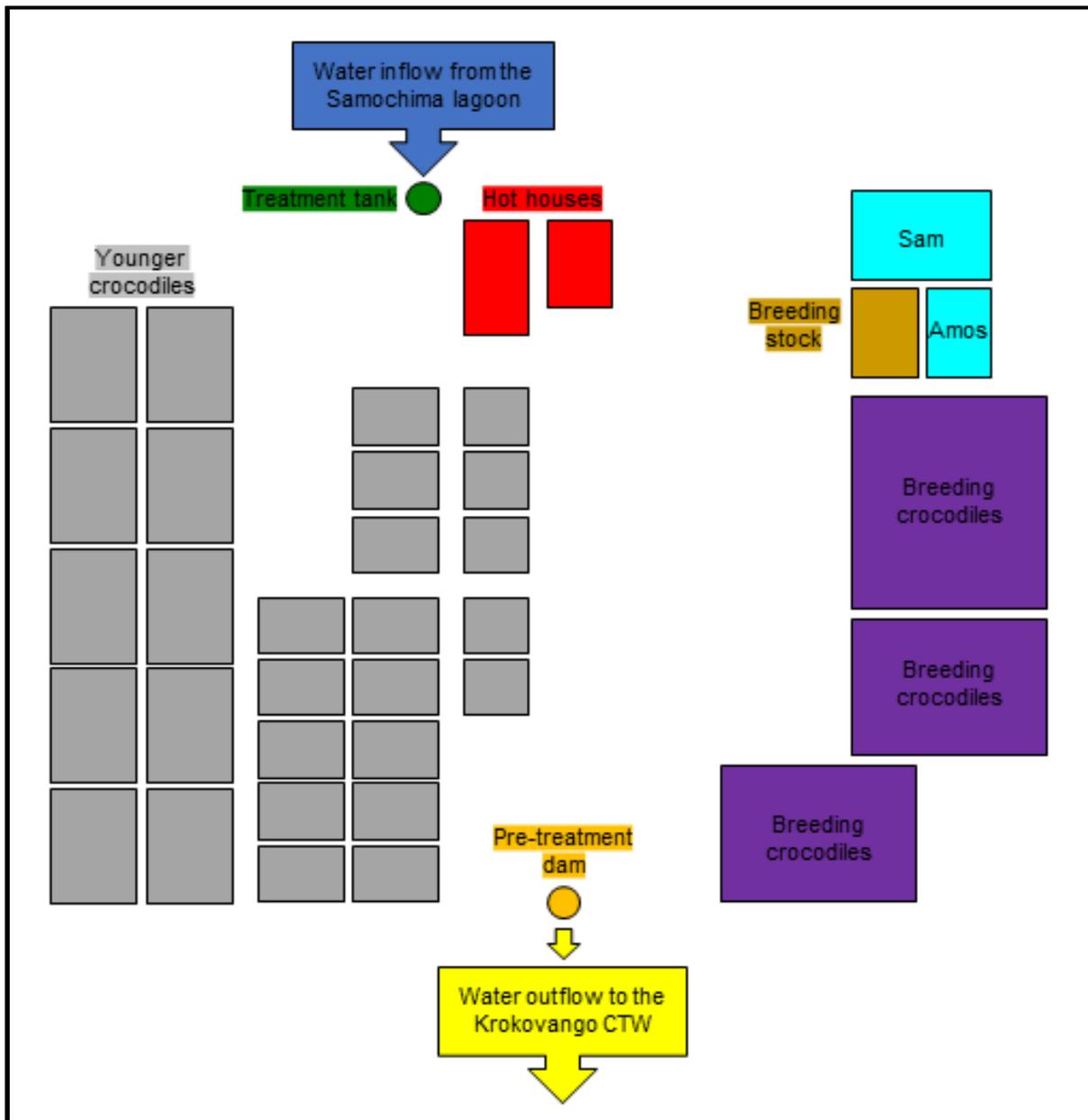


Figure 3.3: Illustrating the basic layout of the crocodile dams of Krovovango Crocodile Farm.

### 3.2: Water supply

Water is pumped by a submerged pump from the Samochima lagoon, through a filter system, into a treatment tank (Fig. 3.3), where effective microorganisms (EM) are added to aid in crocodile digestion and general health. Effective microorganisms are mixed cultures of naturally occurring organisms used to increase microbial activities. This ultimately speeds up the purification process within the CTW. From this point water flows through the Krovovango pipe system by means of gravitation, which is very effective. From the treatment tank, water is distributed to all the crocodile dams.

Cement canals channel all overflowing- or wastewater to a single pre-treatment cement dam (Fig. 3.3), where larger and heavy organic material settle to the bottom. From the pre-treatment cement dam, the nutrient enriched wastewater flows straight to the Krovovango CTW, where the process of water treatment occurs naturally. There is no fixed schedule for the volume of water that enters the CTW over time. From the very beginning of this operation, the water drained from the crocodile dams was rich in nutrients, so pumping the waste back into the lagoon could never have been an option.

### 3.3: Collection and identification of plankton material and other organisms

The present study focussed on microinvertebrates collected from the Krovovango CTW, but birds, insects and plants were also observed and identified. Phytoplankton and zooplankton were collected during July-August 2017 and June-July 2018. The Krovovango CTW was visited at 11:00-13:00 for each day of sampling. Hand-held plankton nets with mesh sizes of 25  $\mu\text{m}$  and 50  $\mu\text{m}$  were swooped horizontally on all levels of the water column and in close proximity to the macrophytes for 25-30 minutes on each sampling trip (Fig. 3.4A). On each sampling trip 2 litres of concentrated plankton samples were collected. Samples were taken to the field laboratory where live observations were made using a compound Nikon Eclipse E200 microscope and a dissection Zeiss Stemi 305 microscope (Fig. 3.4B). Light photomicrographs were taken by the author in the field laboratory. Scanning electron photomicrographs were taken by the author at the Department of Microscopy, University of the Free State. The

### CHAPTER 3: MATERIAL AND METHODS

other photos in this dissertation were taken by members of the Aquatic Ecology group, University of the Free State, South Africa.

Species descriptions were made using applicable literature and these sources were given in the results sections for each taxon. This was done to keep taxa information unified, since it is a crucial part of the result literature.

Zooplankton individuals were counted to 300 individuals for each day of sampling. It is generally suggested to count 300 specimens per plankton sample (Schiebel and Hemleben 2017). The 2 litre concentrated plankton samples ensured that there were more than enough specimens to work with every day. Phytoplankton were transferred from original samples onto microscope slides. The number of individuals within species of phytoplankton per microscope slide were counted to a maximum of 10. Single colonies and -filaments were not counted per individual cell but were considered as one individual. A scale of 1-10 (0-1: very low; 2-6: medium; 7-10: very high) was used to depict abundance. Phytoplankton was not included in statistical analysis, since a different counting technique was used due to their high abundance.

Digital photos were taken using a Zeiss Axiocam ERc 5s on the dissection microscope and a Nikon DS-Fi1 0.7X DMX attached to the compound microscope. Selected specimens of zooplankton were preserved in 70% ethanol, and 4%- and 10% BNF solutions for later processing at the laboratory of the Department of Zoology and Entomology, Bloemfontein, South Africa. All specimens collected were identified using applicable literature listed in Chapter 4 for each taxon. Plankton specimens were identified to genus level and when it was possible, to species level. On each sampling trip birds were observed for one hour. Birds and vegetation were identified to species level and insect larvae that ended up in plankton samples were only identified to order or family level.



**Figure 3.4:** Photos of **A:** Plankton collection from the Krovovango CTW; **B:** Live observations made on the microscope and **C:** Krovovango CTW water quality measurement.

### **3.4: Preparing specimens for the Scanning Electron Microscope (SEM)**

Samples stored in ethanol and BNF were prepared using standard SEM techniques for analysis and photos. Samples fixed in 4% BNF and 10% BNF were washed in water for 15 minutes. Specimens were dehydrated through ethanol concentrations of 30%, 50%, 70%, 80%, 90%, 96% and 100%, critical point dried, mounted on a SEM stub, coated with gold and examined using a JOEL WINSEM JSM 6400 SEM.

### **3.5: Water quality measurements**

Physical water quality parameters were measured by using a portable Hanna HI 9828 multiparameter (Fig. 3.4C). The multiparameter was calibrated before every sampling trip according to the instructions by the manufacturer. For each day of plankton sampling water temperature, dissolved oxygen concentration, pH and conductivity were also measured.

### **3.6: Statistical analysis**

One-way analysis of variance (ANOVA) was used on R to determine whether there were any statistically significant differences between the mean abundance of the four taxa of Protozoa, Rotifera, Cladocera and Copepoda, found.

Abundances of the four taxa were compared over the two-year study period using R. A generalised linear model with two effects (taxon and year) and the interaction were used. It was assumed the data followed a Poisson distribution for discrete counts, because normal distribution could not be obtained. Along with this, a Tukey Honest Significance Difference (HSD) post hoc test was used for pairwise comparisons.

## **CHAPTER 4: RESULTS OF KROKOVANGO CTW – TAXA COLLECTED AND WATER QUALITY**

### **4.1: Phytoplankton**

Phytoplankton are free-floating, single-celled organisms occurring in streams, lakes and oceans. Locomotion occurs passively relying on water currents or actively through flagella. These organisms produce their own food from sunlight through photosynthesis. Phytoplankton can be found almost everywhere where water and sunlight are present (Pal and Choudhury 2014). Thousands of different types of phytoplankton are known, and several main categories are used to classify commonly occurring groups. The genera described below are from the Phyla Chlorophyta, Euglenophyta, Bacillariophyta and Cyanophyta found in the Krokovango-CTW, Botswana. Identification of the material collected during the current survey was based on morphological comparison with known records from published literature, including Komárek and Fott (1983), Fritsch (1948), Schnepf et al. (1980), Komárek and Anagnostidis (1989), Round et al. (1990), Round and Bukhtiyarova (1996), Lange-Bertalot (2001), Wehr and Sheath (2002), Lowe (2003), Marin et al. (2003), Pasztaleniec and Poniewozik (2004), Siver and Baskette (2004), Janse van Vuuren et al. (2006), Luo et al. (2006), Lee (2008), Alves-da-Silva and de Mattos Bicudo (2009), Bellinger and Sigee (2010), Kannan and Lenca (2012), Sili et al. (2012), Novais et al. (2015), Burliga and Kociolek (2016), Cabanelas et al. (2016), Clausen (2017), Watanabe and Lewis (2017), Osório et al. (2018) as well as Guiry and Guiry (2019).

#### **4.1.1: Phylum: Chlorophyta**

Chlorophyta, or green algae are unicellular plants that can either be filamentous or colonial. Furthermore, Chlorophyta can be found swimming, floating or attached to various surfaces in water bodies. The chloroplast of Chlorophyta contain either chlorophyll *a* or *-b* (Clausen 2017), which are responsible for the characteristic green colour. In freshwater, green algae range from unicellular microscopic organisms to large globular colonies and filamentous growths (Bellinger and Sigee 2010).

## CHAPTER 4: RESULTS OF KROKOVANGO CTW - PHYTOPLANKTON

### ***Chlorococcum* sp. Meneghini, 1842**

**(Table 4.1; Fig. 4.1A)**

#### Characteristics:

Vegetative cells solitary or in temporary groups of indefinite form. Cells ellipsoidal to spherical which vary in size (Fig. 4.1A). Cell walls smooth. Parietal chloroplast with or without a peripheral opening and with one or more pyrenoids (Watanabe and Lewis 2017). Cells uninucleate, or multinucleate just prior to zoosporogenesis. Reproduction by zoospores, aplanospores, or isogametes. Motile cells have two equal flagella and remain ellipsoidal for a time after motility ceases (Guiry and Guiry 2019).

#### Ecology:

According to Watanabe and Lewis (2017), specimens of this free-living genus is cosmopolitan. Guiry and Guiry (2019) reported this genus from habitats such as hot springs in Central Asia and soils collected in Antarctica.

### ***Chlorogonium* sp. Ehrenberg, 1836**

**(Table 4.1; Fig. 4.1B)**

#### Characteristics:

Cells are unicellular and elongated, spindle-shaped and pointed at one or both poles (Fig. 4.1B). Two apically inserted, equal flagella occur at the anterior end that are usually shorter than the length of the cell (about half the length the cell) (Bellinger and Sigeo 2010). The single, large, chloroplast is parietal, and may be with or without pyrenoids, depending on the species. In most species, an eyespot (embedded in the chloroplast) is prominent at the cell anterior. Two or more contractile vacuoles generally positioned in both the anterior and posterior halves of the cell but may be distributed in the anterior portion of the cell. The cell wall is delicate. Asexual reproduction is by zoospore formation, but they can also reproduce sexually. Species are distinguished by the presence or absence of pyrenoids (Janse van Vuuren et al. 2006).

### Ecology:

*Chlorogonium* is thought to be a cosmopolitan species (Guiry and Guiry 2019). It is a widespread freshwater species and often occurs in small temporary pools rich in humus, or pools containing decaying leaves, eutrophic lakes and soil. According to Janse van Vuuren et al. (2006), blooms are rarely formed.

### ***Cosmarium* sp. Corda ex Ralfs, 1848**

**(Table 4.1; Fig. 4.1C)**

### Characteristics:

Very diverse morphology (Osório et al. 2018). Cells solitary, tiny to large with shallow to deep median constriction (isthmus) (Fig. 4.1C). Semi-cells round, reniform, pyramidate, quadrate with entire or undulate margin; subcircular to elongate-oval (biradiate) in apical view. Triradiate forms known to occur in certain cultures. Cell wall smooth with scattered pores or ornamented with small or large granules, emarginate verrucae, round or triangular pits, or short spinules. Central and marginal ornamentation different or identical. Mucilaginous sheath, secreted through cylindrical cell wall pores, often surrounds cell. Chloroplasts one to several per semi-cell, axial or parietal, each with one to several pyrenoids per chloroplast. Nucleus in isthmus (Guiry and Guiry 2019).

### Ecology:

*Cosmarium* is a cosmopolitan species found in lentic environments (Osório et al. 2018).

### ***Micractinium* sp. Fresenius, 1858**

**(Table 4.1; Fig. 4.1D)**

### Characteristics:

Colonies triangular to pyramidal forming clusters of 4 (mostly) to 64 cells. Cells spherical or broadly ellipsoid (Fig. 4.1D). Each cell contains 1-8 (up to 18 in some cases) long, tapering spines that may be ten times longer than cell. Spines are clearly distinguishable, thin and needle-like (Schnepf et al. 1980; Janse van Vuuren et al.

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2006). Spines can lock with spines of other cells to form large, compound colonies of up to 128. A single, cup-shaped chloroplast with one pyrenoid is present inside each cell. Cell walls are thin and smooth. Autospores form during asexual reproduction (Janse van Vuuren et al. 2006). Formation of bristles can be triggered by substances produced by certain grazers, such as the rotifer *Brachionus* (Luo et al. 2006).

### Ecology:

Organisms of the genus *Micractinium* frequently (sometimes abundantly) occurs in stagnant waters of ponds and lakes, but also commonly occurs in rivers. These free-floating colonies particularly favour eutrophic waters as was described by Janse van Vuuren et al. (2006).

### ***Pandorina* sp. Bory, 1824**

**(Table 4.1; Fig. 4.1E)**

### Characteristics:

Colonies ovoid or ellipsoidal, containing 8 - 32 cells arranged radially in a gelatinous matrix (Fig. 4.1E). Cells cup-shaped, ovoid or spherical, each with two equal flagella (Bellinger and Sigee 2010), a stigma, two contractile vacuoles at base of flagella, and a massive cup-shaped chloroplast with one basal or multiple pyrenoid (species dependent). Stigmata in anterior cells larger than in posterior cells. In asexual reproduction, autocolony fragmentation occurs with each cell dividing completely to form a plakea. The plakea inverts to become a daughter colony. Sexual reproduction occurs when cells escape from the gelatinous matrix and become isogametes. Walled aplanozygotes are formed. Single biflagellate gone cells arise upon germination of the zygotes (Guiry and Guiry 2019).

### Ecology:

*Pandorina* spp. occur in freshwater pools, ponds and ditches. Kumar (2015) also found that organisms of this genus flourishes in the early rainy season. Coleman (1959) considered *Pandorina* as a cosmopolitan freshwater species.

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### ***Scenedesmus* spp. Meyen, 1829**

(Table 4.1; Fig. 4.1F)

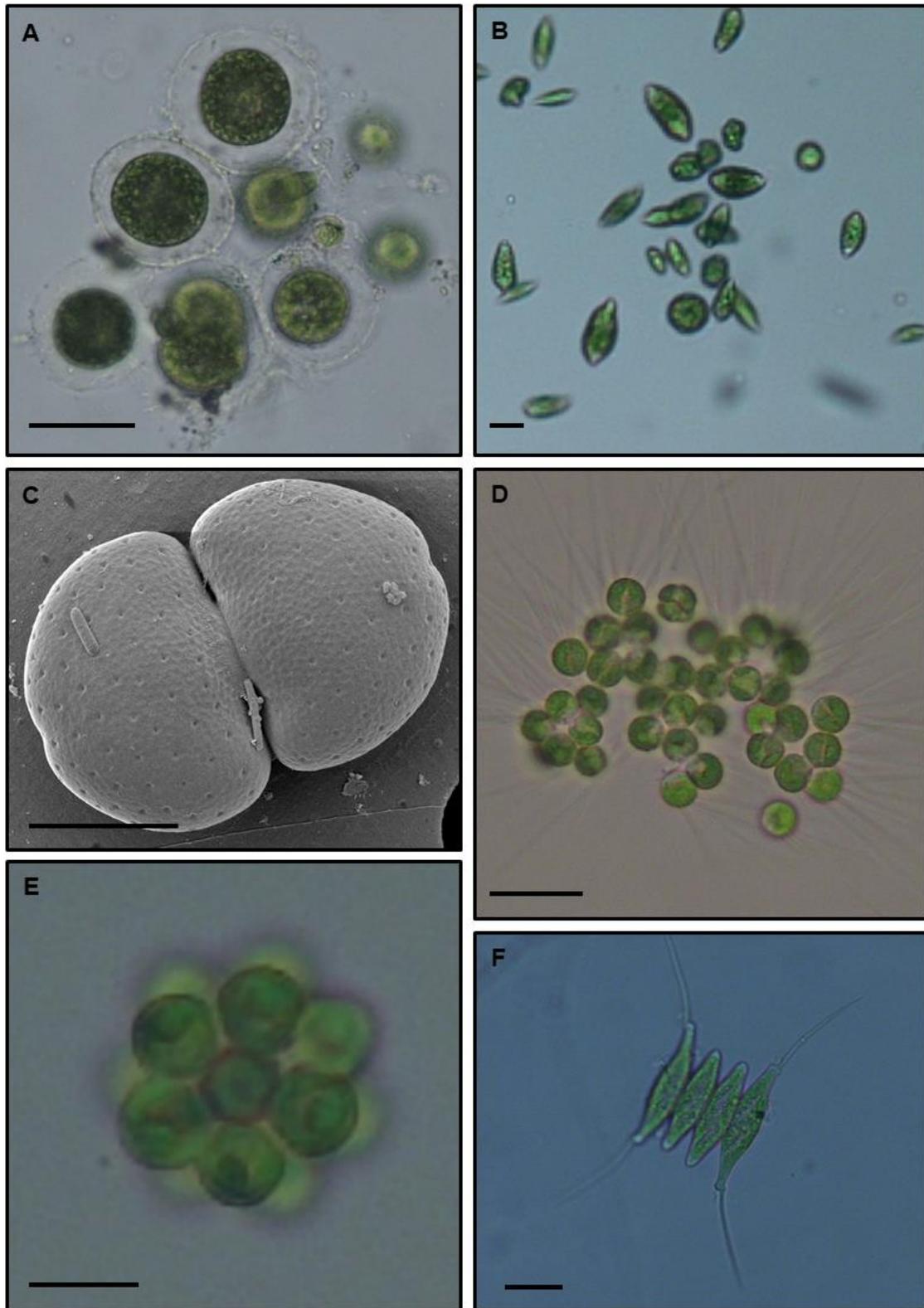
#### Characteristics:

According to Komárek and Fott (1983), the thalli are single celled or colonial, forming 2- to 32-celled, but usually 4 (Fig. 4.1F) or 8-celled coenobia; surrounding mucilaginous matrix present or absent. Cells arranged linearly, alternating in 2-3 rows, touching with the lateral walls or in subpolar region only. Cells nearly spherical to ellipsoidal, elongate or fusiform to elongate fusiform; cell poles capitate, obtuse, acute or long tapering. Cell wall with hemicellulosic and sporopolleninic layer, usually smooth, granulations or dents are visible as ribs under light microscopy. Cells spineless, with sporopolleninic spines. Proteinaceous bristles present. Cells uninucleate; chloroplast single and parietal with single pyrenoid (Guiry and Guiry 2019).

#### Ecology:

An et al. (1999) noted that *Scenedesmus* species are commonly found in fresh- and brackish water, particularly flourishing under nutrient-rich conditions. *Scenedesmus* species are pollution tolerant and are often used as pollution indicators (Brettum and Andersen 2005; Phinyo et al. 2017).

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**Figure 4.1:** Light (A, B, D, E and F) and scanning electron (C) photomicrographs of **A:** *Chlorococcum* sp.; **B:** *Chlorogonium* sp.; **C:** *Cosmarium* sp.; **D:** *Micractinium* sp.; **E:** *Pandorina* sp. and **F:** *Scenedesmus* sp. collected from Krokovango CTW, Botswana. Scale bars: **A, C, D, E:** 5  $\mu\text{m}$ ; **B, F:** 10  $\mu\text{m}$ .

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### ***Stigeoclonium* sp. Kützing, 1843**

**(Table 4.1; Fig. 4.2A)**

#### Characteristics:

Phytoplankton with variously developed prostrate and erect systems of uniseriate filaments (Fig. 4.2A). Erect filaments alternately, oppositely, or dichotomously branched. Branches whorled or irregular, with tips pointed, narrowly obtuse or bearing a multicellular hyaline hair (Fritsch 1948). Prostrate filaments form creeping or rhizoidal system, occasionally aggregated into pseudoparenchymatous disc. Cells uninucleate, thick or thin-walled, cylindrical or swollen, each with a single parietal chloroplast and one to several pyrenoids. Asexual reproduction occurs by quadriflagellate zoospores of two sizes, micro- and macrozoospores. Sexual reproduction occurs isogamous, by biflagellate-orquadriflagellate gametes (Guiry and Guiry 2019).

#### Ecology:

*Stigeoclonium* species commonly occurs in freshwater systems and can grow on different kinds of surfaces (Guiry and Guiry 2019). *Stigeoclonium* species are known to occur over a wide range of nutrient concentrations (Francke and den Oude 1983).

### ***Pediastrum* sp. Meyen, 1829**

**(Table 4.1; Figs. 4.2B, C)**

#### Characteristics:

All *Pediastrum* species form plate-like colonies (Figs. 4.2B, C) (Bellinger and Sigeo 2010). Thalli colonial, comprised of 4-64 (up to 128) celled coenobia, arranged in a flat, circular to oval plate, one cell thick. If 16 or more cells occur, cells tend to be in concentric rings; each ring with definite number of cells; disc continuous or with perforations between cells. Ring development is dependent upon zoospore behaviour at coenobial formation. Cell with highly variable shape, interior cells typically polyhedral with four to many sides; peripheral cells similar or with one or two horn-like processes. Peripheral cells often with bristles not found in central cells. Cell walls

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smooth, finely reticulate, or highly granulate. Cells multinucleate; diffuse chloroplast single and parietal; pyrenoid one or more per cell (Guiry and Guiry 2019).

### Ecology:

*Pediastrum* species can be found in many kinds of fresh water, but commonly occur in nutrient rich lakes, ponds, and slow flowing rivers (Bellinger and Sigeo 2010). In oligotrophic waters *Pediastrum* species occur in the littoral zone (Komárek and Fott 1983).

### Remarks:

Two *Pediastrum* species were identified from Krokovango CTW, Botswana. Pasztaleniec and Poniewozik (2004) identified the following morphological characteristics to distinguish the two *Pediastrum* species from each other:

*Pediastrum boryanum* (Turpin) Menegh. var. *tongicome* Reinsch (Fig. 4.2B) have coenobia without holes, 32 celled 39.5-170 µm in diameter. Marginal cells with two long processes slightly curved, 6.2 - 12.3 µm wide, 10 - 15 µm long. Inner cells: 7.4 - 34 × 4.9 - 22.5 µm. Cell walls scarcely granular. Incision shallow.

*Pediastrum duplex* (Meyen) var. *duplex* (Fig. 4.2C) have circular coenobia, 44.5 - 222.2 µm in diameter with regularly distributed holes, with 32 - 64 cells arranged concentrically. Incision between processi V-shaped. Marginal cells 7.4 - 19.8 × 10 - 24.7 µm, Inner cells almost quadratic, their diameter 10 - 12.5 µm. Cell walls with very fine sculpture visible under immersion.

### ***Volvox* sp. Linnaeus, 1758**

**(Table 4.1; Fig. 4.2D)**

### Characteristics:

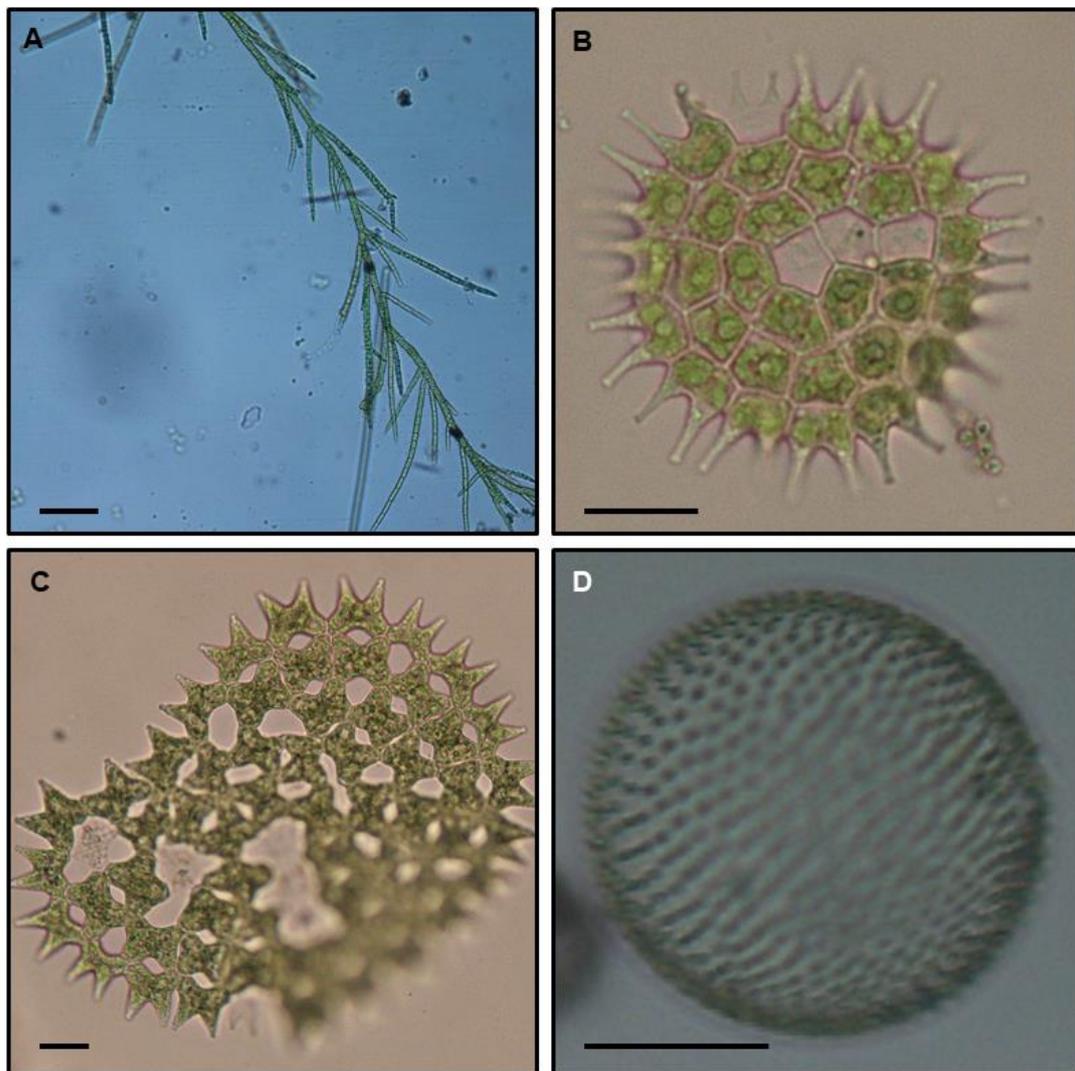
According to Bellinger and Sigeo (2010), the chloroplasts are green, and the storage product is starch. Colonies spherical, subspherical, ellipsoidal or ovoid, containing 500- 50 000 cells arranged at the periphery of a gelatinous matrix, forming a hollow sphere (Fig. 4.2D). Two to 50 large reproductive cells (gonidia) situated in posterior 1/2 to 2/3 of colony. Each cell enclosed by gelatinous sheath which is distinct or

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confluent (species dependent). Somatic cells spherical, ovoid, or star-shaped, each with two equal flagella, a stigma, two contractile vacuoles at base of flagella, and a cup-shaped chloroplast with single pyrenoid. Cytoplasmic strands between cells are thick, thin, or absent and this is species dependent (Guiry and Guiry 2019).

##### Ecology:

Kirk (2005) mentioned that *Volvox* is cosmopolitan in freshwater. Within a few days after the warm rains of early summer months, *Volvox* can occur in great numbers within water bodies and pools formed by the rain (Kirk 2005).



**Figure 4.2:** Light photomicrographs of **A:** *Stigeoclonium* sp.; **B:** *Pediastrum boryanum* var. *tongicome*; **C:** *Pediastrum duplex* var. *duplex* and **D:** *Volvox* sp. collected from Krokovango CTW, Botswana. Scale bars: **A, B, C:** 10  $\mu$ m; **D:** 50  $\mu$ m.

### 4.1.2: Phylum: Euglenophyta

Wolowski (2002) mentioned that Euglenoids have about 44 free-living genera and more than 800 species. Most are colourless, unicellular organisms with phagotrophic or heterotrophic methods of nutrition (Conforti 1998). About 1/3 are green and phototrophic. Euglenoids are largely solitary and motile flagellates, though a few species form non-motile, branched colonies when in the actively growing condition. Cells are ovoid to spindle-shaped and most are naked with plasma membrane surrounded by an often-prominent pellicle with helical striations. Certain forms are radially symmetrical (e.g. *Gyropaigne kosmos* Skuja 1939), others bilaterally symmetrical. The pellicle ranges from being very flexible to semi- or completely rigid and usually striated (Wolowski 2002).

#### ***Euglena* sp. Ehrenberg, 1830**

**(Table 4.1; Fig. 4.3A)**

#### Characteristics:

*Euglena* spp. (Fig. 4.3A) have bright green chloroplasts (Bellinger and Sigeo 2010). This is not an exception. Janse van Vuuren et al. (2006) reported that colourless cells also occur within the genus. The euglenids are part of an extremely diverse lineage of flagellate protists (Marin et al. 2003). The cells are normally motile, using flagella or through metaboly, the ability of the body to change shape. Approximately 1/3 of the euglenoids photosynthesise and are classed within algae. The rest are usually placed amongst the Protozoa, being colourless and either heterotrophic or phagotrophic. Pigmentation is closely related in photosynthetic organisms, carotenoid pigments not always present, routinely causing colours to vary from fresh green to yellow-brown (Bellinger and Sigeo 2010).

#### Ecology:

Bellinger and Sigeo (2010) noted that euglenoids are more abundant in environments with decaying organic matter, given the heterotrophic nature of these organisms.

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### ***Phacus* spp. Dujarin 1841**

**(Table 4.1; Figs. 4.3B, C)**

#### Characteristics:

Cells are solitary, oval or ellipsoidal, pear- or spindle-shaped, often twisted along the longitudinal axis and very flattened (Fig. 4.3B). Cells are round at the anterior end, with a straight or slightly bent tail of variable length (depending on the species) at the posterior end (Bellinger and Sigeo 2010). Most species have discoidal chloroplasts that are small, numerous without pyrenoids, or large and discoidal with pyrenoids. An eyespot may or may not be present. Like other freshwater euglenoids, *Phacus* cells have contractile vacuoles. Cells are free-swimming by means of a single emergent flagellum which arises from an anterior invagination. As in *Euglena* species, a second shorter flagellum is non-emergent. The pellical strips are, unlike that of *Euglena*, longitudinal (stretching from pole to pole) and rigid (for this reason cells cannot change shape). Reproduction takes place by the longitudinal division of cells (Janse van Vuuren et al. 2006).

#### Ecology:

According to Pereira (2003), *Phacus* species are widespread and found in similar habitats to *Euglena* spp., except for stagnant environments, where they are not as common. This free-living genus occurs in freshwater habitats such as swamps, ditches, ponds and lakes. Cells are common in nutrient-enriched water (Janse van Vuuren et al. 2006).

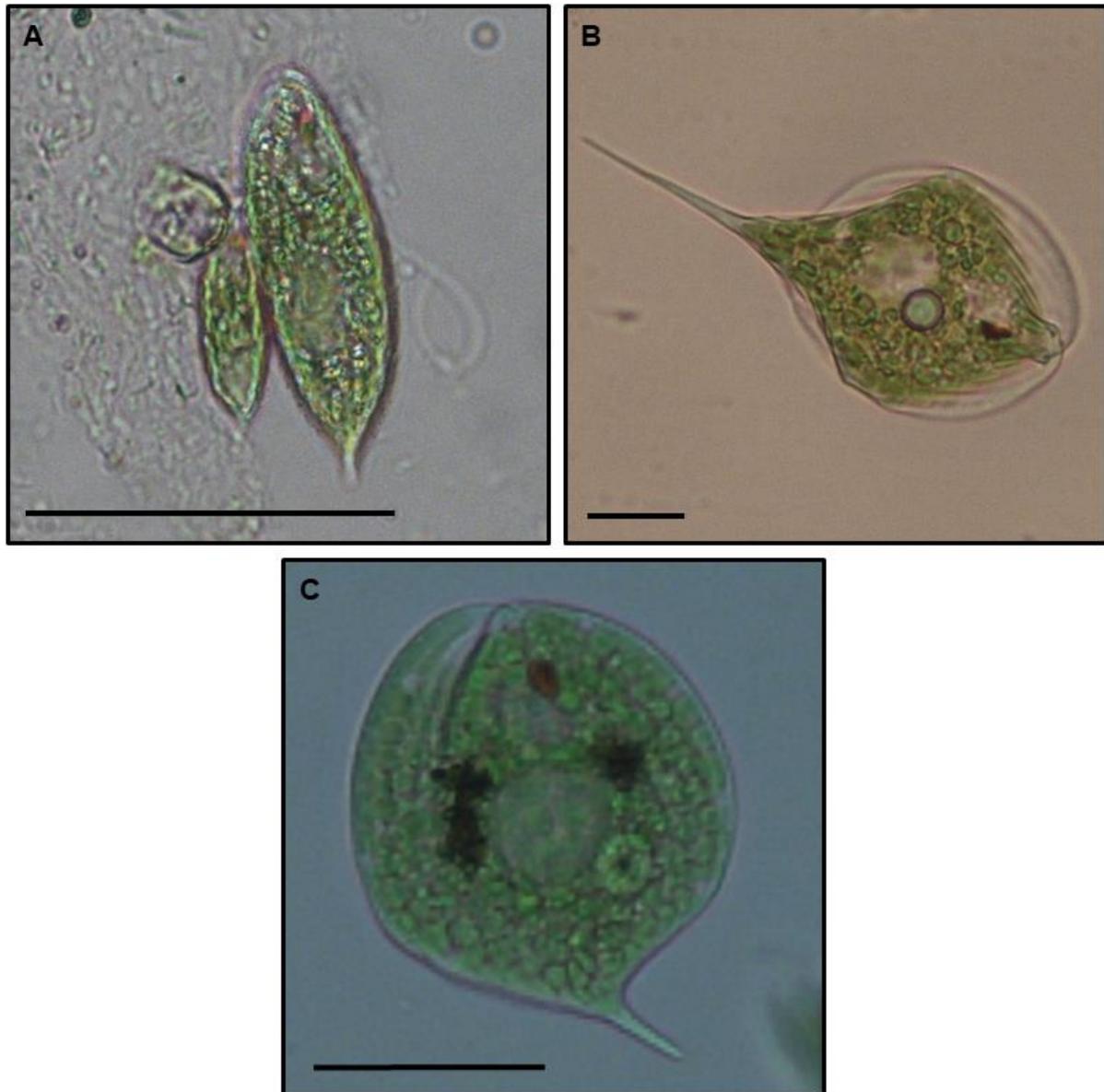
#### Remarks:

Two *Phacus* species were identified from Krokovango CTW, Botswana. Alves-da-Silva and de Mattos Bicudo (2009) identified the following morphological differences to distinguish the two *Phacus* species from each other.

*Phacus longicauda* (Ehrenberg) Dujarin var. *longicauda* (Fig. 4.3B) have half a cell torsion at the base of the caudis. Transverse striae in between the longitudinal ones are absent in var. *longicauda*.

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*Phacus longicauda* (Ehrenber) var. *tortus* Lemmermann (Fig. 4.3C) have a complete torsion at the cell mid region. Transverse striae in between the longitudinal ones are present in var. *tortus*.



**Figure 4.3:** Light photomicrographs of **A:** *Euglena* sp.; **B:** *Phacus longicauda* var. *longicauda* and **C:** *Phacus longicauda* var. *tortus* collected from Krokovango CTW, Botswana. Scale bars: **A, C:** 5  $\mu$ m; **B:** 10  $\mu$ m.

### 4.1.3: Phylum: Bacillariophyta

Diatoms are usually yellow to light brown in colour (Bellinger and Sigeo 2010). Janse van Vuuren et al. (2006) mentioned that all diatoms are microscopic and can occur as single cells, colonies or filaments. Some diatoms lack chlorophyll, making them heterotrophs, but most are autotrophic. Chloroplast containing diatoms use Chlorophyll *a*, Chlorophyll *c* and fucoxanthin. Chrysolaminarin and oil droplets are the storage products in diatoms (Kelly and Haworth 2002), with the oil droplets aiding in buoyancy (Janse van Vuuren et al. 2006). Diatoms have distinctive siliceous cell walls. The pattern (pores and striations) of the frustule is used to classify many different species.

There are two major morphological groups in which diatoms are classified, known as pennate and centric diatoms. Centric diatoms are unable to move around willingly, while some pennate diatoms have raphe, which are slit-like structures along the surface of both valves (Bellinger and Sigeo 2010). The raphe secretes polysaccharides, which enables the diatom to move through water columns with gliding movements when in contact with the substrate. Diatoms primarily reproduce asexually through cell division and lack flagella (except for male gametes). They are commonly found in freshwater- and marine environments where they can be free-floating or attached to substrates and form the base of aquatic food webs. They are the largest contributors to global primary production (Kelly and Haworth 2002). Fritz et al. (2011) mentioned that over time, diatoms have also proven to be powerful ecological tools to monitor environmental conditions, and past conditions by examining fossils of diatoms in various sediments.

#### ***Achnanthisdium* sp. Lowe, 1839**

**(Table 4.1; Fig. 4.4A)**

#### Characteristics:

The valve view of *Achnanthisdium* species usually have linear, linear-elliptical or linear-lanceolate frustules; while the girdle view has undulate-rectangular and bent frustules. In valve view (Fig. 4.4A) the cells are symmetrical, but if viewed from the side, this is not the case (Wehr and Sheath 2002). One of the valves (called the raphe valve) has

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true raphe and the other valve (called the rapheless valve) has pseudoraphe. The end of the tips broadly round to sub-capitate. The pseudoraphe and raphe are in a central position. Striations are parallel to nearly radial and each valve may have a different density and patterns. Each cell may have two to many chloroplasts present (Janse van Vuuren et al. 2006).

### Ecology:

According to Janse van Vuuren et al. (2006), *Achnanthydium* species only occurs in freshwater to brackish environments. Normally *Achnanthydium* spp. occur in oligo- to mesotrophic environmental conditions; however, some taxa also flourish in eutrophic and organic-rich waters. Organisms of this genus mostly occurs in benthic forms attached to various substrates with mucilage stalks (Novais et al. 2015).

### Remarks:

Identifying species of this genus under the compound light microscope can be difficult due to their small size. Smaller species within the genus of *Achnanthydium* are often confused with smaller *Navicula* species. *Achnanthydium* species can be distinguished from *Navicula* species by looking at the girdle view, which shows a bent valve in the case of *Achnanthydium*. The valve of *Navicula* is straight (Janse van Vuuren et al. 2006).

### ***Amphipleura* sp. Kützing, 1844**

**(Table 4.1; Fig. 4.4B)**

### Characteristics:

*Amphipleura* species may occur as individual cells, or they may be protected in diffuse, gelatinous tubes. The valve outline is linear, linear-lanceolate or spindle-shaped in valve view (Fig. 4.4B). Striae are composed of punctae that are extremely fine (Burliga and Kociolek 2016). A simple, narrow, median rib is evident on the internal valve face, except near the poles. At the poles, the median rib is split into two, forming apparent "needle eyes" in which the raphe is located. The raphe is short compared to other naviculoid genera. Living cells contain one central H-shaped chloroplast (Janse van Vuuren et al. 2006).

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### Ecology:

*Amphipleura* species are not abundant in rivers, but they are widely distributed in sedimental habitats of standing slow-flowing waters. The cells of *Amphipleura* species may occur over a wide pH spectrum and are usually found in alkaline- (predominantly) and calcareous waters (Janse van Vuuren et al. 2006).

### ***Frustulia* sp. Rabenhorst, 1853**

**(Table 4.1; Fig. 4.4C)**

### Characteristics:

Pair of ribs are thick, siliceous and parallel. One pair of ribs is present on each side of the raphe that extend from the central region to both poles. Ribs are mostly fused centrally to the nodule and apically with a helictoglossae (internal thickened structure on the distal end of the raphe) (Round et al. 1990). Valves are linear-lanceolate–rhomboidal in shape (Fig. 4.4C) and perforated with numerous small simple areolae that open externally, closing internally by hymen coverings that are often slightly convex (Lange-Bertalot 2001). The external openings are most often circular or slit-like, and the areolae are aligned along both transverse and apical axes. Although other conditions are possible, most species have T- or Y-shaped raphe fissures on both proximal and distal ends of the raphe. Round et al. (1990) found that some species possess relatively complex folded valvocopulae (girdle bands) that opens to the outside via a slit and to the inside by elongated pores.

### Ecology

*Frustulia* species primarily occurs in freshwater habitats (Round et al. 1990) and according to Siver and Baskette (2004), are often abundant in acidic conditions. Bellinger and Sigee (2010) mentioned that *Frustulia* spp. are benthic.

***Navicula* sp. Bory, 1822**

**(Table 4.1; Fig. 4.4D)**

Characteristics:

According to Round et al. (1990), *Navicula* species are mainly naviculoid (boat-shaped) or cigar-shaped (Fig. 4.4D) and the ends of the cell may be round, acute or capitate. Both valves have a raphe present. Striae are composed of elongate (linear) punctae. These striae are usually not visible when live specimens are examined. In girdle view the cells are rectangular. All species have two chloroplasts, one on each side of the cell when seen in valve view (Janse van Vuuren et al. 2006).

Ecology:

Round (1981) mentioned that *Navicula* species can be found in both marine- and freshwater habitats. In fresh water their habitats range from oligotrophic to eutrophic. The cells can be found among plankton or benthos. In benthos, organisms of this genus may occur as single cells, as colonies with a mucilage tube, or in films on submersed substrates and sediments. Raphe-bearing diatoms like *Navicula* secrete mucilage from these structures to enable the cells to make gliding movements along a substratum. Known to clog filters at water treatment plants (Janse van Vuuren et al. 2006).

***Nitzschia* sp. Hassall, 1845**

**(Table 4.1; Fig. 4.4E)**

Characteristics:

*Nitzschia* species are commonly solitary but may also occur in mucilage tubes (Janse van Vuuren et al. 2006). In valve view the cells are linear, elliptical or sigmoid and each of the valves has a raphe present. Figure 4.4E depicts a *Nitzschia* sp. in oblique view, the raphe is displaced to one margin, but the raphes of each valve are diagonally opposite as described by Lowe (2003). The raphe structure itself is supported by bars (fibulae) that appear as dots along the margin of the valve under light microscopy. The valve is decorated with transverse striae of punctae right across the valve. The

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punctae composing the striae may be fine or coarse. Two large chloroplasts are present (Janse van Vuuren et al. 2006).

### Ecology:

*Nitzschia* is an ecologically versatile genus occurring mostly in the benthos, but planktonic taxa are also present (Janse van Vuuren et al. 2006). Round (1981) determined that *Nitzschia* species can occur in temperate lakes and waters of high alkalinity. It is a popular genus used as an indicator of nutrient enriched conditions in the environment. Some species are also used as indicators of high salinity levels. This genus also occurs in oligotrophic waters, but in these types of environments they do not play a major role (Janse van Vuuren et al. 2006).

### ***Pinnularia* sp. Ehrenberg, 1843**

**(Table 4.1; Fig. 4.4F)**

### Characteristics:

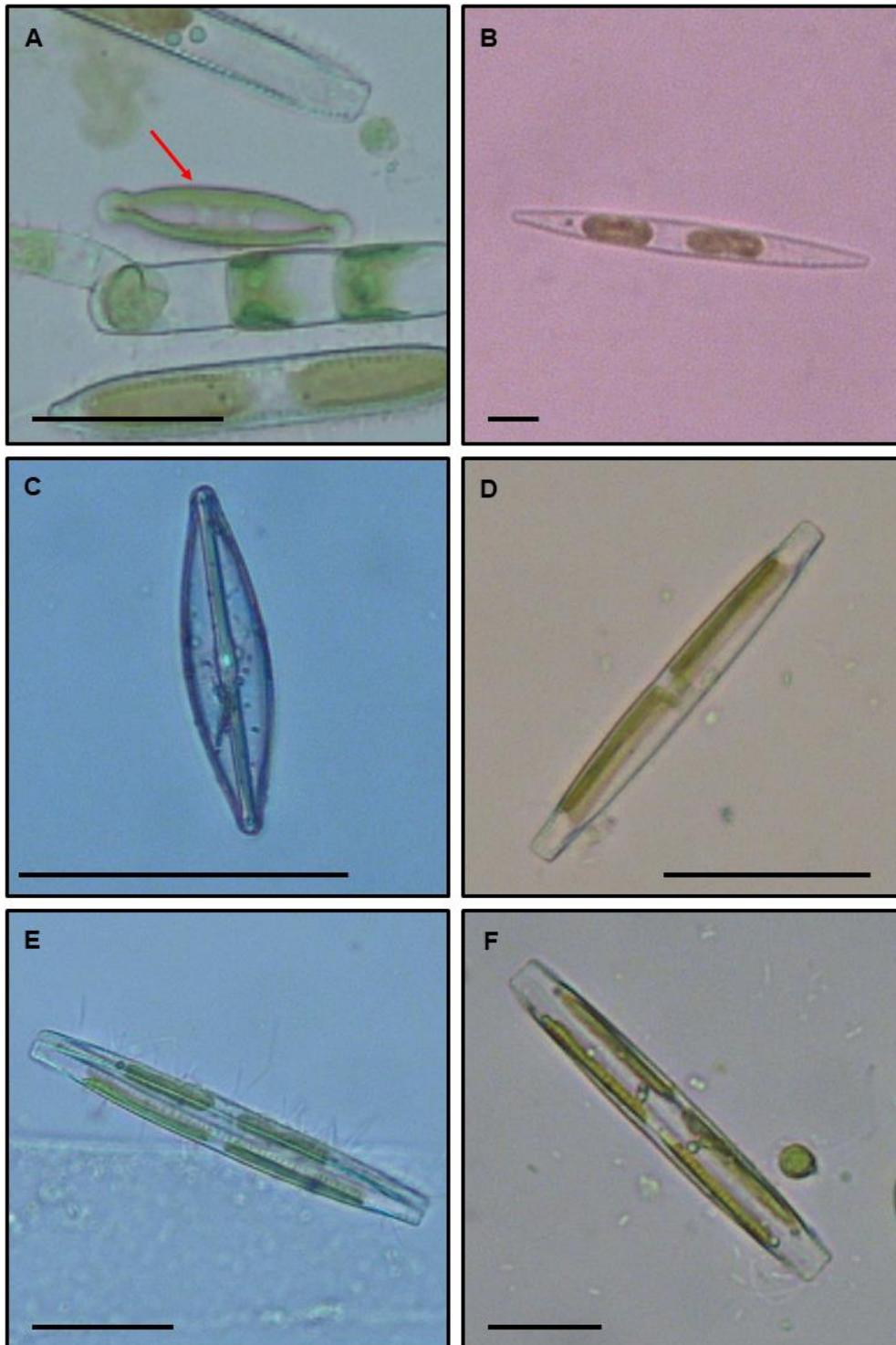
According to Lee (2008), cells are linear to elongate-elliptical in shape with broadly rounded, rostrate or capitate poles (Fig. 4.4F). Frustules are biraphid with the raphe positioned centrally. The raphe fissure may be straight or curved. Centrally the raphe ends usually turn to the same side. There are two plate-like (flattened) chloroplasts. In girdle view the frustules are rectangular with truncate poles (Janse van Vuuren et al. 2006).

### Ecology:

According to Prasad and Nienow (1986), *Pinnularia* is a very common benthic genus found mainly in fresh water where they live on stones and in sediment. Organisms of this genus is often found flourishing among other plankton in various environmental conditions (Janse van Vuuren et al. 2006).

Remarks: Some species within the genus *Pinnularia* are known for their large cell size, for example *P. gigas* can have lengths up to 297  $\mu\text{m}$ . *Pinnularia* have heavy bar-like striae and round ends and they resemble enlarged naviculoid species (Janse van Vuuren et al. 2006).

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**Figure 4.4:** Light photomicrographs of **A:** *Achnantheidium* sp. indicated by the red arrow; **B:** *Amphipleura* sp.; **C:** *Frustulia* sp.; **D:** *Navicula* sp.; **E:** *Nitzschia* sp. and **F:** *Pinnularia* sp. collected from Krovovango CTW, Botswana. Scale bars: **A, D, E:** 5 µm; **B, C, F:** 10 µm.

#### 4.1.4: Phylum: Cyanophyta

Cyanobacteria are also known as blue-green algae. According to Whitton (2002), the colour depends on the relative proportions of the photosynthetic pigments, including chlorophyll (green), phycocyanin (blue), phycoerythrin (red), and sheath pigments (brown). They have bacteria-like cell structure and can occur in colonies or unicellularly. The filaments can be simple or branched. No chloroplasts are present and rather than being distributed in the membrane, the pigment is distributed throughout the cell. This is the primary characteristic separating Cyanobacteria from Chlorophyta (Clausen 2017).

##### ***Anabaena* sp. Bory ex Bornet et Flahault, 1886**

**(Table 4.1; Fig. 4.5A)**

##### Characteristics:

This genus of Cyanobacteria has unbranched filaments or trichomes, which can be curved, coiled or straight. The filaments may occur solitary or in clusters forming a gelatinous mass (Bellinger and Sigee 2010). Filaments uniform in width and consist of cylindrical, spherical or ellipsoidal cells (Fig. 4.5A). Often, the filaments have the appearance of a string of beads. Gas vacuoles in the cells of *Anabaena* spp. assist in buoyancy (Janse van Vuuren et al. 2006).

##### Ecology:

*Anabaena* is a widespread and common fresh- and saltwater genus depicting seasonal abundance in summer months. This genus often grows in association with *Microcystis*. Planktonic forms are more abundant in lakes, ponds, wetlands and ditches (lotic environments), than in slow flowing waters (Bellinger and Sigee 2010). *Anabaena* species rarely occur in faster flowing waters. Other epiphytic species occur on -and within damp soil. *Anabaena* species also occupy submersed substrates forming gelatinous masses (Janse van Vuuren et al. 2006).

***Arthrospira* sp. Stizenberger, 1852**

**(Table 4.1; Fig. 4.5B)**

Characteristics:

The unbranched trichomes of *Arthrospira* species usually form screw-like coils, resulting in a spiral (Fig. 4.5B). Trichomes may be long or short and are usually cylindrical and isopolar (Sili et al. 2012). Apical cells are round, thickened cell walls may occur. They are usually non-motile; can be observed moving through water with gliding-rotating movements. Gas vacuoles are present in most species. In most cases the filaments do not have a mucous sheath and if present, the filaments are very thin and inconspicuous. Species are mainly differentiated by size and by the form of the spiral (Janse van Vuuren et al. 2006).

Ecology:

According to Vonshak and Tomaselli (2007), *Arthrospira* is a cosmopolitan genus occurring in fresh- and saltwater. Habitats include freshwater ponds and lakes and saline inland and coastal waters, among others. In its environment, *Atrthrospira* are often entangled in other algae or free-floating. *Arthrospira* populations can increase rapidly in very mineralised, alkaline and warm waters where other organisms would find it difficult to do so. The filaments can also form slimy benthic clumps which can be olive-green, blue-green or reddish-brown in colour (Janse van Vuuren et al. 2006).

***Merismopedia* sp. Meyen, 1839**

**(Table 4.1; Fig. 4.5C)**

Characteristics:

*Merismopedia* species are distinguished by small round cells forming colonies (Fig. 4.5C) (Janse van Vuuren et al. 2006). According to Komárek and Anagnostidis (1989), the cells only divide through fission and in two directions, forming flat rectangular plate-like colonies. In time the large mother colony fragments into smaller daughter colonies. *Merismopedia* cells occur in multiples of four and are arranged in a single layer in perpendicular rows. The mucilage covering the cells are colourless and structureless (Janse van Vuuren et al. 2006).

## CHAPTER 4: RESULTS OF KROKOVANGO CTW - PHYTOPLANKTON

### Ecology:

*Merismopedia* spp. occur in freshwater habitats which include ponds, lakes, bogs and slow rivers and are also found in marine habitats. Colonies can be free-floating or may form thin films on bottom sediments. Across temperate regions several common species occur in eutrophic and mesotrophic waters (Shen et al. 2018).

### ***Microcystis* sp. Lemmermann, 1907**

**(Table 4.1; Fig. 4.5D)**

### Characteristics:

The cells of *Microcystis* are initially spherical (Fig. 4.5D), but with time grow into irregular or perforated shapes. The cells are arranged in colonies grouped tightly or sparsely and positioned within a fine colourless colonial mucilage (Kannan and Lenca 2012). Colonies of this genus might be microscopic, but larger colonies can be viewed with the naked eye. Thousands of spherical to sub-spherical individual cells without individual mucilage sheaths form colonies of *Microcystis* (Bellinger and Sigeo 2010). The protoplast is a pale-green colour, however when the cells are viewed through a light microscope the protoplast often appears black because of gas vacuoles within the cells (Janse van Vuuren et al. 2006). These gas vacuoles give buoyancy to the colony, allowing it to be more mobile in water to find sunlight (Bellinger and Sigeo 2010). Gas vacuoles can also appear reddish due to the reflection of light (Janse van Vuuren et al. 2006).

### Ecology:

*Microcystis* species form part of phytoplankton, although it may also form granular clumps on bottom substrates (Janse van Vuuren et al. 2006). Organisms of this genus usually colonise enriched lakes, ponds and reservoirs and slow-moving eutrophic rivers (Levy 2017). *Microcystis* spp. thrives in high water temperatures and when nutrient supply is adequate, it can form blooms. When blooms do occur, it is visible as a blue-green tinge on the surface of the water. This genus can also be quite successful being completely dominant over other forms of cyanobacteria in areas where they bloom (Janse van Vuuren et al. 2006).

### ***Oscillatoria* sp. Vaucher ex Gomont, 1892**

(Table 4.1; Fig. 4.5E)

#### Characteristics:

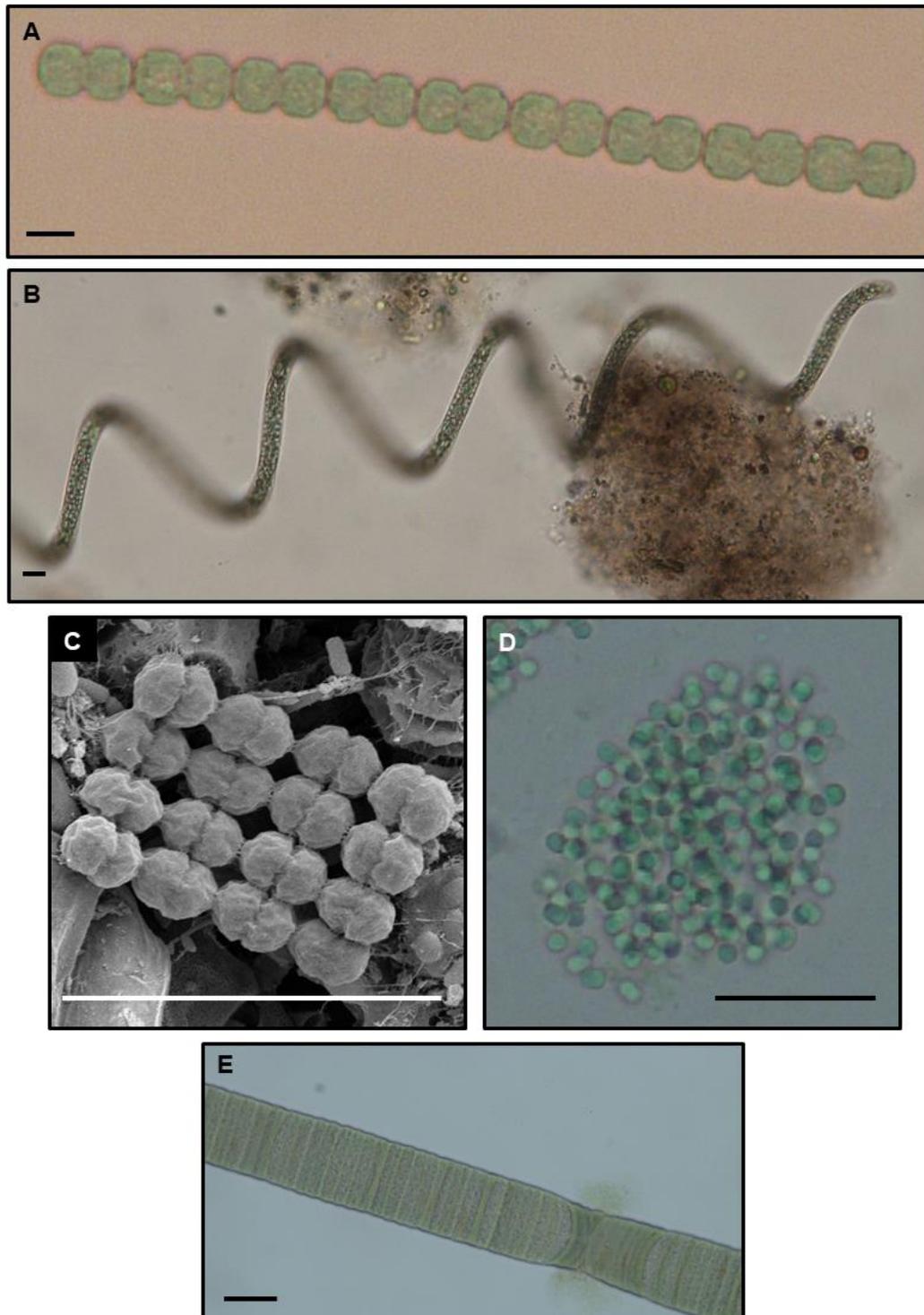
Trichomes of *Oscillatoria* spp. are cylindrical and unbranched and may be straight or slightly wavy and often very long (Fig. 4.5E). Cells of trichomes are shorter than broad and discoid. The edges of the trichome normally form unbroken parallel lines, but some species do show constrictions at cross walls (Janse van Vuuren et al. 2006). A true sheath is absent (Kannan and Lenca 2012), although a thin film might be formed by parallel filaments (Janse van Vuuren et al. 2006). Stressful environmental conditions, such as desiccation or hyper salinity may cause the occasional formation of mucilage sheaths. Planktonic *Oscillatoria* spp. commonly form gas vacuoles. When trichomes come into contact with solid substrate, organisms of the genus is known to create a characteristic oscillating movement (Kannan and Lenca 2012). As it glides through water, the trichomes leave a thin mucilaginous trail, despite it having no mucilage sheath (Janse van Vuuren et al. 2006).

#### Ecology:

*Oscillatoria* is a common and widespread genus found in a variety of habitats. This genus can occur in freshwater, marine waters and hot springs (Kannan and Lenca 2012). *Oscillatoria* spp. can be found both in water and on moist sub-aerial substrates. It is free-floating or entangled with other filamentous algae when in water. Dense, slimy mats are formed on substrates when they are benthic. Large mats commonly dislodge and float to the surface (Wehr and Sheath 2002). *Oscillatoria* spp. are common in waters of sewage treatment, such as farm ponds. Being tolerant to high levels of organic pollution is characteristic of some *Oscillatoria* species, and in high nitrogen conditions the trichomes are often found associated with *Euglena* species (Janse van Vuuren et al. 2006). Some species have adjusted to surviving in the shade by managing the levels of chlorophyll a and by using accessory pigments to assist in low light conditions. This ability allows them to survive below barriers like blooms of green algae or other macrophytes restricting sunlight (Foy et al. 1976). Some species of *Oscillatoria* also use buoyancy to position themselves in the water column while others

#### CHAPTER 4: RESULTS OF KROKOVANGO CTW - PHYTOPLANKTON

can produce toxins including neurotoxins and hepatotoxins (Janse van Vuuren et al. 2006).



**Figure 4.5:** Light (A, B, D, E) and scanning electron (C) photomicrographs of A: *Anabaena* sp.; B: *Arthrospira* sp.; C: *Merismopedia* sp.; D: *Microcystis* sp. and E: *Oscillatoria* sp. collected from Krokovango CTW, Botswana. Scale bars: A, B, E: 10  $\mu$ m; C: 1  $\mu$ m; D: 5  $\mu$ m.

## CHAPTER 4: RESULTS OF KROKOVANGO CTW - PHYTOPLANKTON

### 4.2: Phytoplankton abundance

Different magnifications of the microscope were used to count different species of phytoplankton. Individuals were counted per microscope slide and averages were determined for all species. The number of individuals within species per slide were counted to a maximum of 10. Single colonies and -filaments were not counted per individual cell but were considered as one individual organism for the sake of this dissertation. A scale of 1-10 (0-1: very low; 2-6: medium; 7-10: very high) was used to depict abundance of the different genera of phytoplankton collected from Krokovango CTW for the years of 2017 and 2018 (Table 4.1).

**Table 4.1:** Abundance scale and genus dimensions of the phytoplankton collected from the Krokovango CTW compared to literature dimensions.

Species	Abundance scale		Cell length ( $\mu\text{m}$ ) of specimens collected from the Krokovango CTW	Cell dimensions ( $\mu\text{m}$ ) according to literature			Reference
	2017	2018		Diameter	Length	Width	
<b>Phylum: Chlorophyta</b>							
<i>Chlorococcum</i> sp.	3.4	2.4	3-5	-	1-15.2	-	Klochkova et al. (2006); Cabanelas et al. (2016)
<i>Chlorogonium</i> sp.	3.5	2.9	10-15	-	14-170	1,5-17	Janse van Vuuren et al. (2006); Bellinger and Sigee (2010)
<i>Cosmarium</i> sp.	0	0.1	13	-	10-200	6-140	Janse van Vuuren et al. (2006)
<i>Micractinium</i> sp.	3.2	2.5	2-3; Spines: 10-12	3-10; Spines: 10-35	-	-	Janse van Vuuren et al. (2006); Bellinger and Sigee (2010)
<i>Pandorina</i> sp.	0	1.2	4-5	5-25; Colonies: 60-200	-	-	Janse van Vuuren et al. (2006)
<i>Scenedesmus</i> spp.	1.1	2.3	15-20	-	5-30	2-10	Janse van Vuuren et al. (2006)
<i>Stigeoclonium</i> sp.	5	5.1	Branches: 15-20	-	2-5 times as long as broad	8-25	Janse van Vuuren et al. (2006)
<i>Pediastrum</i> spp.	0.7	2	Colonies: 35-110	8-32; Colonies: 200	-	-	Janse van Vuuren et al. (2006)
<i>Volvox</i> sp.	1.8	1.2	Colonies: 100-150	4-8; Colonies: 0,5-1,5 mm	-	-	Janse van Vuuren et al. (2006)
<b>Phylum: Euglenophyta</b>							
<i>Euglena</i> sp.	3.3	2.9	5-10	-	20-540	5-50	Janse van Vuuren et al. (2006)
<i>Phacus</i> spp.	2.2	2.4	15-50	-	10-140	5-10	Janse van Vuuren et al. (2006)
<b>Phylum: Bacillariophyta</b>							
<i>Achnanthyidium</i> sp.	4.1	4.9	10-15	-	10-20	<5	Round and Bukhtiyarova (1996); Janse van Vuuren et al. (2006)
<i>Amphipleura</i> sp.	2.4	4.1	50-60	-	80-140	7-10	Janse van Vuuren et al. (2006)
<i>Frustulia</i> sp.	4.7	5.4	10-20	-	40-160	5-30	Siver and Baskette (2004)
<i>Navicula</i> sp.	5.6	4	12-20	-	6-42	4-12	Janse van Vuuren et al. (2006)
<i>Nitzschia</i> sp.	6.2	5.1	15-20	-	5-100 (exceptionally 600)	2,5-12	Janse van Vuuren et al. (2006)
<i>Pinnularia</i> sp.	4.2	2.1	30-40	-	24-110	5-18	Janse van Vuuren et al. (2006)
<b>Phylum: Cyanophyta</b>							
<i>Anabaena</i> sp.	2.3	3.9	10	7-12	-	-	Janse van Vuuren et al. (2006)
<i>Arthrospira</i> sp.	4.5	5.3	Trichome width: 10	-	-	Trichomes: 8-10	Janse van Vuuren et al. (2006)
<i>Merismopedia</i> sp.	0.8	4.4	1-5	1-10	-	-	Janse van Vuuren et al. (2006)
<i>Microcystis</i> sp.	5	5.2	Colonies: 10-20	0,5-9	-	-	Janse van Vuuren et al. (2006)
<i>Oscillatoria</i> sp.	6	6.5	Trichome width: 10-20	Trichomes: 8-30	-	-	Janse van Vuuren et al. (2006)

### 4.3: Protozoa

Protozoans are unicellular or colonial eukaryotes that can be found in all aquatic or moist environments (Fenchel 1987). These organisms might exist individually or as members of a loose-knit colony (Taylor and Sanders 2001). According to Patterson (2018), protozoans obtain energy and nutrients through heterotrophy, which distinguishes them from algae.

Protozoans, along with other plankton groups, are often overlooked as valuable bioindicators. Belonging to the kingdom Protozoa, protozoans play an important role in freshwater ecosystems (Radhakrishnan and Jayaprakas 2015). Flagellated protozoans are known to feed on picoplankton. Feeding predominantly on bacteria and small cyanobacteria makes flagellated protozoans valuable role players in the “microbial loop” within lakes, rivers and wetlands (Taylor and Sanders 2001).

According to Kuikman (1990), protozoans contribute to the metabolism of aquatic and terrestrial habitats and the importance of protozoans in their environment is closely related to the fact that they mainly consume bacteria as food. Many flagellates are mixotrophic and can utilise both types of nutrition. Several heterotrophic protozoa have photosynthetically active endosymbionts (Patterson 2018).

Patterson (2018) also mentioned that amoeboid, flagellated and ciliated Protozoa are all capable of heterotrophic nutrition. Amoebozoa and Ciliophora were the only two phyla collected from the Krovovango CTW. Identification of the material collected during the current survey was based on morphological comparison with known records from published literature, including Pritchard (1842), Randall (1957), Brieger (1963), Bick (1972), Andresen (1973), Curds (1975), Wichterman (1986), Roberts and King (1987), Anderson (1988), Caprette and Gates (1994), Jordan and Verma (2001), Beale and Preer (2008), Lynn (2008), Serrano et al. (2008), Lynn (2017), Cai et al. (2018) and Patterson (2018).

### 4.3.1: Phylum: Amoebozoa

Amoebozoa normally have no defined shape and change shape constantly by extension of pseudopodia (Schilde and Schaap 2013). According to Meisterfeld et al. (2012), the Phylum Amoebozoa include amoebas, pelobionts, slime moulds and several less-familiar forms. The typical amoebozoan would, at least at some stage of their life cycle, perform the typical amoeboid motion with lobe-like pseudopodia. Meisterfeld et al. (2012) mentioned that electron microscopy and molecular studies indicated that ancestors of amoebozoans had a cilium. Some living forms still retain this feature, with fewer having two cilia or are multiciliate (Schilde and Schaap 2013).

#### ***Amoeba* spp. Bory de Saint-Vincent, 1822**

**(Table 4.2; Figs. 4.6A-D)**

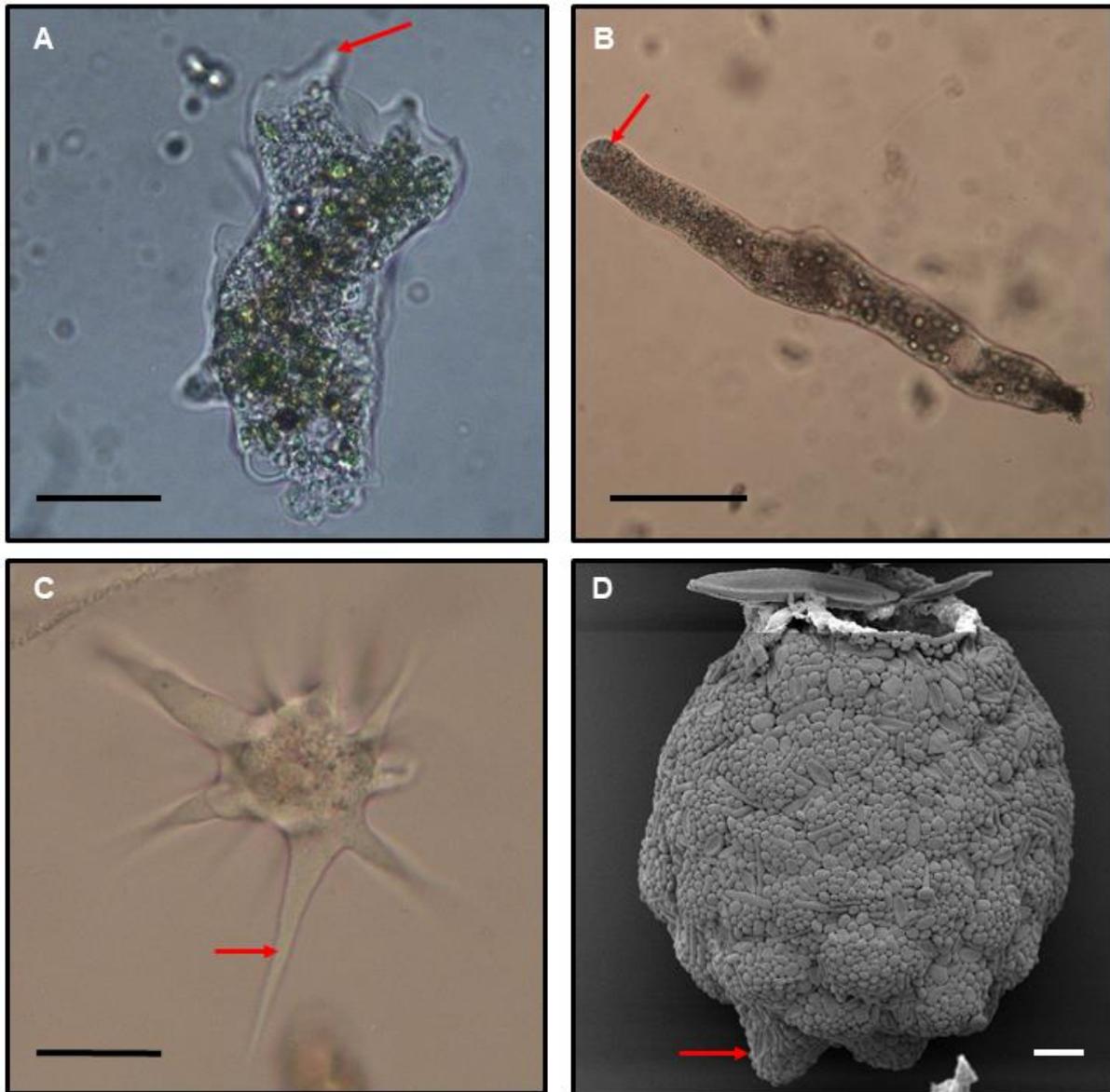
#### Characteristics:

The ability to form pseudopodia is well known among eukaryotes. Amoebae can either have several pseudopodia (polypodial) (Fig. 4.6A) or they move as one pseudopod (Fig. 4.6B) with one advancing point. Pseudopodia may be broad and rounded (Fig. 4.6D). Watery leading margins, called hyaline caps, are usually present. Other forms include conical- and thread-like pseudopodia (Andresen 1973). *Amoeba radiosa* (Fig. 4.6C) must not be perceived as a species of amebas but can rather be seen as the floating form characteristic to many amoebae. Amoebae adopt this form when detaching from substrate. Normally pseudopodia taper from a broad base to a narrow tip. Normal locomotive form develops again within minutes of settling against substrate (Patterson 2018).

#### Ecology:

Smyth (1994) mentioned that *Amoeba* is a cosmopolitan genus found in almost every type of life-supporting habitat, including soil, water, decaying plant material and sewage.

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**Figure 4.6:** Light (A, B, C) and scanning electron (D) photomicrographs of A, B, D: *Amoeba* spp. and C: *Amoeba radiosa* collected from Krokovango CTW, Botswana. Red arrows: Illustrating different forms of pseudopodia. Scale bars: A, B, D: 10  $\mu$ m; C: 5  $\mu$ m.

### 4.3.2: Phylum: Ciliophora

Ciliophora, also called ciliates, are one of the largest groups of protozoans. There are approximately 8000 species of described ciliates and they are mainly known for having hair-like cilia used for locomotion (Shin et al. 2000). According to Brieger (1963) and Lynn (2017), Ciliophora are also characterised by nuclear dimorphism or having two kinds of nuclei, i.e. micronucleus and macronucleus. When metazoans are absent, large-celled ciliates become the top heterotrophs in microbial food webs feeding on bacteria and smaller protists (Lynn 2017).

#### ***Frontonia* sp. Ehrenberg, 1838**

**(Table 4.2; Fig. 4.7A)**

##### Characteristics

According to Serrano et al. (2008), *Frontonia* species are ovoid and flattened with an anterior or middle oral cavity (Fig. 4.7A). *Frontonia* spp. have single contractile vacuoles forming collecting channels which radiate to most parts of the body (more clearly seen from the *Paramecium* specimen). Macronucleus roughly in the middle of the cell. Trichocysts located at the edge of the cell. *Frontonia* species mostly contains symbiotic green algae (Patterson 2018).

##### Ecology

Patterson (2018) mentioned that *Frontonia* species are commonly found in various aquatic habitats associated with detritus or the substrate and occasionally they migrate through the water column. Unlike *Paramecium* spp., *Frontonia* species feed on larger particles of food, such as diatoms (Fenchel 1987).

#### ***Paramecium* sp. Müller, 1773**

**(Table 4.2; Fig. 4.7B)**

##### Characteristics

Beale and Preer (2008) mentioned *Paramecium* (Fig. 4.7B) to be single celled, containing a macronucleus and a spherical adpressing micronucleus. Contractile

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vacuoles present. Cell evenly covered with cilia, caudal tuft of longer cilia. Mouth forms a channel packed with a dense line of cilia. Two contractile vacuoles, each with radiating food collecting canals. Macronucleus and trichocysts present. Body shaped to form a channel from the anterior end of the cell to the buccal cavity (Wichterman 1986). The compound ciliary organelles lie within the buccal cavity, creating currents of water to filter food particles. Food vacuoles form at the cytostome at the base of the buccal cavity (Patterson 2018).

### Ecology

According to Görtz (2012), the ecology of *Paramecium* species has been understudied because of rather being used as tools to investigate fields such as competition and predator-prey interactions in environments. *Paramecium* is a cosmopolitan species, usually occurring in warm stagnant water bodies (Moon et al. 1951).

### ***Spirostomum* sp. Ehrenberg, 1833**

**(Table 4.2; Fig. 4.7C)**

### Characteristics

Randall (1957) noted that *Spirostomum* species (Fig. 4.7C) are distinguished by the compact macronucleus containing smaller, dense particulate objects variable in shape and size. Cell cylindrical, with a posterior contractile vacuole. The adoral zone is covered with membranelles leading to the cytostome. Rigid surface body with locomotor cilia (Patterson 2018).

### Ecology

According to Laybourn-Parry (1992), *Spirostomum* species show a preference for deeper sites below the thermocline. *Spirostomum* spp. migrate up from the bottom of water bodies into the water column of the hypolimnion (Bark 1981). Patterson (2018) stated that this genus is often present in polluted water bodies, or in waters with little or no oxygen present.

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### ***Epistylis* sp. Ehrenberg, 1830**

**(Table 4.2; Fig. 4.7D)**

#### Characteristics

*Epistylis* is a genus belonging to the group of sessile peritrichs forming arborescent (tree-like) colonies (Fig. 4.7D) (Patterson 2018). Large peristomal lip present (Serrano et al. 2008). The membranes lack a contractile spasmoneme in the stalk, making their stalks unable to contract like in *Vorticella* spp. Instead, the cell bodies contract individually. Feeding cilia located around the aboral end of the cell (Patterson 2018).

#### Ecology

Cosmopolitan aquatic species found in fresh- and marine water and in terrestrial habitats (Utz et al. 2014). *Epistylis* species can form epizoitic growths on the surface of other larger zooplankton like copepods. According to Patterson (2018), this relationship is normally facultative, which means the ciliate gains access to food through the movement and feeding currents of the organism that it is attached to.

### ***Vorticella* sp. Linnaeus, 1767**

**(Table 4.2; Fig. 4.7E)**

#### Characteristics

*Vorticella* (Fig. 4.7E) is a solitary-, and usually social species. A long and highly contractile stalk attaches *Vorticella* species to submerged objects and substrate. An asymmetrical and inverted bell-shaped body is attached to the contractile stalk (Jordan and Verma 2001). Feeding cilia found around the anterior end of the cell (Patterson 2018). *Vorticella* is often found in large groups, but individuals still are free and independent of each other (Jordan and Verma 2001). Being anchored by stalks makes them vulnerable to predation. A spirally contractile spasmoneme in the stalk provides some protection by contracting when threatened (Patterson 2018).

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### Ecology

*Vorticella* is a cosmopolitan genus, found among aquatic vegetation in freshwater ponds, rivers and streams. *Vorticella* species favour stagnant water, rich in decaying organic matter, where they feed predominantly on bacteria (Jordan and Verma 2001).

### ***Stentor* sp. Oken, 1815** **(Table 4.2; Fig. 4.7F)**

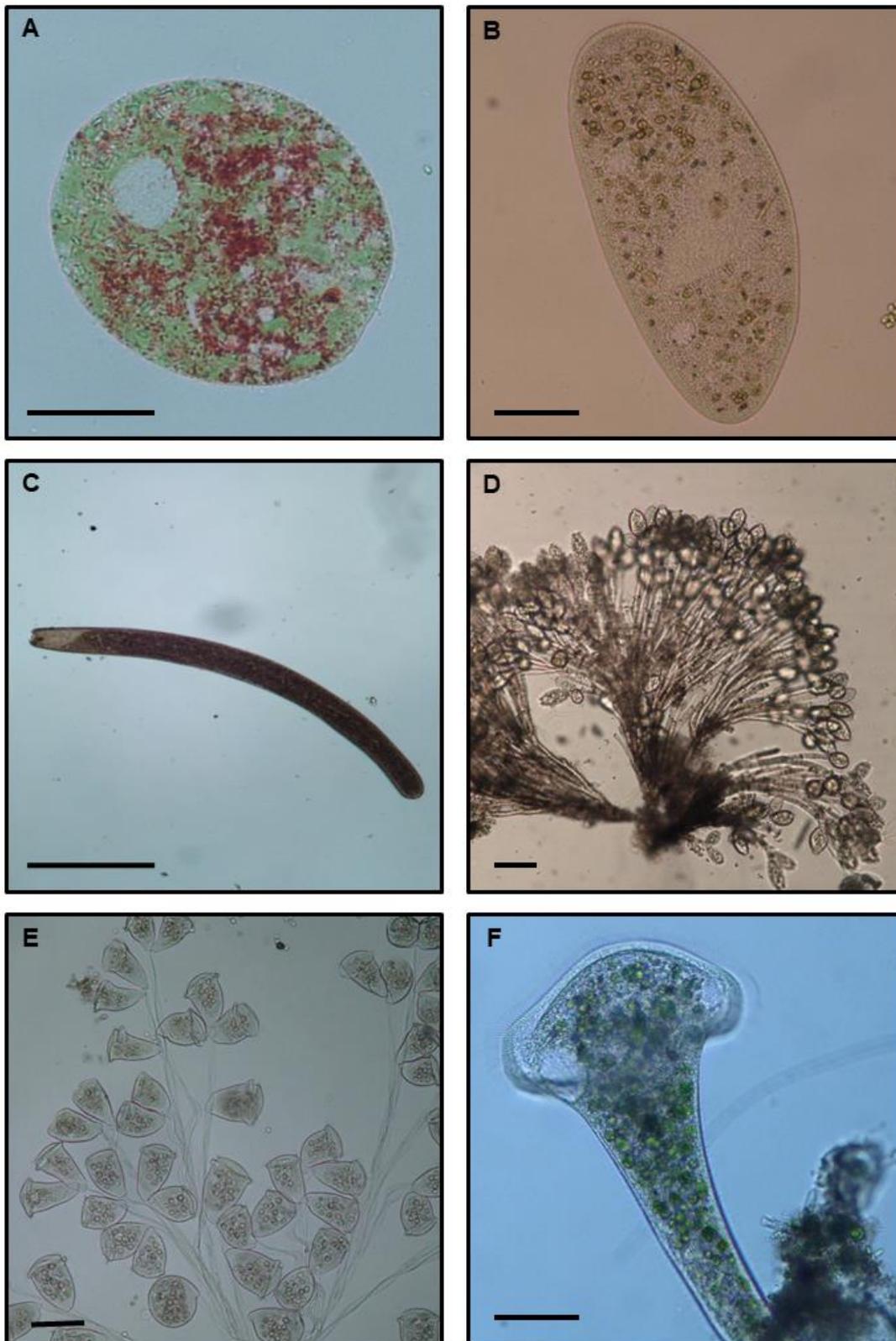
### Characteristics

All species can attach to substrates with a holdfast (Patterson 2018). According to Bick (1972), *Stentor* species are trumpet shaped in resting and feeding stasis (Fig. 4.7F). They feed with membranelles which run along the flattened anterior end of the cell. Contractile vacuole positioned adjacent to the cytostome. The thin part of the cell may be seen with several somatic cilia protruding (Patterson 2018).

### Ecology

According to Thorp and Rogers (2015), *Stentor* species are common worldwide in various freshwater habitats. Most *Stentor* species can be found in normal-, dystrophic- and slightly brackish waters (Foissner and Wölfl 1994).

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**Figure 4.7:** Light photomicrographs of **A:** *Frontonia* sp.; **B:** *Paramecium* sp.; **C:** *Spirostomum* sp.; **D:** *Epistylis* sp.; **E:** *Vorticella* sp. and **F:** *Stentor* sp. collected from Krokovango CTW, Botswana. Scale bars: **A, B, C:** 25  $\mu$ m; **D, E, F:** 50  $\mu$ m.

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### ***Euplotes* sp. Ehrenberg, 1830**

**(Table 4.2; Fig. 4.8A)**

#### Characteristics

*Euplotes* (Fig. 4.8A) is a highly differentiated and speciose genus of hypotrich ciliates (Lynn 2008). The ventral cirri of hypotrichs are used for movement over substrates. The membranelles on the adoral zone forms a collar around the front of the cell. These adoral membranelles cause a current of water under the cell towards the cytostome. The undulating membrane is positioned next to the mouth. Locomotor cilia occur in several clusters. Transverse cirri are the most notable. Other cirri include frontoventral cirri at the adoral surface of the cell, and the caudal cirri at the ventral surface of the cell (Curds 1975; Caprette and Gates 1994; Patterson 2018).

#### Ecology

Widespread and commonly found in aquatic environments (Dini and Nyberg 1999). Zhao et al. (2018) mentioned that ecological studies on the genus are restricted due to a lack of understanding of species delimitation.

### ***Stylonychia* sp. Ehrenberg, 1830**

**(Table 4.2; Fig. 4.8B)**

#### Characteristics

According to Bick (1972) the size of the genus is very variable. This genus contains some of the larger-in-size hypotrich species (Fig. 4.8B). Rigid cell body, with well-developed adoral zone of membranelles, forming a collar around the front of the cell. Lipel leading to the cytostome on the ventral surface. Often distinguished by the three long caudal cirri. Locomotor cilia (cirri) visible on the ventral surface. Most or all cirri in clusters, including marginal cirri at the lateral margins, transverse cirri at an angled line near the posterior end of the cell and frontoventral cirri running from the anterior part of the body and down to the ventral surface (Patterson 2018).

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### Ecology

*Stylonychia* species are widespread, confined to freshwater habitats and may occur in various aquatic conditions, depending on the species (Kumar and Foissner 2016).

### ***Podophyra* sp. Ehrenberg, 1838**

**(Table 4.2; Fig. 4.8C)**

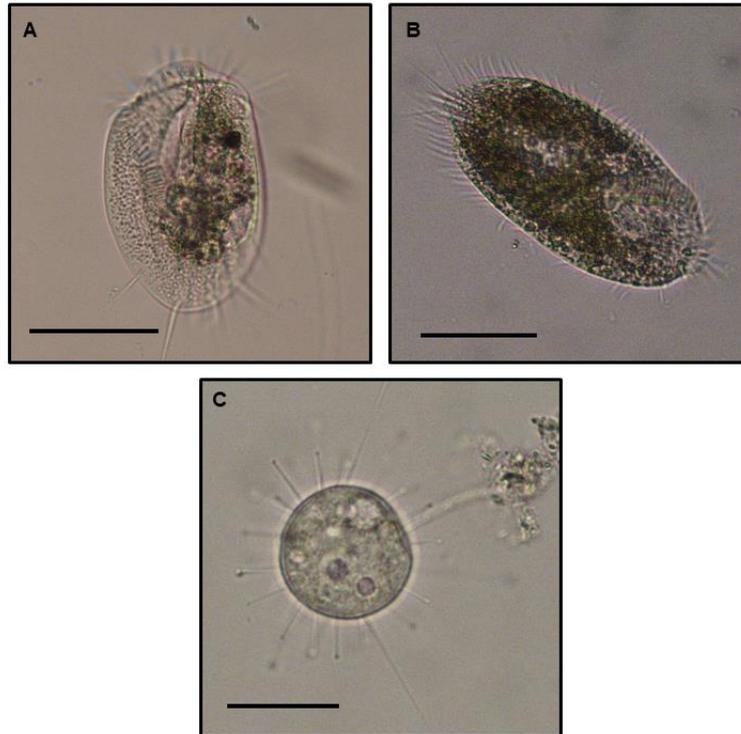
### Characteristics

*Podophyra* (Fig. 4.8C) is a single celled suctorian ciliate with no cilia or teeth (Patterson 2018). Pritchard (1842) referred to *Podophyra* species as the pedicled and rayed animalcules. Spherical, free bodies covered with setaceous tentacles/arms. Knobs can be observed at the tips of the tentacles, which are the mouths of the cell. Mouths are truncate and contain numerous extrusomes (membrane bound structures) used to hold prey (Patterson 2018). Body attached to a stiff stalk, might also be free-floating, without a stalk present (Roberts and King 1987).

### Ecology

Cosmopolitan species found in various freshwater habitats. Several species are endo- or ectoparasites of other ciliated protozoa and in certain cases, the species can only be distinguished by its relationship with a specific host (Curds 1986).

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**Figure 4.8:** Light photomicrographs of **A:** *Euplotes* sp.; **B:** *Stylonychia* sp. and **C:** *Podophyra* sp. collected from Krokovango CTW, Botswana. Scale bars: **A, B:** 50  $\mu\text{m}$ ; **C:** 25  $\mu\text{m}$ .

**Table 4.2:** Summary of literature on genus dimensions of Protozoa collected from the Krokovango CTW, Botswana.

Species	Cell length ( $\mu\text{m}$ ) of specimens collected from the Krokovango CTW	Cell length ( $\mu\text{m}$ ) according to literature	Reference
<b>Phylum: Amoebozoa</b>			
<i>Amoeba</i> spp.	35-50	50-600	Andresen (1973); Lynn (2017)
<b>Phylum: Ciliophora</b>			
<i>Frontonia</i> sp.	65-150	220-350	Cai et al. (2018)
<i>Paramecium</i> sp.	130-150	20-300	McKinney (2015)
<i>Spirostomum</i> sp.	80-200	up to 4000; 800-1000	Lynn (2008); McHenry (2016)
<i>Epistylis</i> sp.	40-50	50-150	McHenry (2016)
<i>Vorticella</i> sp.	50-60	50-150	McHenry (2016)
<i>Stentor</i> sp.	450-500	500-2000	McHenry (2016)
<i>Euplotes</i> sp.	75-90	80-100	McHenry (2016)
<i>Stylonychia</i> sp.	110-120	100-300	Bick (1972)
<i>Podophyra</i> sp.	25-30	10-30	Anderson (1988); McHenry (2016)

### 4.4: Rotifera

Rotifers are known for their unique ciliation, microscopic size and the beating of their ciliated head-crowns. These mouth structures very closely resemble turning wheels – hence the name Rotifera (Hudson and Gosse 1889). They are commonly referred to as wheel animals. Rotifers use the head crowns to circulate food particles into the mouth. These acoelomate worms share many structural features with nematodes, turbellarians and gastrotrichs. According to Brain (2002), wheel animals can have sizes of about 40  $\mu\text{m}$  to about 2 mm in length. Wheel animals are ubiquitous, meaning that they can be found in almost all types of freshwater habitats. These wheel animals can congregate in densities of up to 1000 individuals per liter (Segers 2008). Rotifers are very sensitive towards changes in water quality; hence they have a high population turnover rate (Tasevska et al. 2010).

Wheel animals can serve as valuable indicators of water quality and trophic status (Chung et al. 1991). Studying rotifer community structures over time, assists in determining levels of pollution and -changes in environmental conditions (Marneffe et al. 1998). According to Marneffe et al. (1998), rotifers have been used for chronic toxicity tests for several years.

According to Wallace and Snell (2010), rotifer species' physiological tolerances are determined by upper and lower lethal environmental ranges and the environmental optimum. This indicates a species' preferred range at which they achieve maximum reproductive and survival rates. Only a few species have been critically studied for their environmental tolerances (Wallace and Snell 1991).

Several different rotifer classification systems are presently used. According to Wallace and Snell (1991), there are three basic groups of wheel animals, namely Seisonidea, Bdelloidea and Monogononta and these groups are treated differently in classification depending on the system used. Brain (2002) and Segers (2008) argue that there are two classes within the Phylum Rotifera – Monogononta and Digononta. Brain (2002) estimated that there are approximately 120 genera and 1800 to 2000 rotifer species found world-wide. Rotifers in this dissertation were classified according to the system of Wallace and Snell (1991). Identification of the specimens collected during the current survey was based on morphological comparison with known records

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from published literature, including Edmondson and Hutchinson (1934), Ahlstrom (1943), Carlin (1943), Ruttner-Kolisko (1974), Koste (1978), Koste and Shiel (1990), Roche (1993), Segers et al. (1993), Ricci and Melone (2000), Sharma and Sharma (2000), Smith (2001), Brain (2002), Khan (2003), Varghese et al. (2006), Wallace and Snell (2010), Trinh Dang et al. (2013), Kriska (2014), Mekong River Commission Environment Programme (2015), West (2016), Manickam et al. (2019) and Tausz et al. (2019).

### **4.4.1: Class: Monogononta**

According to Brain (2002), more than 80% of known species of wheel animals belong to the class of Monogononta. This class consist of three orders i.e. Ploima, Flosculariacea and Collothecacea (Brain 2002). Wallace and Snell (1991) suggested that there are 95 genera and more than 1600 species of rotifers within the class Monogononta. Due to a lack of taxonomic information and the occurrence of cryptic speciation among this class, Segers (2008) believed that numbers may be much higher.

Female rotifers of the class Monogononta have single ovaries with a vitellarium. Wallace and Snell (1991) mentioned that males were smaller than females and structurally more simplistic. Males have only been observed for a few species and are usually only present for a few days or weeks annually (Wallace and Snell 1991).

### **Order: Ploima Hudson and Gosse, 1886**

The order Ploima represents most of the rotifer species found in the Krovovango CTW. According to Hochberg (2006), this order is generally the largest and most diverse group of monogononts.

**Family: Asplanchnidae Eckstein, 1883**

***Asplanchnopus* sp. De Guerne, 1888**

**(Table 4.3; Fig. 4.9A)**

Characteristics:

Wallace and Snell (2010) determined that *Asplanchnopus* spp. (Fig. 4.9A) are usually large, transparent and sac-shaped with variable morphology. The soft body can change in shape, as internal and external organs move around. A well-developed and incurved corona forms a circumapical ring of cilia. No intestine or anus present. Species are often viviparous (Wallace and Snell 2010). Ruttner-Kolisko (1974) indicated that the foot of *Asplanchna* sp. (a closely related species) is absent and that *Asplanchnopus* spp. have a reduced foot.

Ecology:

*Asplanchnopus* are an understudied genus and are less common than species of the genus *Asplanchna*. Since *Asplanchna* spp. have no foot, they are completely adapted to floating, while *Asplanchnopus* species are semi-pelagic (Ruttner-Kolisko 1974).

**Family: Brachionidae Ehrenberg, 1838**

***Brachionus* sp. Pallas, 1766**

**(Table 4.3; Figs. 4.9B-F)**

Characteristics:

*Brachionus* species (Figs. 4.9B-F) are known for its constant structure of trophi within species. Short and broad fulcrum. Rami externally broad and rounded and have conspicuous anterior structures (Brain 2002). Membranous filaments are attached to these structures between the rami and unci. Zigzag-like structures cover the median edge of the rami. These consist of fused teeth-like projections. The rami are hollow, each containing two cavities. Uncus are more or less pentagonal in shape, bearing almost fused, subequal teeth. Subuncus also present. The distal part of the unci fit in cup-shaped articulations of the manubria. A membrane covers cavities in the proximal part of the manubria. External margin of the manubria is sickle-shaped and bent

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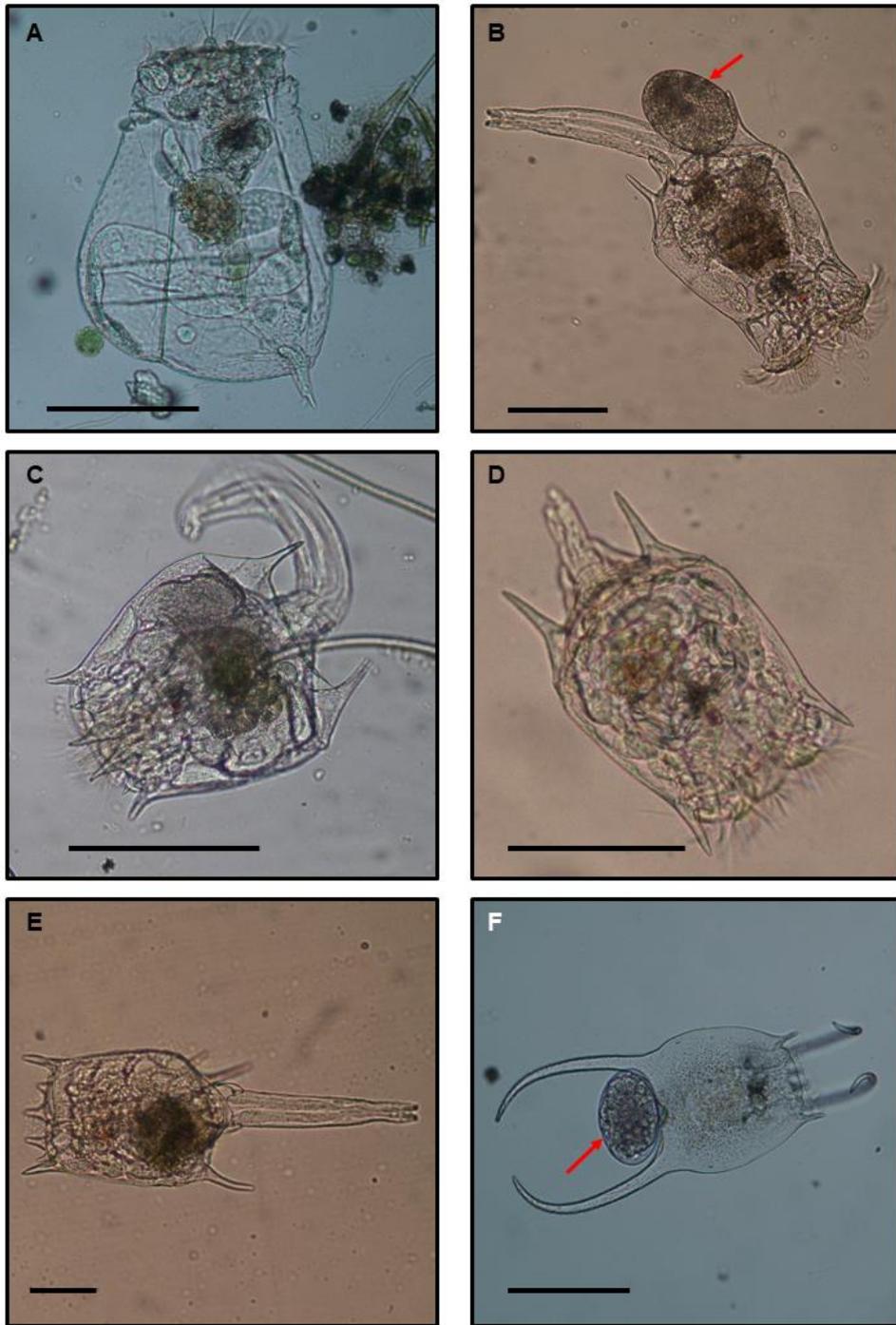
inwards distally (Segers et al. 1993). Figure 4.9B shows a *Brachionus* species with one egg sack. The other egg sack already detached.

*Brachionus falcatus* Zacharias, 1898 (Fig. 4.9F) have a firm lorica which is dorso-ventrally compressed. Six spines on antero-dorsal margin. Intermediate spines much larger than laterals and medians. The intermediate spines also curve laterally outwards or ventrally towards the head. Median spines can be smaller than lateral spines, but mostly equal of length. Widely separated and long posterior spines are present, that can either be parallel or bow outwards and twist towards apices. Foot opening between bases of posterior spines (Varghese et al. 2006). Figure 4.9F shows a specimen with one egg sack.

#### Ecology:

Species of the genus *Brachionus* is very diverse. Green (2003) mentioned that this is a cosmopolitan species, commonly found in plankton samples.

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**Figure 4.9:** Light photomicrographs of **A:** *Asplanchnopus* sp.; **B-E:** *Brachionus* spp. and **F:** *Brachionus falcatus* collected from Krokovango CTW, Botswana. Red arrows: Indicating egg sacks. Scale bars: 100 µm.

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### ***Keratella* sp. Bory de St. Vincent, 1822**

**(Table 4.3; Fig. 4.10A)**

#### Characteristics:

The dorsal surface of the lorica in *Keratella* spp. has a pattern of polygonal facets. One, two or no posterior spines may be present with six short to medium anterior spines. *Keratella* species are known to have a hard lorica. Dorsal plate with sculptural pattern. Areolate network, postulation or fine spines on dorsal plate might be present (Tausz et al. 2019).

Based on studies conducted by Carlin (1943), Ahlstrom (1943), Ruttner-Kolisko (1974) and Koste (1978), the morphology of *Keratella quadrata* (Müller, 1786) is extremely variable (Fig. 4.10A). According to Edmondson and Hutchinson (1934), *Keratella quadrata* are distinguished by the posterior breadth of lorica being slightly smaller than the maximum breadth of the lorica; and the presence of two subequal posterior spines.

#### Ecology:

Most species within *Keratella* occur in fresh water, with only a few species occurring in brackish water. According to Ruttner-Kolisko (1974), they have wide environmental tolerances with a cosmopolitan distribution. *Keratella quadrata* is a euplanktonic and perennial species, having a wide range of tolerance to mineralisation and temperature (de Manuel Barrabin 2000; Inaotombi et al. 2016).

### ***Platyias* sp. Haring, 1913**

**(Table 4.3; Figs. 4.10B, C)**

#### Characteristics:

In the genus *Platyias* the dorsal and ventral plates of the lorica are completely fused laterally. Four or six spines are usually present on the anterior dorsal margin of the lorica. Body somewhat dorso-ventrally flattened. The foot is segmented and retractile. Two toes are present (Sharma and Sharma 2000).

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*Platyias patulus* Müller, 1786 (Figs. 4.10B, C) have a rigid foot, four anterior spines and ten anterior spines. Lorica dorso-ventrally flattened, foot opening ventral (Mekong River Commission Environment Programme 2015).

### Ecology:

According to Ruttner-Kolisko (1974), *Platyias* species are sporadically widespread in warmer climates. They are normally found in the littoral zone of fresh water to slightly saline environments where they occur amongst macrophytes. In their environment they have never been documented to occur in large numbers (Ruttner-Kolisko 1974). *Platyias patulus* is a cosmopolitan species (Houssou et al. 2018).

### **Family: Lecanidae Bartos, 1959**

***Lecane* sp. Nitzsch, 1827**

**(Table 4.3; Figs. 4.10D-F)**

### Characteristics:

*Lecane* species are monogonont rotifers with dorso-ventrally compressed loricate bodies. Heads are retractable. A flexible membrane connects the dorsal and ventral plates. Foot protruding from an indentation in the anterior part of the ventral plate. Two short joints on the foot with two well-formed toes often ending in a claw (Wallace and Snell 2010).

### Remarks:

According to Koste and Shiel (1990), *Lecane bulla* (Gosse, 1851) have a stiff, smooth, elongated and ovate lorica with an anterior margin (Fig. 4,10D). Ventral surface with complete transverse fold. Ventral margin with deep, rounded anterior sinus. Concave and U-shaped dorsal margin. Convex dorsal plate without spines. Pseudo-segmented foot. Long, slender toe, approximately half the body length, ending in a long, fused pseudo claw (Mekong River Commission Environment Programme 2015).

*Lecane papuana* Murray, 1913 (Figs. 4.10E, F) have a nearly subcircular lorica and a straight anterior margin. Ventral margin with a V-shaped sinus and undulate sides. Dorsal plate slightly broader than the ventral plate. Posterior segment small and

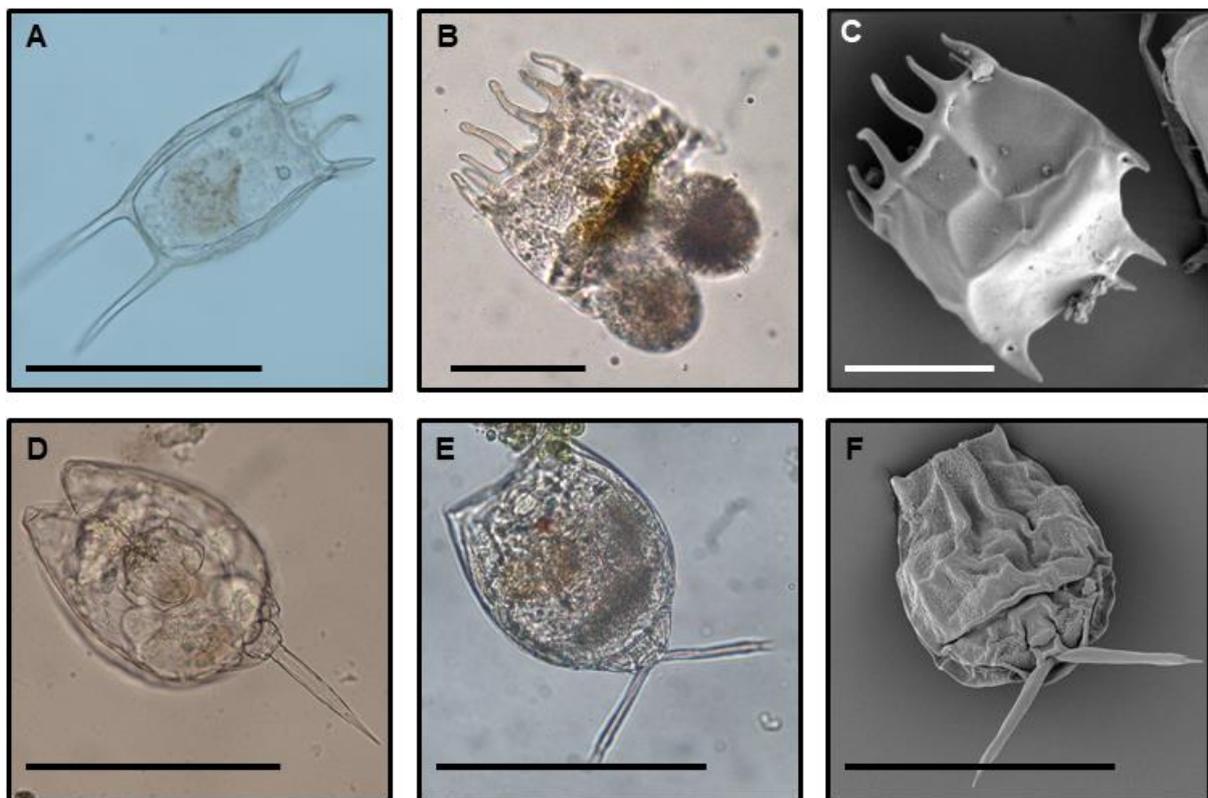
#### CHAPTER 4: RESULTS OF KROKOVANGO CTW - ROTIFERA

rounded. Pyriform first joint of the foot and robust second joint. Stout and pointed claw with basal spicules at the end of slender toe (Khan 2003).

##### Ecology:

According to Walsh et al. (2009), *Lecane bulla* is littoral and widespread, occurring in ponds, rivers and lakes from plains to mountains with a cosmopolitan distribution.

*Lecane papuana* has a wide distribution range and is classified as a warm stenothermal species (Segers 1995). Green (2003) and West (2016) found specimens from various locations in the Okavango Delta, Botswana.



**Figure 4.10:** Light (A, B, D, E) and scanning electron (C, F) photomicrographs of A: *Keratella quadrata*; B, C: *Platyias patulus*; D: *Lecane bulla* and E, F: *Lecane papuana* collected from Krokovango CTW, Botswana. Scale bars: 100  $\mu$ m.

**Family Lepadellidae Haring, 1913**

***Lepadella* sp. Bory de St. Vincent, 1822**

**(Table 4.2; Figs. 4.11A-C)**

Characteristics:

Within *Lepadella* spp. (Figs. 11A-C), the dorsal and ventral plates form the lorica, which is dorso-ventrally compressed. At the edges the dorsal and ventral plates are rigidly united. The head protrudes through an anterior opening and a well-developed foot protrudes through a large posterior opening. The foot is divided into 3 - 4 segments with two moderately long and slender toes. Head with one pair of lateral eyes. Mastax with malleate trophi (Smith 2001; Wallace and Snell 2010).

Remark:

According to Trinh Dang et al. (2013), *Lepadella desmeti* Segers and Chittapun, 2001 (Fig. 4.11C) is distinguished by its convex dorsal plate, having two pairs of rounded, longitudinal ridges.

Ecology:

According to Segers and De Smet (2009), species within *Lepadella* are benthic-littoral or psammon-inhabiting (occurring in sandy environments), with a majority found in oligo- to mesotrophic, slightly acidic, soft waters.



**Figure 4.11:** Light (A, C) and scanning electron (B) photomicrographs of A, B: *Lepadella* sp. and C: *Lepadella desmeti* collected from Krokovango CTW, Botswana. Scale bars: 100  $\mu$ m.

***Trichocerca* sp. Lamarck, 1801  
(Table 4.2; Figs. 4.12A-D)**

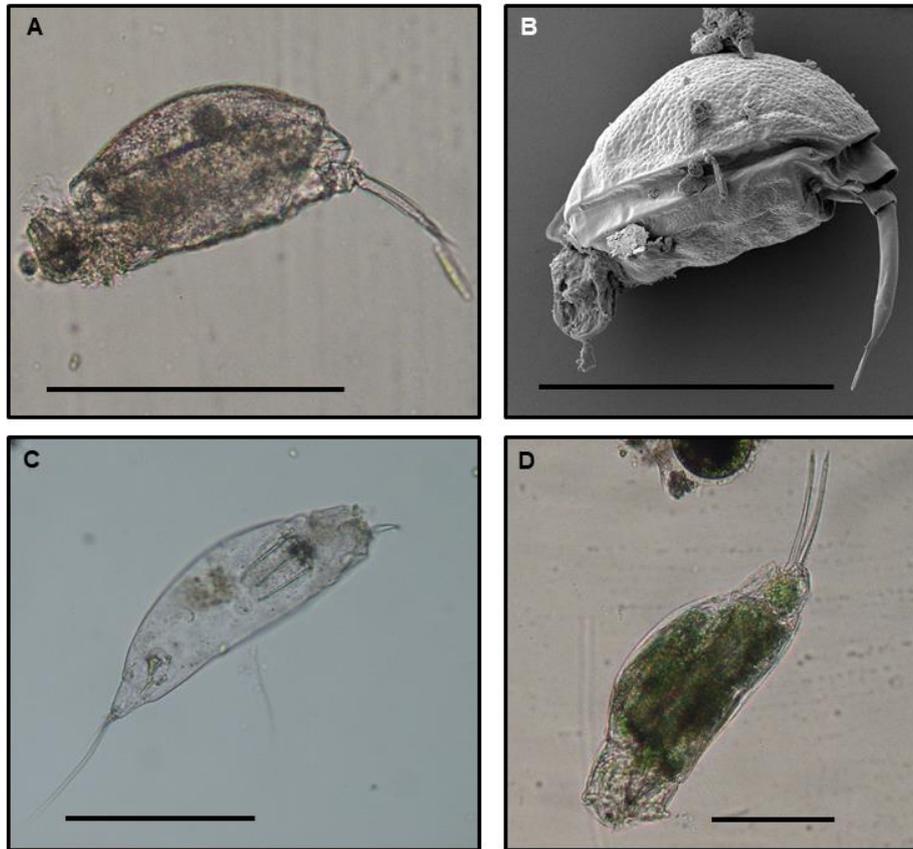
Characteristics:

The lorica of *Trichocerca* species (Figs. 4.12A-D) consists of a single cylindrical piece. The lorica often contains teeth and longitudinal grooves or ridges. Body cylindrical, asymmetrical and slightly curved. Spine-like toes of equal- or unequal (left toe longer than the right) length, with small spinules at their base. Asymmetrical trophi. The trunk is usually cylindrical and is often arched (Smith 2001).

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### Ecology:

According to Segers (2003), *Trichocerca* spp. are ecologically diverse, occurring in fresh- and marine environments. They can also be pelagic, littoral or psammobiotic (Segers 2003).



**Figure 4.12:** Light (A, C, D) and scanning electron (B) photomicrographs of A-D: *Trichocerca* spp. collected from Krokovango CTW, Botswana. Scale bars: 100  $\mu$ m.

### 4.4.2: Class: Digononta

Digononta is a class of wheel animals that can be distinguished by paired ovaries. In Digononta males and females share similar morphology and size. Both sexes have a functional gut and paired gonads (Wallace and Snell 1991). There are two orders within the class of Digononta, namely Bdelloidea and Seisonidea. Seisonidea contains a single genus known as *Seison*, Grube, 1861 (Brain 2002). This unusual taxon is very understudied in Rotifera literature. This group was not found in the Krokovango CTW, while specimens of one Bdelloidea genus was collected.

**Order: Bdelloidea Hudson, 1884**

Bdelloidea is the second order within the class of Digononta and is comprised of 18 genera and more than 360 species (Wallace and Snell 1991). No Bdelloid male rotifers have ever been found, which implies that reproduction only occurs through parthenogenesis (Wallace and Snell 1991; Brain 2002). All bdelloid wheel animals have paired ovaries with vitellaria, ramate trophi, more than two pedal glands and a corona with either a modified ciliated field or two trochal discs. Many bdelloids have vermiform shapes and pseudo-segmentation which enable them to 'telescope' by shortening and lengthening their bodies. Bdelloid rotifers are mainly found on the surface of aquatic plants, -in sediments, -in the capillary water films in soils, or on mosses. Dense aquatic vegetation eases the collection of bdelloids with plankton nets (Wallace and Snell 1991).

**Family Philodinidae Ehrenberg, 1838**

***Rotaria* sp. Scopoli, 1777**

**(Table 4.2; Figs.4.13A, B)**

Characteristics:

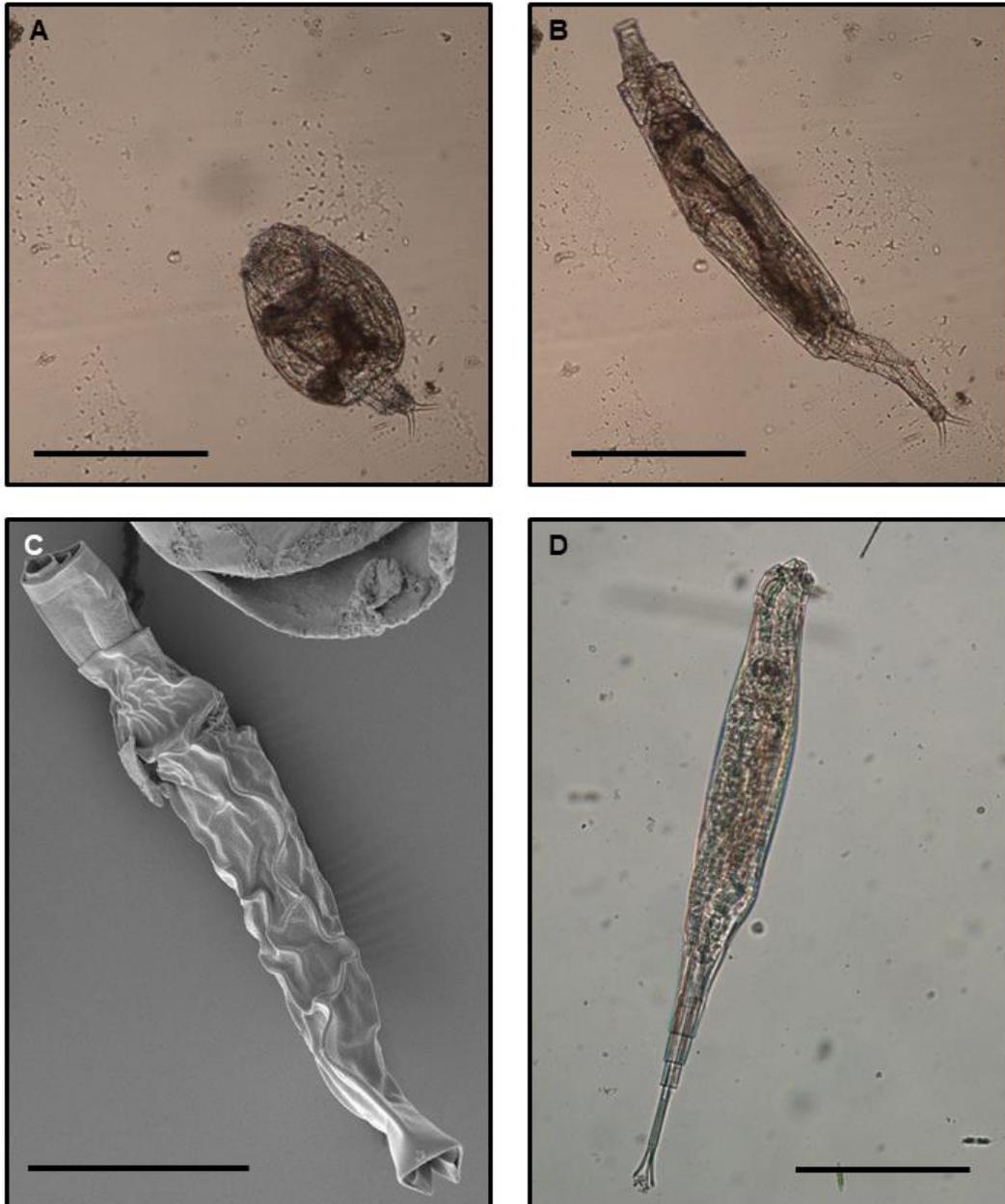
Due to pseudo-segmentation, *Rotaria* spp. can lengthen and shorten their bodies in order to move around and feed (Figs.4.13A-C). In this genus, all parts of the body are extended. Most notably, the foot, spurs and toes, might be lengthened (West 2016). *Rotaria* species have a well-developed rostrum and corona. The corona can always be retracted into the mouth. Three plain toes can be observed in all species, one dorsal and two terminal. The eyes, if present, are usually paired and narrow and located on the rostrum. Species are usually littoral, with some being epizotic. All species within the genus of *Rotaria* are viviparous (Smith 2001).

*Rotaria neptunia* Ehrenberg, 1832 (Fig.4.13D) have long, slender and fusiform bodies. Two eyes on the rostrum. Antenna on the first neck segment are palp-like. Long trunk gradually narrowing. Very long foot with five joints, about half the length of the body. Equal, jointed and pointed spurs occur in one pair. Three slender and equal toes form the last foot joint. Small corona with two bristle-bearing trochal discs (Sharma and Sharma 2000).

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### Ecology:

*Rotaria neptunia* is widely distributed in fresh water. Common in ponds, lakes and rivers. They are helioplanktonic, meaning that they are typically found in small bodies of still fresh water. Also being polysaprobic, they are found in habitats rich in decomposable organic matter with low oxygen concentrations (de Manuel Barrabin 2000).



**Figure 4.13:** Light (A, B, D) and scanning electron (C) photomicrographs of A, B, C: *Rotaria* sp. and D: *Rotaria neptunia* collected from Krokovango CTW, Botswana. Scale bars: 100 μm.

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**Table 4.3:** Body lengths of Rotifera species collected from Krokovango CTW, Botswana, compared to literature reports.

Species	Body length (µm) of specimens collected from the Krokovango CTW	Body length (µm) according to literature	References
<b>Family: Asplanchnidae</b>			
<i>Asplanchnopus</i> sp.	200-300	400-2000	Wallace and Snell (2010)
<b>Family: Brachionidae</b>			
<i>Brachionus</i> sp.	250-300	100-340	Manickam et al. (2019)
<i>Brachionus falcatus</i>	230-240	277-435	Ahad and Roa (2017)
<i>Keratella quadrata</i>	115-130	100-150	Roche (1993)
<i>Platyias patulus</i>	140-200	up to 350	Kriska (2014)
<b>Family: Lecanidae</b>			
<i>Lecane bulla</i>	180-190	180-186	Koste and Shiel (1990)
<i>Lecane papuana</i>	100-120	90-170	Khan (2003)
<b>Family: Lepadellidae</b>			
<i>Lepadella</i> sp.	120-160	60-180	Smith (2001)
<i>Lepadella desmeti</i>	100-110	80	Sharma and Sharma (2015)
<i>Trichocerca</i> sp.	120-300	100-500	Brain (2002)
<b>Family: Philodinidae</b>			
<i>Rotaria</i> sp.	340-400	up to 1,5mm	Brain (2002)
<i>Rotaria neptunia</i>	400-550	150-1600	Ricci and Melone (2000)

### 4.5: Cladocera

Cladocera are also known as water fleas inhabiting various kinds of aquatic habitats including reservoirs, rivers, ponds, lakes, oxbows, river floodplains, pools, bogs, ditches, canals, swamps, moorland pools, dune depressions, flooded grasslands and clay pits (Balcer et al. 1984; Błędzki and Rybak 2016a). Cladocerans are phytophilic, benthic and there are only a few identified marine species. In plankton communities, cladocerans fulfill roles as algae grazers (filtrators) and predators (raptors), feeding on particles such as bacteria, detritus, algae and other cladocerans. Predatory species include *Leptodora* and *Pseudochydorus* (Smirnov 2013). In their respective habitats, cladocerans play a critical role in the energy flow of pelagic food webs. Cladocera are very effective indicators when short- and long-term environmental changes are monitored as well as top-down or bottom-up processes (Davidson et al. 2011). This is because of their ecological position in the middle of the food web (Kattel and Sirocko 2011), where they form a link between primary producers and higher trophic levels. These organisms consist of a mixture of littoral, benthic, pelagic and plant-associated taxa, which respond sensitively towards rapid environmental changes (Błędzki and Rybak 2016a).

Fryer (1987) divided Cladocera into four different orders, i.e. Anomopoda, Ctenopoda (also known as Sididae), Haplopoda and Onychopoda. According to Seaman et al. (1999), most species occur in fresh water with pH values between 6.5 and 8.5, with only a few opportunist species found in brackish water. Identification of the specimens collected during the current survey was based on morphological comparison with known records from published literature, including Pérez et al. (1996), Green et al. (1997), Seaman et al. (1999), Mergeay et al. (2005), Sinev (2009), Fernandez et al. (2012), Fuentes-Reinés (2014), Nautiyal et al. (2015) and Rogers et al. (2019).

#### 4.5.1: Family Chydoridae Dybowski & Grochowski, 1894

In this family, species have compound eyes and the bodies are spherical in shape (Nautiyal et al. 2015). The rostrum and fornix jointly form a rounded beak, projecting ventrally in front of the antennules. Both branches of the antennae are three-segmented. On the side of the head, the first antennae are at least partly covered with flanges (Green et al. 1997). Species within this family are benthic (Seaman et al. 1999).

##### ***Alona affinis* Leydig, 1860**

**(Table 4.4; Figs. 4.13A, B)**

##### Characteristics:

*Alona affinis* (Figs. 4.13A, B) have robust, distally broader post-abdomens with parallel margins (Rogers et al. 2019). Distal denticles not conspicuously larger than marginal denticles. Terminal claw has a slender basal spine (Seaman et al. 1999). Distal part of the head shield forms an elongated angle. Two connected head pores present. The inner distal lobe limb number 1 is covered with strong and hook-like setae (Sinev 2009; Rogers et al. 2019). Exopodite of limb bilobed, with seven setae. Exopodite of limb is bilobed. Three setae present on the filter plate of limb number 5. Well-developed thoracic limb number 6 present (Sinev 2009).

##### Ecology:

According to Evans and Stewart (1977), this genus is benthic, occurring in the littoral zone of water bodies. According to Sinev (1997), this species occurs in all continents except for Antarctica. Rogers et al. (2019) mentioned that this species can also be Palearctic.

#### 4.5.2: Family Daphniidae Strauss, 1820

Antennules are usually rudimentary or small. When they are large, they do not protrude from the anterior extremity of the head (Seaman et al. 1999). The first antennae are three times as long as wide (Green et al. 1997). The dorsal rami are four-segmented, and the ventral rami three-segmented. The rostrum in this species is

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prominent, except for in the case of the genus *Ceriodaphnia*. Daphniidae have non-convoluted intestines with two caeca. One or two eggs are normally present in the ephippium. Species within this family are limnetic (Seaman et al. 1999).

### ***Daphnia barbata* Weltner, 1897**

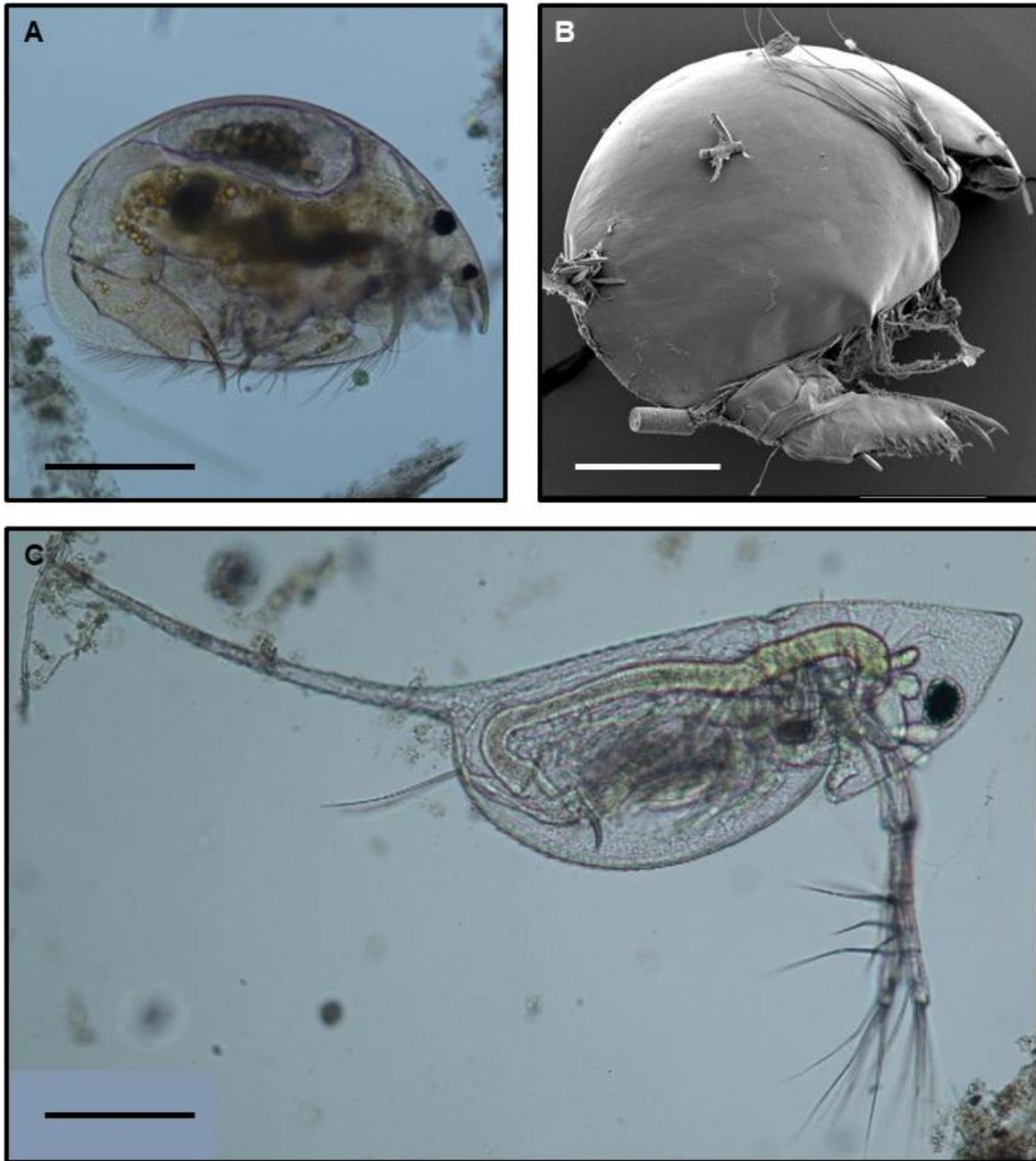
**(Table 4.4; Fig. 4.13C)**

#### Characteristics:

*Daphnia barbata* (Fig. 4.13C) possess a row of spines forming a “moustache” on the rostrum. Mid-dorsal extension of the carapace ending in a slightly expanded button. A ridge is visible running down from the apex of the head to close to where the antennae protrudes (Seaman et al. 1999). At the ventral side, the two valves of the ephippium are always open. This reveals the internal cuticula surrounding the embryos, when viewed from the ventral angle (Mergeay et al. 2005).

#### Ecology:

According to Dumont (1979), Green and Kling (1988) and Green (1990), *Daphnia barbata* is widespread in sub-Saharan Africa but does not occur in the central African tropical rainforests. Dumont (1979) also mentioned that this species was collected from the Nile system as well as from a few North African water bodies.



**Figure 4.13:** Light (A, C) and scanning electron (B) photomicrographs of A, B: *Alona affinis*; C: *Daphnia barbata* collected from Krokovango CTW, Botswana. Scale bars: 100  $\mu$ m.

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### ***Daphnia laevis* Birge, 1879**

**(Table 4.4; Figs. 4.14A, B)**

#### Characteristics:

*Daphnia laevis* (Figs. 4.14A, B) have a crested head which is longer than broad. The ventral margin of the head is nearly straight in lateral view. The posterodorsal margin of the head is concave and the setae of antennules do not reach the tip of the rostrum (Seaman et al. 1999). In females the second abdominal process is much smaller than the first, about  $\frac{1}{4}$  of the length of the first antennule. Very long carapace length, about  $\frac{3}{4}$  the length of the valves or more (Green et al. 1997).

#### Ecology:

According to Mergeay et al. (2005), *Daphnia laevis* is normally found in clear water bodies. *Daphnia laevis* is thought to be cosmopolitan, occurring in North- (Taylor et al. 1998), Central- (Collado et al. 1984) and South America (Paggi 1977), Brazil (Rocha et al. 2011) and various localities all over the world, including the African continent.

### ***Ceriodaphnia dubia* Richard, 1894**

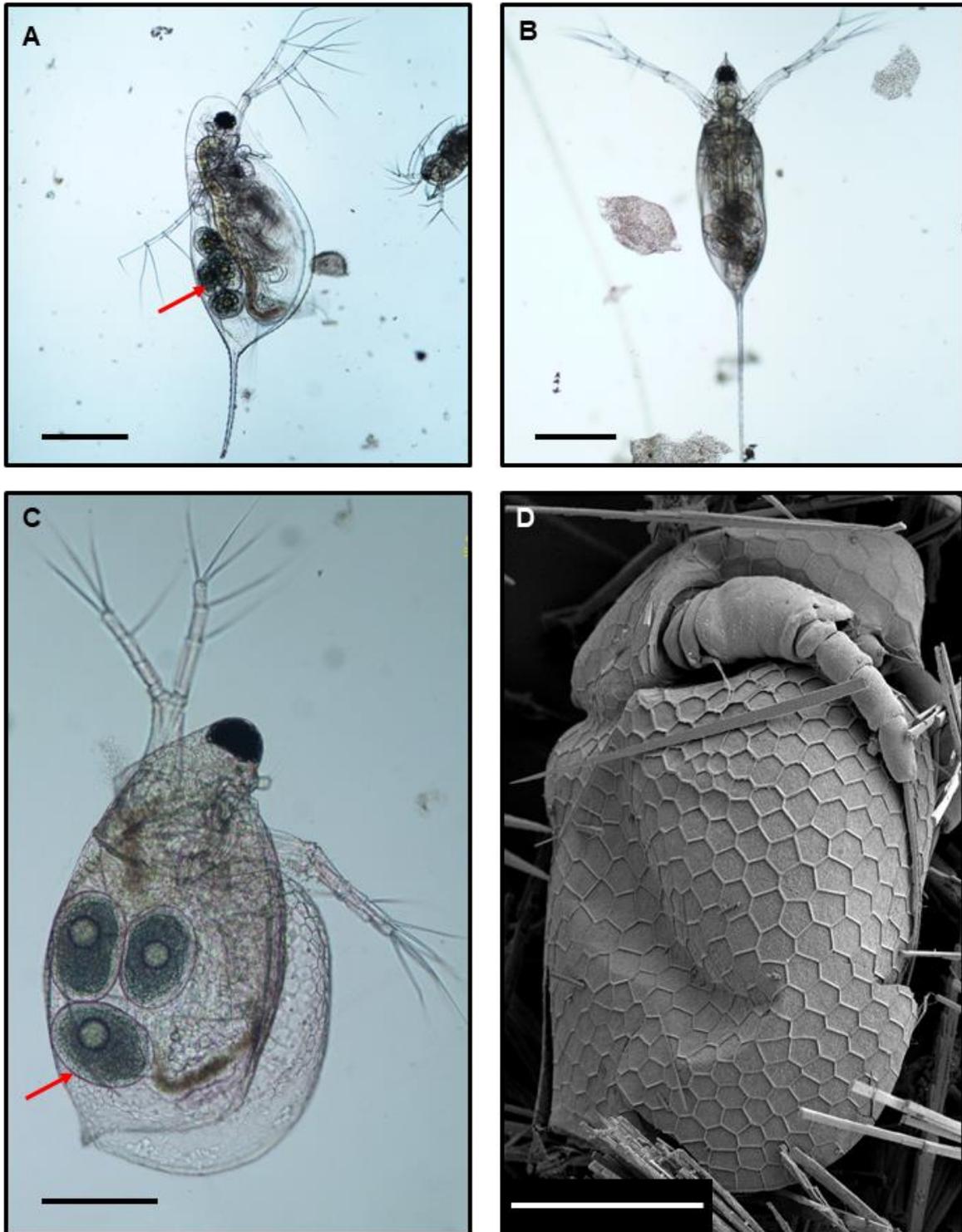
**(Table 4.4; Figs. 4.14C, D)**

#### Characteristics:

*Ceriodaphnia dubia* (Figs. 4.14C, D) have approximately ten anal spines present. Posterior angle of carapace slightly dorsal of the body's midline. The posterior angle normally points directly backwards, rather than upward (Seaman et al. 1999). The central pecten have nearly parallel sides with 8 to 16 narrow teeth with sharp tips (Green et al. 1997). A female carrying three egg sacks can be observed in Fig. 4.14C.

#### Ecology:

According to Rogers et al. (2019), *Ceriodaphnia dubia* is a cosmopolitan species, but revision is needed. *Ceriodaphnia dubia* normally occur in the littoral zone of lakes, ponds and marshes throughout most of the world (Lauridsen et al. 1999).



**Figure 4.14:** Light (A, B, C) and scanning electron (D) photomicrographs of A, B: *Daphnia laevis*; C, D: *Ceriodaphnia dubia* collected from Krokovango CTW, Botswana. Red arrows: Indicating egg sacks. Scale bars: 100  $\mu$ m.

**4.5.3: Family Macrothricidae Norman & Brady, 1867**

Species from this genus have no prominent head. Anteroventrally inserted, large, moveable and inconspicuous antennules at or near the anterior end of the ventral surface of the head (Green et al. 1997; Seaman et al. 1999) (except for *Ilyocryptus*), with simple post-abdominal setae. Distal bident tooth on postabdominal margin absent (Green et al. 1997). Members of this family are usually found in vegetated littoral areas, where they occur near the bottom of water bodies (Seaman et al. 1999).

***Macrothrix spinosa* King, 1853**

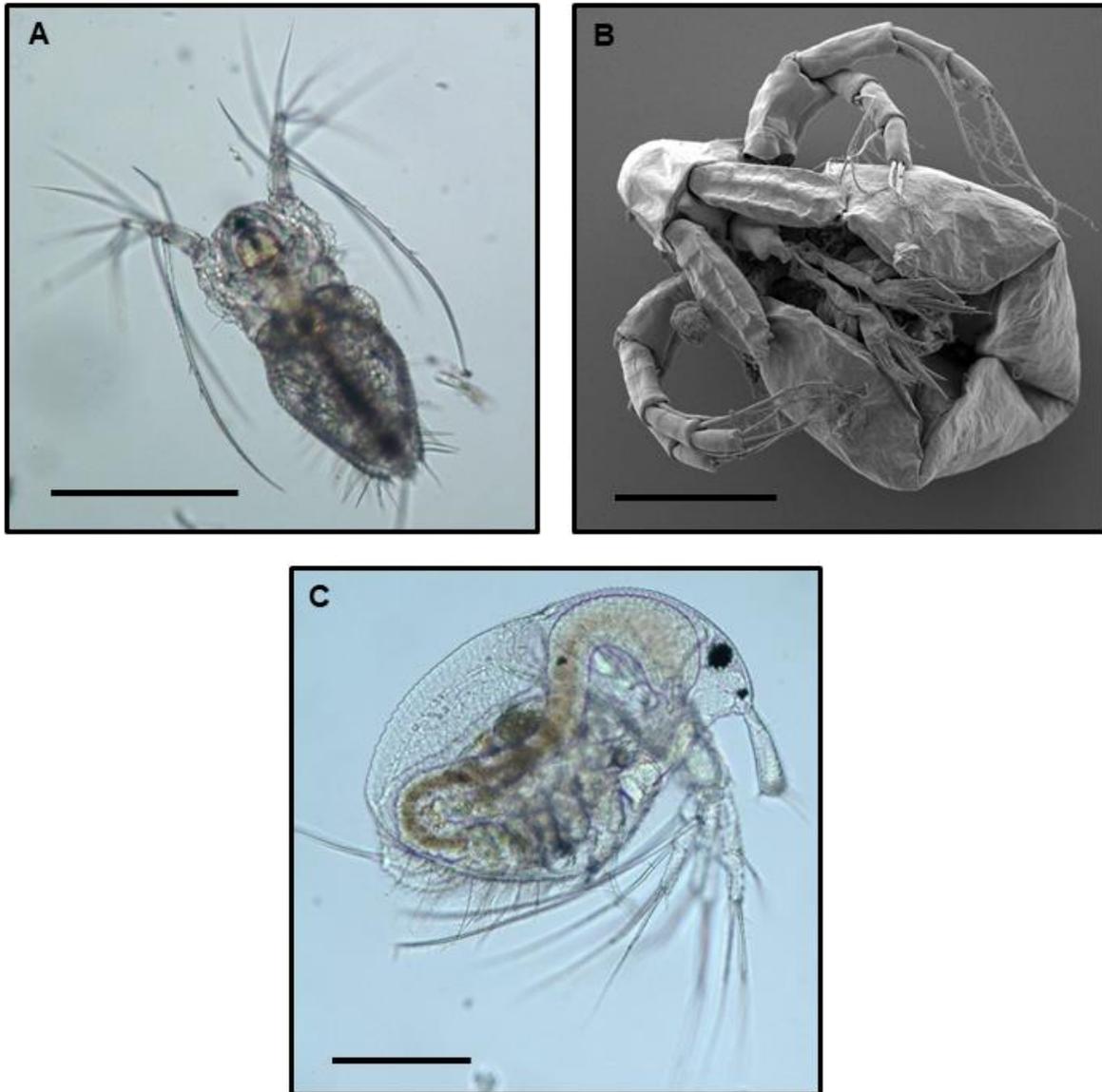
**(Table 4.4; Figs. 4.15A-C)**

Characteristics:

*Macrothrix spinosa* (Figs. 4.15A, B, C) is easily identifiable by the suboval and elongated body (Pérez et al. 1996), antennule dilating distally, with serrations along the dorsal part of the valve (Fuentes-Reinés 2014). The surface of the carapace is covered with closely set squamous ridges (Seaman et al. 1999). Valves on the head are without lateral bulges. Small and serrated head pore with squamose pattern. Head laterally depressed (Pérez et al. 1996).

Ecology:

According to Rogers et al. (2019), *Macrothrix spinosa* occur in central and southern Asia, northern Africa and China, while also being pantropical.



**Figure 4.15:** Light (A, C) and scanning electron (B) photomicrographs of A-C: *Macrothrix spinosa* collected from Krovovango CTW, Botswana. Scale bars: 100  $\mu\text{m}$ .

## CHAPTER 4: RESULTS OF KROKOVANGO CTW - CLADOCERA

**Table 4.4:** Body lengths of cladoceran species collected from Krokovango CTW compared to body lengths from relevant literature.

Species	Body length ( $\mu\text{m}$ ) of specimens collected from the Krokovango CTW	Body length ( $\mu\text{m}$ ) according to literature	Reference
<b>Family Chydoridae</b>			
<i>Alona affinis</i>	300-400	800-1150	Sinev (2009)
<b>Family Daphniidae</b>			
<i>Daphnia barbata</i>	500-600	females up to 1800	Seaman et al. (1999)
<i>Daphnia laevis</i>	400-600	Mean body length: 1300	Gillooly and Dodson (2000)
<i>Ceriodaphnia dubia</i>	300-400	45-747	Fernandez et al. (2012)
<b>Family Macrothricidae</b>			
<i>Macrothrix spinosa</i>	200-350	about 500	Seaman et al. (1999)

## 4.6: Copepoda

### 4.6.1: Order: Cyclopoida Burmeister, 1835

According to Humes (1994) and Balian et al. (2008), there are approximately 2814 freshwater copepod species in the world. Cyclopoida is the most abundant and successful order among the Copepoda (Rayner 2001). The other two orders are Harpacticoida and Calanoida, but these were not collected in the Krokovango CTW. Cyclopoid copepods are primarily benthic (Reid 2001), occurring in all types of habitats such as temporary pools, ponds, lakes, streams, rivers and wells (Rayner 2001). Cyclopoid biodiversity seems to be the highest in the littoral zone when they inhabit larger water bodies. In shallower water bodies, such as wetlands and ponds, their diversity and abundance can also be high (Reid 2001). Cyclopoids have a prostome comprising of a cephalostome, and four free pedigerous somites and an urostome with six somites in both sexes (Rayner 2001). According to Rayner (2001), the female antennules do not exceed 17 segments in free-living freshwater genera. Male antennules are bilaterally geniculate, which is the main distinguishing factor between the sexes. The antennae are uniramous, swimming legs 1-4 are biramous, with the fifth pair being uniramous. The females carry paired egg sacks, compared to females in calanoids and harpacticoids, usually bearing one egg sack (Rayner 2001). Since the 18<sup>th</sup> century, free-living freshwater copepods have been studied intensively and several different classification systems have been proposed since (Błędzki and Rybak 2016b). Only one family was collected from the Krokovango CTW – the Cyclopidae. Identification of the specimens collected during the current survey was based on morphological comparison with known records from published literature, including Fiers and Van de Velde (1984), Van de Velde (1984), Jeje (1988), Baribwegure et al. (2001), Rayner (2001) and Dela Paz et al. (2016).

### **Family Cyclopidae Rafinesque, 1815**

The order Cyclopoida contains 18 families and the Cyclopidae is one of them. This family contains about 35 genera and more than 600 known species have been described worldwide (Hairston and Bohonak 1998). This estimation includes a few marine species, but most genera within Cyclopidae inhabit either brackish- or freshwater habitats (Karaytug 1999).

#### ***Mesocyclops major* Sars, 1927**

**(Fig. 4.16A)**

##### Characteristics:

Female antennules have 17 segments. Spines are absent from the front side of the antennules; basipodite proximal to the implementation of the exopodite seta. The inner distal margin of the basal joint of leg 1 bears no spine (Van de Velde 1984). The 5<sup>th</sup> leg's segment is inserted nearly apically (Dela Paz et al. 2016), bearing armature with three long seta and a long spine on the last segment (Van de Velde 1984). Spine formula of 2:3:3:3 (Rayner 2001). The seta and the spine arise at different levels (Rayner 2001). Setules cover the last thoracic segment laterally. Setules absent from the inner margin of the furcal rami. A row of spines is present on the ventral distal margin of the last thoracic segment, visible between the right- and left 5<sup>th</sup> legs (Van de Velde 1984). This row of spines connect lamella 4 (bearing setules) on the caudal side. Short setules cover the dorsal surface of the genital segment (Van de Velde 1984). *Mesocyclops major* (Fig. 4.16A) individuals collected from the Krovovango CTW averaged 1300 µm in body length. Based on measurements by Rayner (2001), average body lengths can be between 1200-1580 µm.

##### Ecology:

According to Reid et al. (2002), *M. major* are commonly collected from permanent waters such as reservoirs and man-made impoundments. Van de Velde (1984) mentioned that *M. major* is a very successful copepod amongst the species of Africa, and it occurs in all climatic belts. It occurs south of the Sahara, in the Tassilin-Ajjer (Algeria) and in Kufra oasis (Libya) (Van de Velde 1984). According to Rayner (2001),

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*M. major* occurs throughout southern Africa, including Eastern Cape, KwaZulu-Natal, Namibia, Zimbabwe, Cape Peninsula, Free State and Gauteng. This species is also distributed in the Okavango Delta, including permanent off-channel lagoons-, side channels- and rain-filled floodplain wetlands connected to the Okavango river (Hart et al. 2003).

### ***Thermocyclops neglectus* Sars, 1909**

**(Fig. 4.16B)**

#### Characteristics:

Female antennules in *T. neglectus* (Fig. 4.16B) have 17 segments. The 5<sup>th</sup> leg's armature has three long seta and a long spine on the last segment. The seta and the spine arise apically with a spine formula of 2:3:3:3 (Rayner 2001). The basal- and front part of the external margin is covered with spines. The caudal side near the external margin is covered by a row of spines (Fiers and Van de Velde 1984). The inner spine of 3<sup>rd</sup> edopodite; 4<sup>th</sup> swimming legs is less than half the length of the outer most. The receptaculum seminis (spermatheca) has slender lateral arms that are extremely curved on the lateral margin (Jeje 1988). Fig.4.16C shows the bilaterally geniculate pair of antennules characteristic to all male cyclopoid copepods. This is also the main distinguishing factor between the sexes. *Thermocyclops neglectus* individuals collected from the Krokovango CTW averaged 1300 µm in body length. Based on measurements by Baribwegure et al. (2001), average body lengths between 700-773 µm.

#### Ecology:

*Thermocyclops neglectus* is a common species to inhabit African water bodies (Dussart and Defaye 1985). Hart et al. (2003) reported specimens collected from various localities from the Okavango Delta system, including Guma lagoon, Xakanaxa and different rain-filled floodplains connected to the system. Gras and Saint-Jean (1976) and Irvine and Waya (1999) also reported this species from Lake Chad and Lake Malawi, respectively.

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**Figure 4.16:** Light photomicrographs of **A:** *Mesocyclops major* female with *Epistylis* sp. (indicated by the red arrow) attached to the carapace; **B:** *Thermocyclops neglectus* female; **C:** *Thermocyclops neglectus* male with bilaterally geniculate antennules, collected from Krokovango CTW, Botswana. Scale bars: 500  $\mu\text{m}$ .

In the Krokovango CTW the organisms mentioned in this chapter contribute in the breakdown of excess organic material flowing in from the crocodile dams, preventing the wetland from becoming too organically enriched.

### 4.7: Birds, plants and insects recorded from the Krokovango CTW

In CTWs, *Cyperus papyrus* are very effective in the removal of pollutants, while *Phragmites australis* are known to retain more solids (García-Ávila et al. 2019). The oxygen leakage from roots occurring in *Phragmites australis*, stimulates both aerobic decomposition of organic matter and the growth of nitrifying bacteria (Brix 2003). *Typha* species such as *Typha latifolia* have been studied for their capabilities for the removal of biological oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (TSS), nitrogen, phosphorous and pathogens (Coleman et al. 2001; Ciria et al. 2005). *Wolffia arrhiza* is also a useful plant species to use for decontamination of waters from phthalates and other organic compounds (Kotowska et al. 2018). Quantitative analysis was not carried out on the Krokovango wetland's vegetation, but it was clear that the most abundant species observed during sampling were *Phragmites australis*, *Typha capensis* and *Cyperus papyrus*, in that order with approximate wetland coverage of 50%, 30% and 20% respectively. *Wolffia arrhiza* covered about 80% of the water surface (see table 4.5).

Ultimately wetland vegetation play a critical role in wastewater treatment efficacy by maintaining a wetland's treatment capability, especially for systems with high organic matter and ammonia-N content (Ciria et al. 2005).

The study focussed on the microinvertebrates collected from the Krokovango CTW, but the following birds, plants and insects were recorded and identified to acquire a more complete picture of the wetland's biodiversity. Eleven different bird species were observed during the survey period, of which a flock of marabou storks dominated (see table 4.6). Seven different orders and 19 families of insects were recorded from the Krokovango CTW (see table 4.7).

The presence of birds not only adds to the aesthetic value of the Krokovango CTW. Both birds and insects are an indication of the ecological status of the wetland, since both groups are also used as biological indicators (Resh and Cardé 2009; Egwumah et al. 2017).

## CHAPTER 4: RESULTS OF KROKOVANGO CTW – BIRDS, PLANTS & INSECTS

**Table 4.5:** Plant species from the Krokovango CTW identified from Butchard (2016) and Blackmore (2018). Visual estimations of wetland coverage for each plant species were as follow: *Phragmites australis* – 50%; *Typha capensis* – 30%; *Cyperus papyrus* – 20% and *Wolffia arrhiza* covered approximately 80% of the water surface.

Vegetation	Common names
<i>Cyperus papyrus</i>	Papyrus
<i>Phragmites australis</i>	Common reed
<i>Typha capensis</i>	Bulrush
<i>Wolffia arrhiza</i>	Duckweed

**Table 4.6:** Birds from the Krokovango CTW identified using Chittenden (2007). Daily average counts for birds are also provided in the table.

Birds	Common names	Average daily counts
<i>Actophilornis africanus</i>	African Jacana	4.2
<i>Centropus senegalensis</i>	Senegal Coucal	1.7
<i>Ceryle rudis</i>	Pied Kingfisher	0.4
<i>Charadrius tricollaris</i>	Three-banded Plover	5.7
<i>Dicrurus adsimilis</i>	Fork-tailed Drongo	3.6
<i>Haliaeetus vocifer</i>	African Fish Eagle	1.6
<i>Laniarius atrococcineus</i>	Crimson-breasted Shrike	3
<i>Leptoptilos crumeniferus</i>	Marabou Stork	246.3
<i>Scopus umbretta</i>	Hamerkop	5.9
<i>Threskiornis aethiopicus</i>	African Sacred Ibis	28.5
<i>Vanellus armatus</i>	Blacksmith Lapwing	3.6

**Table 4.7:** Insect larvae identified from the Krokovango CTW. Insect larvae were identified from Gerber and Gabriel (2002).

Insect larvae	Common names
Baetidae	Small minnow mayflies
Belostomatidae	Giant water bugs
Ceratopogonidae	Biting midges
Chironomidae	Midges
Coenagrionidae	Narrow-winged damselflies
Coleoptera	Beetles
Corixidae	Water boatmen
Culicidae	Mosquitoes
Diptera	Flies
Dytiscidae	Predacious diving beetles and other species
Elmidae	Riffle beetles and other species
Ephemeroptera	Mayflies
Gerridae	Pond skaters

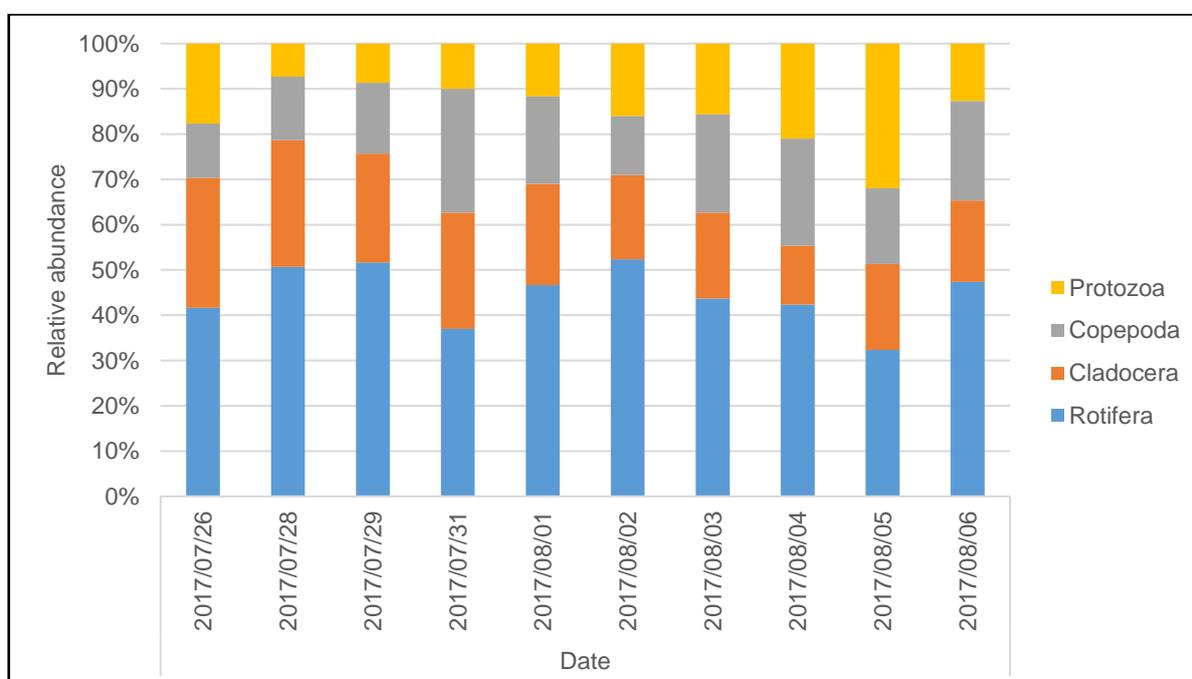
Insect larvae	Common names
Gyrinidae	Whirligig beetles
Hemiptera	True bugs
Hydrophilidae	Water scavenger beetles
Libellulidae	Dragonflies
Naucoridae	Creeping water bugs
Notonectidae	Backswimmers
Odonata	Dragonflies
Perlidae	Stoneflies
Plecoptera	Stoneflies
Pleidae	Pygmy backswimmers
Psychodidae	Moth flies
Tabanidae	Horse flies
Zygoptera	Damselflies

## 4.8: Results on zooplankton abundance

There was a general similarity observed in the taxa relative abundance between the two years of plankton collection. During the 2017 collection Rotifera was the most abundant zooplankton taxa collected from the Krokovango wetland, followed by Cladocera, Copepoda and Protozoa (Fig. 4.17). In 2018, the taxa relative abundance depicted the same hiermethodarchy, except for Copepoda, showing the lowest occurrence, instead of Protozoa (Fig. 4.18).

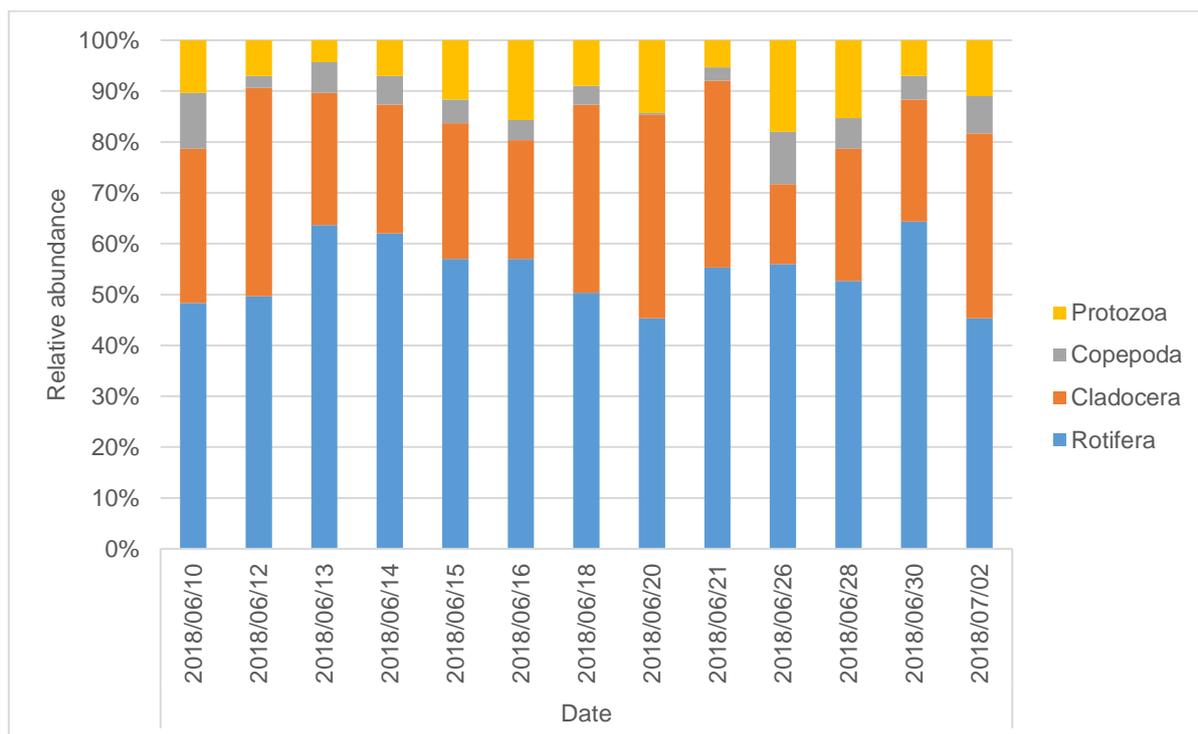
Abundance values of the four taxa over the two years indicated that Rotifera (2017: 44.57%; 2018: 54.38%), Cladocera (2017: 21.63%; 2018: 29.87%) and Protozoa (2017: 15.27%; 2018: 10.46%) populations remained quite similar, while copepod abundance decreased by 13.25% (2017: 18.53%; 2018: 5.28%).

During the 2017 survey, *Brachionus* species were the most abundant wheel animalcules and zooplankton genus collected, depicting a percentage of 13.23%. *Alona affinis* was the most abundant cladoceran found in 2017, with a relative abundance value of 11%. In the same year the highest relative abundance percentage amongst Protozoans was represented by *Paramecium* sp., with a value of 4.63%.



**Figure 4.17:** Relative abundances of Rotifera, Cladocera, Copepoda and Protozoa collected from the Krokovango CTW, Botswana, during 26 July – 6 August 2017.

During the 2018 survey, the most abundant rotifer species collected was *Platyias patulus* with a percentage of 13.95%. *Alona affinis* was the most abundant Cladoceran species with a value of 11.79%. Copepod numbers drastically decreased from 2017 to 2018. *Thermocyclops neglectus* was the most abundant copepod species collected during the survey, showing a decrease from 10.1% to 3.26% from 2017 to 2018. *Paramecium* sp. was the most abundant protozoan collected during the 2018 survey as well, with a value of 3.46%.



**Figure 4.18:** Relative abundances of Rotifera, Cladocera, Copepoda and Protozoa collected from the Krokovango CTW, Botswana, during 10 June - 2 July 2018.

To compare the taxa, Tukey’s Honest Significant Difference test was used to acquire a significance value of smaller than 0.0001 ( $p < 0.0001$ ), which is below 0.05 and, therefore, there was a statistically significant difference in the mean abundance between the different taxa. Comparing taxa and year, a significance value of  $p < 0.0001$  was obtained, also indicating changes of taxa over the course of the study.

Based on Tukey’s Honest test, there were statistically significant differences observed in abundance between the following taxa during the 2017 survey: Cladocera vs Copepoda ( $p < 0.0001$ ); Cladocera vs Protozoa ( $p < 0.01$ ); Copepoda vs Protozoa

( $p < 0.0001$ ) and Copepoda vs Rotifera ( $p < 0.0001$ ). Cladocera vs Rotifera had a significance value of  $p = 0.6724$ , indicating no significant differences between these taxa during 2017.

Within 2018, the following statistically significant differences in abundance were observed between taxa: Cladocera vs Copepoda ( $p < 0.05$ ); Cladocera vs Protozoa ( $p < 0.0001$ ); Copepoda vs Rotifera ( $p < 0.0001$ ) and Protozoa vs Rotifera ( $p < 0.0001$ ). In 2018 no significant differences were observed between Cladocera vs Rotifera and Copepoda vs Protozoa, having significance values of  $p = 0.8986$  and  $p = 0.5839$  respectively.

Comparing data from the two years, no statistically significant differences were observed for Protozoa ( $p = 0.9859$ ), Cladocera ( $p = 0.3614$ ) and Rotifera ( $p = 0.4424$ ), but Copepoda had a significant value of  $p < 0.0001$ , indicating that abundance changed for this taxon.

## 4.9: Physical water quality

(Table 4.7)

The average values for dissolved oxygen saturation decreased from 2017 (34.94%) to 2018 (12.4%). The pH values remained stable throughout the study ranging between 6.18 and 7.21. Electrical conductivity ranged between a minimum value of 20.7 mS/cm<sup>-1</sup> and a maximum value of 63.9 mS/cm<sup>-1</sup>. Average temperature measured during 26 July – 6 August 2017 was greater than the average temperature measured during 10 June – 2 July 2018. West et al. (2015) measured water quality in the upstream region of the Okavango Delta and these measurements are given in Table 4.7 below, along with measurements of the Krokovango CTW.

**Table 4.7:** Physical water quality range values of the Krokovango CTW from 2012 to 2018 and Okavango Delta up until 2015.

Physical water quality parameters	Krokovango CTW				Okavango Delta (Upstream region) West et al. (2015)
	2012 (Van As and Van As 2016)*	2016 (Van As and Van As 2016)*	2017	2018	-2015
			Current study		
Dissolved oxygen (%)	15	42	8.5-86	10.1-16.7	18.4-179.5 (July 2008)
pH	6.6	6.2	5.56-7.12	6.18-7.21	6.09-7.38
Conductivity (µS/cm <sup>-1</sup> )	36.9	16.0	20.7-63.9	24.8-35.2	27.0-59.0
Temperature (°C)	18	18	14.92-26.17	12.49-17.76	14.91-27.27 (July 2008)

\*Data from unpublished reports

## CHAPTER 5: DISCUSSION

### 5.1: CTWs and the Krovovango CTW

According to Gopal and Junk (2000), the biodiversity of wetlands and other freshwater systems is poorly understood, and this gives rise to various challenges, since many wetland values are linked to biodiversity of wetlands.

The wetland at Krovovango cannot be fully recognised as a true constructed treatment wetland. This is because it does not fit into any of the known criteria for the classes of the different types of CTWs, as described by Kadlec and Wallace (2008). The Krovovango wetland can be described as a vertical surface flow wetland (hybrid system), which is a unique scenario since wastewater enters, but only leaves the system by means of evapotranspiration and filtration to the groundwater table.

According to Nivala et al. (2013), the more aerobic conditions of vertical flow CTWs reduce total nitrogen removal, since denitrification is affected. Despite possible poor nitrogen removal, the removal of suspended solids and organic material are very efficient in vertical CTWs (Brix and Arias 2005). Sellami et al. (2009) mentioned that it is possible for the accumulation of solids near the surface of the bed to reduce the hydraulic conductivity of the media, which might be the reason why vertical flow CTWs are so efficient in suspended solid removal. This might also be part of the reason why the Krovovango CTW is so effective at removing suspended crocodile waste.

It is also important to mention that the Krovovango wetland is built on Kalahari Desert sand, as this might also act as an effective natural filter system. Oxygenation of the system is limited when attached suspended solids are too high in the filtration media, which might decrease organic material- and nitrogen removal, as was explained by Sellami et al. (2009).

In vertical flow constructed treatment wetlands, beds are normally sand based. In Germany, the Langenreichenbach Ecotechnology Research Facility designed four layered sand-based systems to treat wastewater (Nivala et al. 2013). According to Saeed and Sun (2012), the substrate of choice would be gravel, since it increases the

## CHAPTER 5: DISCUSSION – CTWs AND THE KROKOVANGO CTW

hydraulic conductivity of the media. It was, however, confirmed by Ávila et al. (2014), that gravel-based systems were outperformed by sand-based systems for the removal of conventional pollutants and -emerging organic contaminants. Since the Okavango Delta is situated on Kalahari Desert sand, it makes the construction of more artificial wetlands easier and cheaper.

It was only during the 1990s that Europe started recognising vertical CTWs for their oxygenation capacity and higher effluent quality (Cooper 2009). Since then vertical flow CTWs have been implemented all over Europe, especially for domestic wastewater treatment from small communities (Sani et al. 2013). Presently, these systems are used as small secondary treatment of domestic sewage (Cooper 2009).

Zhao et al. (2011) mentioned that clogging tends to be a problem in vertical flow CTWs. Clogging is a seasonal occurrence triggered by increased macrophyte growth because of intensified nutrient uptake. At the end of the growing season senescence also generates extra litter, exacerbating the problem and ultimately changing the performance of the system (Sani et al. 2013). The nature for these systems to clog is, however, the reason for their treatment capabilities and therefore it is important to distinguish between natural clogging and systems in which performance is affected by clogging. According to Pucher and Langergraber (2019), clogging contributes to the growth of microorganisms, but needs to be monitored in order to prevent malfunctioning.

Possible signs of clogging, as described by Petitjean et al. (2016), include low concentrations of nitrate within effluent and lower than usual dissolved oxygen concentrations, along the depth of the bed. The main source of oxygen in these systems is transfer from the atmosphere (Ye et al. 2012). The Krokovango CTW show minor signs of clogging by having low average dissolved oxygen concentrations and the wetland was slightly enlarged in 2018 to prevent overflowing. Organic degradation is the major source of dissolved oxygen consumption in the upper parts of the bed. Subsequently dissolved oxygen is used for nitrification near the roots, and this contributes to the redox potential of the system (Petitjean et al. 2016).

## CHAPTER 5: DISCUSSION – CTWs AND THE KROKOVANGO CTW

The effectiveness of the Krokovango CTW might be improved by removing some of the sand banks and making the water level deeper in certain areas. A depth of 1.5m is recommended by Bremen Overseas Research and Development Association (BORDA) guidelines available from Sasse (1998). The Krokovango CTW is 1.5m deep in certain areas, but other areas are quite shallow ranging from 0.1m to 0.5m.

Based on claims made by Buckley and Arumugam (2016), the hydraulic loading rate of the Krokovango CTW is not constant, causing increased resting periods for the bed. The sandy area ensures longer contact time of the influent with microbial populations because of lower infiltration rate compared to gravel media. This is good, since longer contact time provides microbial populations more time for nutrient transformation (Lee et al. 2009).

Wu et al. (2015) concluded that overall treatment efficiency is mainly dependant on vegetation- and media type. The most reactive zone of a wetland is within the rhizosphere (Stottmeister et al. 2003). An oxidation-reduction opportunity for aerobic dependant reactions are created when plants release oxygen near the roots. These reactions include nitrification, and the uptake of nutrients (Tanner 1996). Nutrient storage, however, is only temporal, since much of these nutrients are often released back into wetlands at the end of a plant's life cycle (Verhoeven and Meuleman 1999).

Since the construction of the Krokovango CTW in 2012, no extra vegetation has been added to the water bodies. In the past few years, a positive change in the aquatic vegetation has been observed, which is an indication of the effectiveness of this artificial wetland. The wetland has been slightly enlarged, since the original construction, to allow more surface area to be exposed. This was done to improve the process of purification. Currently the wetland consists out of three separate water bodies, with an elevated wall, divided by small patches of sand and some terrestrial vegetation.

The wetland at Krokovango is partly covered with common reed (*Phragmites australis*), bulrush (*Typha capensis*), duckweed (*Wolffia arrhiza*) and papyrus (*Cyperus papyrus*). The Okavango River is mostly covered with *Cyperus papyrus*, while the Krokovango CTW have more *Phragmites australis* growing in the shallow

## CHAPTER 5: DISCUSSION – CTWs AND THE KROKOVANGO CTW

waters. *Phragmites australis* are known for their root systems being extensive, and longer lived. The common reed is also very competitively successful compared to other species and they are the most common species of choice for wastewater treatment in constructed treatment wetlands (Tanner 1996; Saeed and Sun 2012). The Krokovango CTW is only partly covered with *P. australis*, and it might be beneficial to add more of this species for improved results. The Krokovango is also close to the Samochima lagoon, where *P. australis* can easily be harvested.

In a study conducted by García-Ávila et al. (2019), *C. papyrus* outperformed *P. australis* in treating municipal wastewater from the city of Santa Isabel, Ecuador. The experiment was conducted on the waste treatment plant of El Guabo, located next to the city, where they used a vertical subsurface flow setup for both plant species. Although *P. australis* is by far the most popular choice for CTWs, the El Guabo study indicated the rising potential of other plant species and the need for research to maximise potential for these systems all over the world.

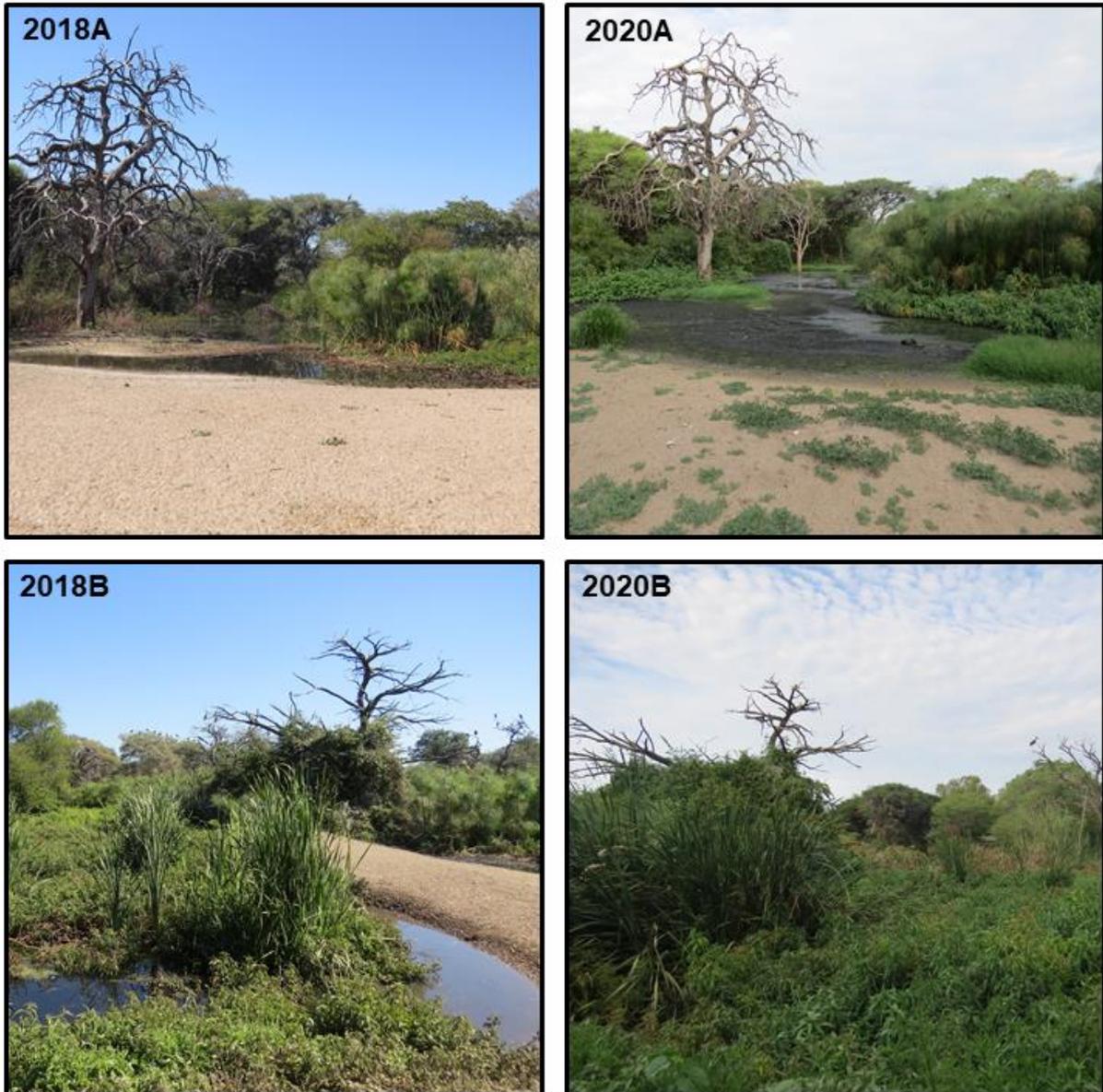
Some small additional maintenance in the Krokovango CTW will be required from time to time. Such as the control of the castor-oil trees (*Ricinus communis*), as they add no value to the effectiveness of the wetland. More space should be created for *C. papyrus*, *T. capensis* and *P. australis*.

During the 2018 survey, a large portion of the water surface of the Krokovango CTW was covered with the smallest known flowering plant, duckweed (*Wolffia arrhiza*). Vegetation covering water bodies prohibits penetration of sunlight into the water column, hindering algal photosynthesis (Altieri 2019). It might be a good idea to monitor and control the duckweed growing on the water surface of the Krokovango CTW from time to time.

Eutrophic conditions are natural in treatment systems such as this but can cause problems in waters containing fish and other organisms sensitive to eutrophication. An extreme example would be the “dead zone” in the Gulf of Mexico, which occurred as a result of excess nutrients flowing in from the Mississippi River causing too much primary production (Altieri 2019).

## CHAPTER 5: DISCUSSION – CTWs AND THE KROKOVANGO CTW

The main supervisor of this dissertation visited Botswana briefly in January 2020 and provided the author with two more recent images of the Krovovango CTW (Fig. 4.19). By comparing it to images of 2018, the 2020 images indicate the extreme dynamics of this ecosystem and the extent to which it is capable of transformation in such a short period of time. It is also worth noting that the more recent images were taken at the start of the summer rainy season.



**Figure 4.19:** Illustrating the Krovovango CTW transformation from 2018 to 2020.

## 5.2: The importance of phytoplankton and microinvertebrates found in the Krovovango CTW

### 5.2.1: Phytoplankton of the Krovovango CTW

From the Krovovango CTW, 22 species belonging to four different phyla were collected. Most of the taxa collected commonly occur in various freshwater systems and many species are common in eutrophic systems like the Krovovango CTW. As primary producers, the study of phytoplankton is crucial to understanding bottom-up and top-down processes occurring in wetlands and other aquatic ecosystems (Stephen et al. 2008; Bellinger and Sigeo 2010). Algae are one of the three major groups of photosynthetic organisms within freshwater environments, where they generate biomass and fix carbon (Bellinger and Sigeo 2010; Khangembam et al. 2018). Size and taxonomy distinguish algae from higher plants or macrophytes, while biochemistry distinguishes algae from photosynthetic bacteria. The level of primary production in freshwater ecosystems varies, depending on trophic status and depth within the water column (Lampert 1978; Bellinger and Sigeo 2010).

According to Lewandowski et al. (2018), primary production is directly influenced by the availability of nutrients. The continuous inflow of nutrient enriched crocodile wastewater into the Krovovango CTW may cause too much primary production. It was observed throughout the study period that phytoplankton were the most abundant organisms, which is not surprising since they form the base of the ecological food web (Humphrey 2019).

Tuchman (1996) mentioned that some algal species have become secondarily heterotrophic, meaning that they can absorb complex organic compounds, either by active ingestion of particulate material or absorption over their outer surface. These secondary heterotrophic algae are often very mobile, ingest organic material and/or lack chlorophyll, resembling protozoans. Examples of secondary heterotrophic algae from the Krovovango CTW include: *Chlorococcum* spp., *Chlorogonium* sp., *Micractinium* sp., *Scenedesmus* sp., *Pediastrum duplex*, *Volvox* sp., *Euglena* sp., *Nitzschia* sp., *Anabaena* sp., *Arthrospira* sp., *Merismopedia* sp., *Microcystis* sp. and *Oscillatoria* sp. (Bellinger and Sigeo 2010).

Presence or absence of certain species can be useful to indicate the ecological status of water bodies. Colonial blue-green algae, such as *Microcystis* and *Anabaena* spp. are particularly useful as indicators of high nutrient status. *Microcystis* species also occur in mesotrophic environments (Bellinger and Sigeo 2010). Based on the occurrence of these species and the fact that the Krovovango CTW receives nutrient rich crocodile wastewater, it can be concluded that the system is, at least partly eutrophic.

According to Basavaraja and Parameswara Naik (2018), blue-green algae are extremely important in the nitrogen cycle, as nitrogen fixers. These cyanobacteria transform atmospheric nitrogen into fixed nitrogen in the form of inorganic compounds used by plants (Gaysina et al. 2019). Cyanobacteria are therefore important in systems such as the Krovovango CTW, as it prevents serious nutrient pollution. Mateo et al. (2015) emphasised that the presence of cyanobacteria should not always be regarded as a sign of ecological decline. Certain species are bioindicators of clean waters and are used to monitor running streams as well (Mateo et al. 2015).

Contemporary green algae are mostly utilised to gather information on physicochemical characteristics of aquatic ecosystems. Environments stressed by eutrophication, acidification and metal contamination will often be a habitat to filamentous green algae, such as *Anabaena*, *Arthrospira* and *Oscillatoria* spp. (Bellinger and Sigeo 2010; Mateo et al. 2015). These species also occurred in the Krovovango CTW. Certain species of the genus *Oscillatoria* are capable of toxin production, including toxins such as microcystins, anatoxina, lipopolysaccharides and aplysiatoxins (Chorus and Bartram 2002).

According to Bellinger and Sigeo (2010), euglenoid algae are not very useful as environmental bioindicators. They also mentioned that yellow-green algae are not a prominent group in the aquatic environment, and this is part of the reason why they are very seldomly used as bioindicators. John et al. (2002) discussed different species that could provide information on ambient conditions, but *Euglena*- and *Phacus* species were excluded from their list. These species might thus not be very useful as bioindicators in the Krovovango CTW.

Palmer (1969) conducted research on four selected organically polluted sites. The first site in the study of Palmer (1969) was a sewage stabilisation pond and the following species were collected from this pond and from the Krovovango CTW: *Euglena*, *Micractinium*, *Nitzschia*, *Phacus* and *Scenedesmus*. The second site was Greenville Creek in Ohio where *Euglena*, *Nitzschia*, *Oscillatoria* and *Navicula* spp. were collected from this site and from the Krovovango CTW. *Navicula* and *Scenedesmus* spp. were collected from Grand Lake, Ohio and the Krovovango CTW. The last site was Lake Salinda in Indiana, but none of the species collected there occurred in the Krovovango CTW, possibly because of the lake showing no organic enrichment at that stage.

Palmer (1969) also made a top ten list of the most pollution tolerant algal genera and the Krovovango CTW contained six of these species, including *Euglena*, *Oscillatoria*, *Scenedesmus*, *Nitzschia*, *Navicula* and *Stigeoclonium* (listed from highest to lowest pollution tolerance).

During the study one specimen of *Cosmarium* sp. was observed from samples collected from the Krovovango CTW, and according to Bellinger and Sigeo (2010), their presence is indicative of mesotrophic conditions. This might indicate that the Krovovango CTW have some mesotrophic characteristics, but since only one specimen was found, the signs of eutrophication are notably stronger.

Bellinger and Sigeo (2010) mentioned that *Pediastrum* and *Scenedesmus* spp. can be found in a wide range of conditions, including eutrophic to hypertrophic conditions and saline waters. *Volvox* spp. commonly occur in eutrophic environments and *Anabaena* spp. can be collected from meso- to eutrophic conditions, commonly occurring in organic debris. (Bellinger and Sigeo 2010).

After phytoplankton, zooplankton forms the second level of the trophic structure within freshwater ecosystems, where they form a link between primary producers and other higher forms. The following part of the discussion will discuss the microinvertebrates collected from the Krovovango CTW.

### 5.2.2: Protozoa of the Krovovango CTW

One genus in the phylum Amoebozoa was collected from the Krovovango CTW, namely *Amoeba*. The other 9 species collected fall under Ciliophora.

According to Laybourn-Parry (1984) and Horan (2003), a wide range of trophic levels are occupied by free-living protozoans. Some unicellular and filamentous forms feed on algae within the herbivore food chain as primary consumers (Pace and Orcutt 1981; Laybourn-Parry 1984). Other protozoans are microbivores and ingest bacteria for sustenance, thus also playing an important role in the processing of dead organic matter and improving water quality (Jagadeeshappa and Kumara 2013). These algae- and bacteria consuming protozoans named above, also serve as food sources to predacious protozoan species (Laybourn-Parry 1984). Protozoans are therefore an important component in the Krovovango CTW, since it assists in organic decomposition and water quality improvement.

According to Nisbet (1984), the volume of available organic matter usually determines the number of organisms and diversity of protozoans found in the water column of freshwater habitats. In eutrophic water bodies richer communities of organisms occur compared to oligotrophic waters. Patterson (2018) mentioned that organism richness increases during the productive seasons. Microbial activity leads to aggregates of bacteria and detritus in more enriched water bodies. In these environments, aggregates might support more protozoan diversity, resembling what is found in and on the benthos area (Sanders 2010; Patterson 2018). The Krovovango CTW comprised of rich organism communities, adding to the evidence of eutrophication due to high volumes of organic matter in the system.

According to Laybourn-Parry and Wadham (2014), autotrophic and heterotrophic flagellates are usually present in the water column, and this was also observed in the Krovovango CTW. Heterotrophic flagellates such as euglenoids mainly consume bacteria, while other flagellates are ingested by the larger ciliates such as *Paramecium* (Nisbet 1984).

Da Anunciação et al. (2019) noted that *Euplotes* sp. might be present in water bodies with low oxygen levels. These species might contain endosymbiotic green algae, which was observed in the *Euplotes* samples collected from the Krovovango CTW. Photosynthates produced by these ingested algae, can be utilised as nutrients by their hosts. The endosymbiotic algae also produce oxygen, which might assist in the survival of the host during environmentally stressful periods (Patterson 2018).

According to Fenchel (1987), some protozoan communities attach themselves to submerged surfaces permanently. These attached species normally use flagella or ciliates to remove food (suspended particles) from their surrounding environment. The flagella or cilia creates a flow of fluid to filter and extract particles such as bacteria and algae. An example collected from the Krovovango CTW includes the peritrich ciliate *Vorticella* sp.

Heliozoa and Suctoria rely on the prey organism's movements. In this case, flagellates and small ciliates are trapped after colliding with the adhesive arms containing extrusomes (Krugens et al. 1994; Verni and Gualtieri 1997). *Podophyra* sp. is a suctorian ciliate collected from the Krovovango CTW. Smaller protozoans, such as flagellates and peritrich ciliates feed on bacteria, while larger forms feed on other protozoa. Hypostome ciliates, hypotrich ciliates as well as bodonid- and euglenid flagellates feed on bacteria, diatoms, algae and filamentous cyanobacteria (Patterson 2018).

Different ecological properties are also represented by the different phyla (Cavalier-Smith 1997). Since amoebozoans are so abundant among soil protozoa, they are considered as a very important group (Berthold and Palzenberger 1995). In this dissertation, one genus, *Amoeba* was identified and since identification did not progress to species level, no accurate ecological assumptions could be made based on their presence in the Krovovango CTW.

Within the benthos, energy is obtained in two ways: organic matter settling from above and from organisms photosynthesising in the sediment (Patterson 2018). In organically rich water bodies such as the Krovovango CTW, the benthos also becomes enriched. These conditions give rise to much higher physiological demands from

organisms, and often species occurring there need to be tolerant to these low levels of oxygen (Covich et al. 1999).

Patterson (2018) mentioned that bacteria and dissolved organic matter are the main food sources in organically rich sediments, therefore most protozoan species in these types of sediments are bacterivores (feeding on bacteria) or osmotrophs (feeding on dissolved organic compounds). Usually protozoan species occurring here can be found in most permanent bodies of water. A good example would be *Paramecium*, also known to grow readily in culture (Patterson 2018).

According to Wilpiseski et al. (2019) microbial communities are essential in organic rich environments, since they degrade organic matter and assist in phosphorus and nitrogen cycling. Microbial communities are dependant on oxygen to carry out these processes (Paerl et al. 2003). Oxygen levels are quite low in the Krovovango CTW and exposing more water surface will allow the system to become more oxygenated, improving microbial activity.

At a certain depth, whether it is above the sediment or many meters below it, the habitat becomes anoxic. In other words, at this point no oxygen is available for microbial respiration (Hershey and Lamberti 2001). At this depth, other compounds are used by microbial communities to act as a terminal electron-acceptor. These compounds include carbon dioxide and various sulphur compounds. Some ciliates collected from the Krovovango CTW, such as *Spirostomum* spp. have adapted to these types of reduced habitats by adopting aerobic metabolism, in order to maintain the energy flow between anoxic- and oxygenated zones as was found by Patterson (2018).

### **5.2.3: Rotifera of the Krovovango CTW**

Twelve species of Rotifera were found from the Krovovango CTW, belonging to 5 different families. Rotifera is one of the most diverse invertebrate phyla (Wallace et al. 2006) and are commonly dominant in eutrophic waters (Apaydin Yağci 2016), as was also determined in the present study at Krovovango. Rotifers are important role players in freshwater systems, where they have high production and short

developmental rate with a fast turnover as they circulate matter, which assists in energy transfer within ecosystems (Li et al. 2005). Within freshwater ecosystems, wheel-animalcules help maintain ecological balances by forming natural food links between primary producers and consumers such as crustacean larvae and small zooplanktivorous fish (Wallace 2002). Wheel animalcules are highly nutritious food sources for these higher organisms (Bhandarkar 2015).

Rotifers constitute a significant planktonic component of secondary production, especially in the structure of lentic ecosystems, where they can occur in pelagic-, periphytonic-, littoral- and benthic forms. Rotifers are ciliary filter feeders and in the trophic food web they primarily feed on phytoplankton (Casanova et al. 2009; Bhandarkar 2015). According to Wallace and Snell (2001) and Wallace et al. (2006), most rotifers are opportunistic feeders, although some species are highly specialised in what they ingest. Species like *Asplanchnopus* are semi-pelagic, where they feed on smaller cladocerans, other rotifers and ciliates (Koste 1978).

According to Yin et al. (2018), rotifers can be characterised by their physical and chemical attributes allowing them to inhabit diverse habitats, while also serving as indicators of nutrient levels. The species composition of wheel animalcules is easily influenced by environmental changes within ecosystems, therefore ecological characteristics of wheel animals can assist in determining water quality (Dodson et al. 1976; Yin et al. 2018).

Arora (1966) mentioned that wheel animalcules are relatively tolerant of minor environmental changes and they are capable of rapid exploitation of suitable environments, making them effective biological indicators. In fluvial drainage systems, rotifers serve as food for higher organisms and can also serve as indicators of nutrient status (Arora 1966; Sládeček 1983; Berzins and Pejler 1989). Trophic status strongly influences population dynamics of wheel animalcules (Duggan et al. 2001).

Salinity is an important factor in determining rotifer community structure (Green 1993). Water temperatures also play a role in community structures, especially in subtropical climates, due to dramatic seasonal changes (Wen et al. 2011). Wen et al. (2011) also mentioned that *Brachionus*, *Lecane* and *Trichocerca* spp. are known to dominate in

tropical climates. All three genera were present in the Krovovango wetland, with *Brachionus* spp. being the most abundant rotifer species collected throughout the study period.

Attayde and Bozelli (1998) mentioned that *Brachionus* species are very useful in monitoring water quality more intensively than most other genera. The community structure of this genus not only assist in determining the level of pollution, but can also show trends in other environmental conditions over certain periods of time (Bhat et al. 2014).

Rotifer abundance and diversity increases with an increase of eutrophication but decrease in hyper-eutrophic conditions (Green 1993; Wen et al. 2011). Food sources such as algae, bacteria, phytoplankton and smaller ciliates are more abundant in slightly eutrophic environments, and for this reason rotifers can flourish under these conditions (Bonecker and Aoyagui 2005; Wen et al. 2011).

Sládecek (1983) mentioned that *Brachionus* and *Trichocerca* abundance can be compared to indicate the trophic status of water bodies, since *Brachionus* species are associated with eutrophic conditions and *Trichocerca* species are associated with oligotrophic conditions. Results of 2017 and 2018 indicated that the Krovovango CTW is far more eutrophic than oligotrophic. Comparing only these two genera with one another, the ratios for “*Trichocerca: Brachionus*” collected from the Krovovango CTW in 2017 and 2018 were 1:2.88 and 1:1.71, respectively. Apaydin Yağci (2016) mentioned *Trichocerca* species to be an indicator of eutrophic conditions, nevertheless.

The ability of *Brachionus falcatus* and *Platyias patulus* to evaluate organic pollution in Ouémé River’s basin (Republic of Benin) were tested in a study conducted by Houssou et al. (2018), where abundances were also high. Since *Brachionus* species were the most abundant wheel animalcules collected from the Krovovango CTW, they serve as a valuable indication of organic enrichment.

### 5.2.4: Cladocera of the Krovovango CTW

It was noted that cladoceran specimens collected from the Krovovango CTW were quite smaller in size than specimens collected from previous studies, seen in Table 4.4. Four cladoceran species from 3 families were collected and identified from the Krovovango CTW. From a global point of view, four is an extremely low species count. *Alona affinis* was by far the most abundant crustacean collected throughout the study. West (2016) noted that *Daphnia* species are relatively absent from the Okavango Delta system while Seaman et al. (1999) mentioned that *Daphnia* is the most speciose in South Africa.

*Daphnia* spp. are important in trophic dynamics of freshwater ecosystems where they consume algae, detritus and bacteria as food sources (Pinto-Coelho 1991; Pinto-Coelho et al. 2003). According to Scavia and Fahnenstiel (1988), the presence of *Daphnia* creates a link to the microbial loop by removing both autotrophic and heterotrophic particulate organic carbon directly from certain microbial trophic levels and transporting it to the conventional food web.

*Daphnia laevis* was collected in low abundance from the Krovovango CTW. Based on observations made by Pinto-Coelho et al. (2003), eutrophication decreases food quality (algae) and this might have been the key factor that influenced low *Daphnia laevis* abundance in the Krovovango CTW. *Daphnia* species are quite large in size, compared to other cladocerans. Large body size usually makes plankton more susceptible to predation by juvenile fish and small fish species. This might also be the reason why they are present, since fish are completely absent from the Krovovango CTW. Seaman et al. (1999) also mentioned that *Daphnia* are limnetic, further increasing predation risks by normally residing in localities with little to no vegetation and thorough light penetration. *Alona affinis* and *Macrothrix spinosa* are also littoral dwellers. *Ceriodaphnia dubia* are littoral at night and move to more open water (photic zone) during the day (Threlkeld 1979; Wright and Shapiro 1990; Smiley and Tessier 1998).

An increase in blue-green algal filaments such as *Anabaena*, can cause a decrease in *Daphnia* occurrence. This may be due to *Daphnia* rejecting food, which

consecutively leads to higher respiration rates and ultimately restraining larger *Daphnia* spp. (Webster and Peters 1978; Porter and McDonough 1984). This phenomenon was observed in the Krovovango CTW, when *Daphnia* populations decreased with an increase in blue green algal filaments such as *Anabaena*, *Arthrospira* and *Oscillatoria* spp. from 2017 to 2018.

Cladocerans have adapted remarkably to eutrophic conditions, and often small *Daphnia* spp. are seen dominating in these ecosystems, which was proved in a study conducted by Haberman et al. (2007). In their study the role of cladocerans in having a trophic status was studied and compared between a moderately eutrophic Lake Peipsi, and a strongly eutrophic lake Võrtsjärv in Estonia, Europe. They found that cladocerans are useful indicators of the efficiency of food webs and trophic status in Estonian large shallow lakes.

Based on a study conducted by Zawiska et al. (2016), *Alona affinis* occurrence ranged from dystrophic (acidic) to neutral waters and this species do not seem to be heavily affected by pH values. According to Nevalainen (2011), they are adapted to survive in medium depth waters, and this might be part of the reason why they thrive in these dystrophic conditions. This was observed in the Krovovango CTW when vegetation growth dramatically increased in 2018, leading to more humus and an increase in *Alona affinis* population.

### 5.2.5: Copepoda of the Krovovango CTW

Only two species (*Mesocyclops major* and *Thermocyclops neglectus*) within one family (Cyclopidae) of Copepoda was collected from the Krovovango CTW. It was noted by Tönno et al. (2016), that cyclopoid copepods are notably prevalent in eutrophic conditions. In aquatic ecosystems, Copepoda form a link in energy transfer to higher trophic groups such as fish larvae (Williams et al. 1994; Dole-Olivier et al. 2000). Cyclopoids are also known as micropredators of small fish larvae, especially early stages of cyprinids (Piasecki et al. 2004).

Fryer (1957) mentioned that some cyclopoid copepod species are carnivorous, and that other species are herbivorous. *Thermocyclops neglectus* and *Mesocyclops major*

both feed on a wide range of planktonic prey, including protozoans, cladocerans and rotifers (Brandl 2005). The ingestion of cladoceran species by some cyclopoid species are usually limited to size ratio of both predator and prey. Adult cyclopoids will feed on small-bodied cladocerans, but as soon as cladoceran species reach adult size, they often coexist (Dodson 1974; Gliwicz and Umana 1994). Herbivorous copepods were not collected from the Krovovango CTW.

The drastic decline in copepod abundance (18.53% - 5.28%) could be attributed to the decrease in available dissolved oxygen concentration (34.94% - 12.4%) in the Krovovango CTW from 2017 to 2018. Decker et al. (2004) studied the effects of total dissolved oxygen concentration on copepods and found that copepod movements drastically decrease with decreasing dissolved oxygen concentrations.

### 5.3: Comparable studies on zooplankton composition and abundance

In the present study Rotifera was the most abundant taxon collected from the Krovovango CTW with abundances of 44.57% and 54.38% in 2017 and 2018, respectively. Cladocera had abundance values of 21.63% in 2017, and 29.87% in 2018. Copepoda comprised of 18.53% of the total zooplankton community in 2017 and declined to 5.28% in 2018. Protozoa represented 15.27% of the zooplankton community in 2017 and decreased to 10.46% in 2018. Combining the two years Rotifera, Cladocera, Copepoda and Protozoa had abundance values of 49.48%, 25.75%, 11.91% and 12.86%, respectively.

In a study carried out by Kamaladasa and Jayatunga (2007), species composition, density and distribution of zooplankton were studied between the restored South West Lake and the non-restored, eutrophic East lakes of Beira Lake, Colombo, Sri Lanka. In the eutrophic conditions of the East Lakes *Brachionus* sp. and *Keratella* sp. were collected among rotifers. *Daphnia* sp. and *Ceriodaphnia* sp. were collected among cladocerans and *Thermocyclops* sp. were collected among copepods. The above-mentioned species were also found in the Krovovango CTW.

In a study of zooplankton community structure conducted by Özdemir Mis and Ustaoglu (2009), *Brachionus* spp., *Keratella quadrata* and a *Trichocerca* species served as biological indicator species of eutrophication in Gölcük Lake. Cladoceran species in their study included *Daphnia* spp., *Ceriodaphnia* sp. and *Alona* sp. These species were also collected from the Krovovango CTW. No copepod species from Gölcük Lake matched species collected from the Krovovango CTW.

Zooplankton composition of the lower eutrophic basin of Yeşilirmak River (northern Turkey) were studied and results indicated that rotifers were the most abundant taxa found and the most abundant species were *Keratella* sp. The most abundant cladoceran species from this study was *Ceriodaphnia* sp. (Bozkurt and Akin 2012). These species were also collected from the Krovovango CTW.

A study was conducted on the polluted lower Sakarya River Basin in Turkey, where *Brachionus* spp. and *Keratella quadrata* were found in abundance, among other

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rotifers. In the study conducted by Dorak (2013), Rotifera showed an abundance of 96.4%, with Copepoda and Cladocera only comprising of 2.7% and 1.0%, respectively. Rotifer species present in their study included *Brachionus* spp., *Keratella quadrata*, *Lecane* spp., *Lepadella* spp., *Platylas* sp., *Rotaria* sp. and a *Trichocerca* species. Cladoceran species from their study included *Alona* spp., *Chydorus* sp. and *Daphnia* sp. Species that were mentioned from their study above also occurred in the Krokovango CTW. Copepod species from the lower Sakarya River Basin did not match the ones collected from the Krokovango CTW during the study period.

In northern Turkey, zooplankton composition was studied in eutrophic Ladik Lake by Apaydin Yağci et al. (2015). In this study Rotifera, Cladocera and Copepoda abundances resulted in 59%, 24% and 17%, respectively. Biological indicator rotifer species from their study included *Brachionus* spp. and *Keratella quadrata* amongst the rotifers, which were also collected from the Krokovango CTW. Species composition of other taxa from Ladik Lake did not match that of the Krokovango CTW. The difference in zooplankton composition could be caused by the difference in the trophic status of the water. The Krokovango CTW is a smaller water body receiving wastewater from crocodiles only, while Ladik Lake receives wastewater from various sources including agricultural, industrial and urban activities (Erdoğan et al. 2019).

Ismail and Adnan (2016) studied zooplankton composition and abundance in two small constructed lakes, namely Harapan and Aman Lakes. In their study, Rotifera showed an abundance of 64%, followed by 29% for Copepoda and 7% for Cladocera. Rotifer composition in their study included *Brachionus* spp., *Brachionus falcatus*, *Lecane* spp., *Lecane bulla*, *Lecane papuana*, *Lepadella* sp. and *Trichocerca* sp., which were also found in the Krokovango CTW.

In a study conducted by Ranrag and Zade (2018), rotifers were studied as indicators of water quality in Ramala Lake, situated in Chandrapur city. Results from their study showed abundances of 33.75%, 24.83%, 18.47% and 10.71% for Rotifera, Cladocera, Copepoda and Protozoa, respectively. From their study pollution indicator species within the Rotifer community included *Brachionus falcatus* and *Lecane bulla*, which were also collected from the Krokovango CTW. No species lists were provided for the other taxa in their study.

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Riato et al. (2014) conducted a study on zooplankton- and epiphytic diatom communities of permanent and temporary freshwater pans in the Mpumalanga Highveld area of South Africa. The pans are extremely threatened by mining and agricultural development. Results from their study indicated a difference in zooplankton- and diatom community compositions between permanent- and temporary pans. Species collected from the freshwater temporary pans included *Mesocyclops major* among copepods, *Daphnia laevis* among Cladocera and *Platyias* sp. among rotifers, which were also collected from the Krokovango CTW. Species collected from the permanent pans included *Ceriodaphnia* sp., *Alona* sp. and *Macrothrix spinosa* among cladocerans, and *Brachionus* spp. among Rotifera, which were also found in the Krokovango CTW. According to Riato et al. (2014), the species collected from the permanent pans can tolerate highly saline conditions.

Hutchinson et al. (1932) and Ferreira et al. (2012) sampled zooplankton from numerous permanent pans in the same region of the Mpumalanga Highveld. Species that were sampled from these studies included *Ceriodaphnia* spp. and *Daphnia laevis* among Cladocera, and *Platyias* sp. among Rotifera. From the temporary pans, *Platyias* sp. and *Ceriodaphnia* sp. were collected from the two studies. Epiphytic diatoms collected from temporary pans by Riato et al (2014), included *Nitzschia* sp. and *Pinnularia* spp. Permanent pans only contained *Nitzschia* sp., with low abundance of *Pinnularia*. The species mentioned from these two studies were also collected from the Krokovango CTW.

By comparing the above-mentioned studies to the Krokovango CTW, it was clear that there was some compositional overlap between the plankton species recorded. Inland freshwater studies on zooplankton- and phytoplankton community structures in South Africa are seriously underrepresented compared to estuarine studies such as the ones conducted by Montoya-Maya and Strydom (2009) and Vezi et al. (2019). More freshwater zooplankton studies are needed in South Africa to expand the cosmopolitan database on this topic.

## **5.4: Water quality of the Krokovango CTW and the Okavango Delta**

Wetland structure and function relies on water chemistry to remain healthy (Mosimane et al. 2017). According to Mitsch and Gosselink (2007), the processes undergone by solute transport and storage in wetlands are important for wetland functions and structure. Interactions between hydrological, biological and geochemical processes cause solutes in wetland systems to undergo a series of transformations (Mitsch and Gosselink 2007).

Ecosystem services rely on sustained healthy ecosystems. Water quality is very important since it affects the ability of aquatic environments to remain healthy (Stark et al. 2000). Physical water quality parameters of the Krokovango CTW were measured to observe whether any major fluctuations in parameters occurred after water reached the wetland. When comparing water quality results of the Krokovango CTW to the water quality parameters of the Okavango Delta, parameters could be seen remaining similar to the main source of water.

In evapotranspiration-dominated wetland systems like the Okavango Delta the input and final fate of solutes are ecologically very important (Eugster and Maglione 1979; Boettinger and Richardson 2001). Since these types of systems lose water rapidly through evapotranspiration, solutes can easily become saturated. These saturated conditions are then eradicated through precipitation (Boettinger and Richardson 2001; Humphries et al. 2010). A good example of such a system from South Africa, would be the lower floodplain of the Mkuze wetland, northern KwaZulu-Natal. In this wetland chemical solutes are permanently removed from the solution by means of chemical precipitation under the influence of evapotranspiration (Humphries et al. 2010). The Krokovango CTW receives a relatively constant inflow of organically rich water. Precipitation, especially in the summer months, assists in ensuring that the Krokovango CTW does not become too organically enriched.

According to Wilson and Dincer (1976) and Gumbricht and McCarthy (2003), approximately 98% of the water that enters the Okavango system leaves the system through evapotranspiration throughout the year. The remaining 2% of the mean inflow

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are drained at the distal edges of the Okavango Delta wetland (Gieske 1996; Wolski et al. 2006). Despite all the water loss through evapotranspiration, the biggest area of the Delta remains to be a freshwater system. The reason for this lies within the presence of the various islands in the wetland. Unidirectional mass transfer is a process whereby solutes are sequestered (isolated) beneath the islands (McCarthy et al. 1993; McCarthy and Ellery 1995). The same process might be taking place on a smaller scale within the Krokvangong CTW, although there is no substantial evidence to prove it.

Vegetation causes the water table to be lowered beneath the islands. This phenomenon allows the Okavango Delta to remain a freshwater wetland system (McCarthy et al. 1986; McCarthy et al. 1993; Bauer 2004; Bauer-Gottwein et al. 2007; Ramberg and Wolski 2008). According to McCarthy and Ellery (1998), about 360 000 tons of dissolved solutes accumulate in the Okavango Delta each year. Mass-balance calculations were carried out to determine this. Solute are utilised or become isolated in highly saline groundwater beneath island centres. The processes responsible for the cycling of solutes in surface- and soil waters of the Delta, are not completely understood (Mosimane et al. 2017).

The total dissolved oxygen saturation within the Krokvangong CTW were very low, with arithmetic mean values of 34.94% (min: 8.5%; max: 86%) and 12.4% (min: 10.1%; max: 16.7%) in 2017 and 2018, respectively. Comparing results from West et al. (2015), July 2008 dissolved oxygen saturation of the Okavango acquired a mean value of 71.4% (min: 18.4%; max: 179.5%) (Table 4.7).

Water temperature plays an important role in aquatic ecosystems. Sudden changes of water temperatures during certain times of the day should not exceed an increase of 10%, since this might harm some aquatic organisms. Seasonal changes in water temperature are a natural occurrence and organisms migrate, enter stages of dormancy, emerge and spawn according to these fluctuations (DWAf 1996a).

Since the current study was not a seasonal study, and both years of data collection occurred during the winter months of June, July and August, low water temperatures were observed with arithmetic mean averages of 18.97 °C (min: 14.92 °C; max: 26.17

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°C) and 14.68 °C (min: 12.49 °C; max: 17.67 °C) for 2017 and 2018, respectively. Comparing these averages to water temperatures of the Okavango Panhandle, results of West et al. (2015) showed an arithmetic mean value of 17.13 °C (min: 14.91 °C; max: 27.27 °C) (Table 4.7).

The temperature differences seen here were expected, since the Krokovango CTW is a completely different system compared to the Okavango Panhandle. There are various factors that might influence dissolved oxygen concentration. Water temperature directly influences oxygen levels, but there are other factors influencing oxygen concentrations as well. The eutrophic nature of the Krokovango CTW and the vegetation also played a notable role. It was speculated by the author that the decrease in dissolved oxygen concentration from 2017 to 2018 were due to the dense cover of *Wolffia arrhiza* on the water surface observed in 2018.

The pH of the Krokovango CTW was close to neutral, ranging between 6.18 and 7.21 during the study period. West et al. (2015) also found the pH to range between 6.09 and 7.38 for most of the panhandle of the Okavango Delta (Table 4.7). West et al. (2015) encountered no noteworthy fluctuations in pH of the Okavango Panhandle and Delta between the different seasons.

The electrical conductivity of the Krokovango CTW ranged between 20.7  $\mu\text{S}/\text{cm}^{-1}$  and 63.9  $\mu\text{S}/\text{cm}^{-1}$  from 2017 to 2018. Electrical conductivity measured by West et al. (2015), ranged between 27.0  $\mu\text{S}/\text{cm}^{-1}$  and 59.0  $\mu\text{S}/\text{cm}^{-1}$  in most areas of the panhandle, including the Samochima lagoon (Table 4.7). These values are below the target water quality range for domestic use set by DWAF (1996b), although more improvements are needed on the Krokovango CTW before water can be utilised directly from the wetland.

### **Concluding remarks**

Constructed treatment wetlands with their microbial assemblages have been proven to be successful all over the world, but more research is needed on the role of the different microorganisms that tend to reside in these systems. The fact that the Krokovango CTW has been in operation for almost a decade without intense maintenance, illustrated that these treatment systems do not have to be complicated for it to serve its purpose of water quality improvement. It is also worth noting that the Krokovango CTW had minimalistic foul odours, since one would expect such a system to smell revolting. We do know what taxa are normally represented in certain water bodies, but more research on species level contribution in breaking down pollutants could be useful in improving the effectiveness of CTWs even more. In Southern Africa, zooplankton- and phytoplankton community structures in inland freshwater studies are underrepresented compared to estuarine studies. More freshwater zooplankton studies are needed in Southern Africa to expand the database on this topic. CTW technology is still developing, and the potential for more of these treatment systems in Southern Africa needs more exploration. In the case of the Krokovango CTW and other successful CTWs, it is ultimately important for water to enter the water cycle again as pure as possible, and there is no better way of achieving this. The information from this study adds to our understanding of plankton community compositions in specifically the Krokovango CTW. It also aids in our understanding within natural treatment systems with a constant inflow of organically enriched wastewater into a small area and how these systems can be improved. As for practical implications, CTWs can be utilised along the banks of the Okavango Delta. Lodges and even local households can make use of these systems to treat wastewater. For a follow up study, I would recommend conducting more extensive water quality tests on both the water from Krokovango CTW and from the Samochima lagoon. These tests should include nitrogen and phosphorus concentration tests, which should provide more substantial evidence of the CTW's effectiveness. Lastly, I would also recommend conducting summer surveys to acquire more complete ecological results.

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APPENDIX 1

APPENDIX 1: ZOOPLANKTON SPECIES COUNTS AND ABUNDANCE 2017

Species	26/07	28/07	29/07	31/07	01/08	02/08	03/08	04/08	05/08	06/08	Total	Abundance
<b>Rotifera</b>												
<i>Asplanchnopus</i> sp.	26	12	5	7	17	16	19	6	3	13	124	4,13
<i>Brachionus</i> spp.	38	45	37	46	34	40	29	39	33	56	397	13,23
<i>Keratella quadrata</i>	-	1	-	4	-	-	3	4	-	-	12	0,40
<i>Platylas patulus</i>	36	51	56	21	39	60	21	19	14	20	337	11,23
<i>Lecane</i> spp.	-	2	4	16	-	1	10	1	21	13	68	2,27
<i>Lepadella</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trichocerca</i> spp.	2	24	19	2	11	9	23	21	17	10	138	4,60
<i>Rotaria</i> spp.	23	17	34	15	39	31	26	37	9	30	261	8,70
<b>Cladocera</b>												
<i>Alona affinis</i>	53	46	58	41	28	33	25	20	7	19	330	11,00
<i>Daphnia barbata</i>	2	-	9	2	-	1	3	10	11	17	55	1,83
<i>Daphnia laevis</i>	8	19	3	3	21	2	6	8	12	1	83	2,77
<i>Ceriodaphnia dubia</i>	-	2	-	-	6	11	-	1	7	-	27	0,90
<i>Macrothrix spinosa</i>	23	17	2	31	12	9	23	-	20	17	154	5,13
<b>Copepoda</b>												
<i>Mesocyclops major</i>	23	6	24	53	25	21	31	28	19	23	253	8,43
<i>Thermocyclops neglectus</i>	13	36	23	29	33	18	34	43	31	43	303	10,10
<b>Protozoa</b>												
<i>Amoeba</i> spp.	-	-	-	-	-	-	1	2	-	-	3	0,10
<i>Frontonia</i> sp.	-	-	-	-	1	2	-	-	1	-	4	0,13
<i>Paramecium</i> sp.	12	8	15	7	20	3	26	21	18	9	139	4,63
<i>Spirostomum</i> sp.	22	12	9	18	3	27	15	20	24	6	156	5,20
<i>Epistylis</i> sp.	8	2	-	-	3	9	-	1	12	3	38	1,27
<i>Vorticella</i> sp.	11	-	-	5	1	2	-	13	22	17	71	2,37
<i>Stentor</i> sp.	-	-	2	-	1	3	5	-	9	2	22	0,73
<i>Euplotes</i> sp.	-	-	-	-	5	2	-	4	6	1	18	0,60
<i>Stylonychia</i> sp.	-	-	-	-	1	-	-	2	4	-	7	0,23
<i>Podophyra</i> sp.	-	-	-	-	-	-	-	-	-	-	-	0,00
Total	300	300	300	300	300	300	300	300	300	300	3000	100,00

- = Not collected

APPENDIX 2

APPENDIX 2: ZOOPLANKTON SPECIES COUNTS AND ABUNDANCE 2017

Species	10/06	12/06	13/06	14/06	15/06	16/06	18/06	20/06	21/06	26/06	28/06	30/06	02/07	Total	Abundance
<b>Rotifera</b>															
<i>Asplanchnopus</i> sp.	3	-	23	14	10	30	21	5	35	7	14	28	19	209	5,36
<i>Brachionus</i> spp.	57	36	50	52	32	23	41	22	15	27	31	39	16	441	11,31
<i>Keratella quadrata</i>	-	1	5	-	-	-	2	-	5	7	-	1	3	24	0,62
<i>Platylas patulus</i>	31	46	26	47	49	53	30	43	70	24	45	32	48	544	13,95
<i>Lecane</i> spp.	10	2	20	13	17	12	12	8	1	18	8	5	2	128	3,28
<i>Lepadella</i> spp.	3	12	6	21	19	13	10	-	4	16	7	16	9	136	3,49
<i>Trichocerca</i> spp.	5	23	29	21	6	11	5	20	13	28	22	34	24	241	6,18
<i>Rotaria</i> spp.	36	29	32	18	38	29	30	38	23	41	31	38	15	398	10,21
<b>Cladocera</b>															
<i>Alona affinis</i>	51	48	46	33	26	31	33	49	54	13	22	35	19	460	11,79
<i>Daphnia barbata</i>	9	8	10	21	4	6	0	6	-	8	2	-	3	77	1,97
<i>Daphnia laevis</i>	16	19	7	3	21	-	22	14	13	17	6	23	28	189	4,85
<i>Ceriodaphnia dubia</i>	2	11	-	12	6	21	13	12	10	-	23	1	14	125	3,21
<i>Macrothrix spinosa</i>	13	37	15	7	23	12	43	39	33	9	25	13	45	314	8,05
<b>Copepoda</b>															
<i>Mesocyclops major</i>	10	3	12	-	3	8	9	-	3	12	4	5	10	79	2,03
<i>Thermocyclops neglectus</i>	23	4	6	17	11	4	2	1	5	19	14	9	12	127	3,26
<b>Protozoa</b>															
<i>Amoeba</i> spp.	-	-	2	2	-	1	-	-	1	3	-	-	-	9	0,23
<i>Frontonia</i> sp.	-	-	-	1	1	3	-	5	-	-	-	1	4	15	0,38
<i>Paramecium</i> sp.	12	8	5	7	16	23	11	2	1	20	13	8	9	135	3,46
<i>Spirostomum</i> sp.	15	4	4	9	3	17	5	19	2	24	10	2	8	122	3,13
<i>Epistylis</i> sp.	-	2	-	-	3	1	1	0	-	5	4	6	2	24	0,62
<i>Vorticella</i> sp.	2	3	-	-	7	2	-	5	9	-	8	-	3	39	1,00
<i>Stentor</i> sp.	-	-	1	1	2	-	5	-	3	-	4	-	7	23	0,59
<i>Euplotes</i> sp.	2	4	-	-	-	-	1	3	-	-	2	4	-	16	0,41
<i>Stylonychia</i> sp.	-	-	1	1	1	-	4	8	-	2	5	-	-	22	0,56
<i>Podophyra</i> sp.	-	-	-	-	2	-	-	1	-	-	-	-	-	3	0,08
Total	300	300	300	300	300	300	300	300	300	300	300	300	300	3900	100

- = Not collected