

# IMPACT OF PIGEON PEA FISH FEED FORMULA ON THE LIMNOLOGY OF SMALL-HOLDER AQUACULTURE SYSTEMS DURING TILAPIA FISH FEEDING TRIALS, VHEMBE DISTRICT, LIMPOPO PROVINCE

SINTHUMULE HANGWELANI, MOKGOEBO MATJUTLA JOHN, GUMBO JABULANI RAY\*  
University of Venda, South Africa

## ABSTRACT

Inland aquaculture systems are on the rise worldwide, including in South Africa to provide affordable fish and promote local economic growth. But the main cost input, is the supply of fish feed. Thus, there is a need to develop local fish feed to offset the rising cost of commercial fish feed. The study was aimed at assessing the effects of a local low-cost pigeon pea feed on the limnology of aquaculture systems using three types of feed, viz. commercial feed (control), pigeon pea feed (one roasted and one raw). The results as computed by the Czekanowski coefficient statistical analysis showed that the commercial and low-cost feed had similar environmental impacts ( $p < 0.05$ ). When environmental factors fluctuated, there was a fluctuation in phytoplankton composition which led to the proliferation of cyanobacteria species in all the aquaculture tanks. A total of 446 phytoplankton species were identified in the commercial feed tank, 601 species in the roasted fish feed and 630 species in the raw fish feed. Phytoplankton spectra were recorded from six taxonomic groups namely: Chlorophyta, Euglenophyta, Dinophyta, Bacillariophyta, Chrysophyta and Cyanophyta (the dominant taxonomic group). Most of the physio-chemical parameters were within the recommended aquaculture guidelines of the Department of Water & Forestry, making the feed suitable for fish feeding. The results show that the three fish feeds (commercial feed, roasted pigeon pea feed and raw pigeon pea feed) all influenced the health of the aquaculture system with both beneficial and harmful algae growing in the system. This shows that the pigeon pea formula has similar impacts on the aquatic health of aquaculture tanks.

**Keywords:** *Cyanobacteria, Cyanotoxin, phytoplankton, water quality, aquaculture, pigeon pea*

## 1 INTRODUCTION

Tilapia fish (*Oreochromis mossambicus*) is one of the most common fishes that is found on abundance worldwide that is enjoyed by many and serves as a staple food for most African homes. As the world's population increases so has the demand for white meat as it is known to be cheaper and much healthier than red meat [1]. Fish farming has been on the rise for the past decade and as such inland aquaculture systems are rising, that is the same case for the Vhembe district in Limpopo Province as the district has fish farmers, some have ponds at their homes [2–3]. Fish farmers who have ponds depend on commercial feed in order to grow their production however the feed is relatively expensive which has a negative impact on their profit which is a major challenge for small-holder aquaculture practitioners [4].

Pigeon pea is plant protein that has been found to replace soya in formulation of local fish feed [5]. A local fish feed was then formulated blending pigeon pea and maize bran and then fed to juvenile tilapia (*O. mossambicus*) [6]. Since we are aware that available nutrients from fish feed may contribute to algae proliferation [3], we assessed the effects of the pigeon pea fish feed formula on the limnology of small-holder aquaculture systems during tilapia fish feeding trials, Vhembe district, Limpopo Province, South Africa.

---

\*ORCID: <http://orcid.org/0000-0001-8967-3858>

## 2 MATERIALS AND METHODS

### 2.1 Location of the study area

The study was done in the north-eastern corner of Limpopo Province, Thohoyandou, in the Vhembe District (Figure 1). The fish tanks were situated on the premises of University of Venda in the School of Agriculture and were populated with *O. mossambicus*. The study area is located at 22°57'30"S and 30°26'15"E.

### 2.2 Sample collection and onsite physical measurements

A total of 36 water samples were collected and of which 12 water samples were collected from each of the fish tanks. The fish tank 1 (a commercial fish feed), tank 2 (ground pigeon pea formula mixed with maize bran) and tank 3 (roasted, ground pigeon pea mixed with maize bran). The study period was 12 weeks, from November 2016 to January 2017. The water samples were collected once a week (on Fridays) using a 200 ml no-transparent sterile water bottles. The water samples were carried in a cooler box with ice inside. The physical water quality parameters: pH and temperature were measured in-situ using a multimeter instrument (Jenway 430, England) and calibrated as per manufacturer's guideline and the Total dissolved solids (TDS) and electrical conductivity were determined with a multimeter instrument (Wasser Profession, Austria) and calibrated as per manufacturer's guideline Turbidity was measured using a turbidity meter (Oakton T-100, Eutech Instruments, USA) calibrated as per manufacturer's guideline. All measurements were in triplicate.

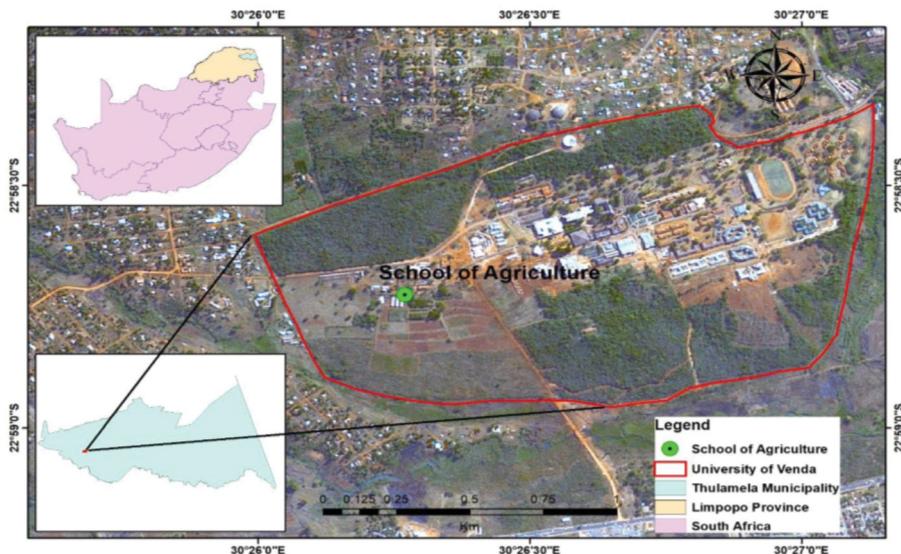


Figure 1: The study site (University of Venda, Vhembe district).

## 2.3 Laboratory analysis

The Ion Chromatography instrument (Metrohm, Germany) was used to determine chloride, sulphate and nitrate and phosphate in the water samples. The ammonium concentration was determined using the photometer and ammonium test kit according to the procedure of Sehnitzler et al. [7]. The metals, zinc and lead were measured in the laboratory using a flame atomic absorption spectrometer (FAAS) with a double beam and deuterium background corrector.

## 2.4 Cyanobacteria and phytoplankton collection

Cyanobacteria and phytoplankton samples were collected 12 times, i.e. once a week over a period of 12 weeks (November 2016 to January 2017) using a phytoplankton net with mesh size of 25 µm. The samples were collected on the fifth day of each week (every Friday) because water quality parameters were needed to determine their effects on cyanobacteria. The cyanobacteria and phytoplankton samples were collected at the depth of 5–90 cm, using a hand held phytoplankton net with a 10-cm radius with a mesh size of 25 µm net placed inside the tank and moved in a zig-zag motion (moved from the middle of the tank to the left end of the tank, then moved to the right end of the tank via the middle of the tank, in order to collect specimen that dwell at the tank end and the middle of the tank) as the tanks size was 1.2 m which is big enough for phytoplankton to freely swim around in it, 200 ml dark non-transparent bottles were used to collect the samples. Rocks, leaves and other debris were taken out of the collected sample.

## 2.5 Cyanobacteria and phytoplankton analysis

Laboratory analysis consisted of two parts which were the analysis of soft algae and analysis of hard algae. For the soft algae analysis, organisms were enumerated in a settling chamber using an inverted microscope with 10x and 40x objectives. A sample of 30 ml was extracted from each of the 100 ml collected samples without using a syringe filter, the samples settled for 24 h before analysis. Another 30 ml was extracted from the 100 ml containers and filtered with a 45-µm filter and the samples settled for 24 h before being analysed using the flow cam. Enumeration on the flow cam was carried out by running 6 ml of the filtered samples through the enumeration chamber, each sample was run 3 times which meant the 18 ml of each sample was enumerated

For hard algae analyses, 30 ml from each of the collected samples were poured into an enumeration chamber and allowed to settle for 24 h and enumerated using a compound Amscope IN480 TC-10M microscope at 1250x magnification. The abundance of the specimen was weighed by manually counting the number of sampled species (per 6 ml analysed). The abundance of both soft and hard algae was done by using an elimination process where identified specimen were recorded as either being present or absent in each sample/tank, the genera/species which were not identified in other tanks were recorded as 0 and the identified ones were recorded using any number >0 (depending on the number of identified species number), the specimen were identified by using a chart adopted from Smithsonian Environmental Research Centre [8]. Cyanobacteria were identified by using a cyanobacterial identification

kit by Janse et al. [9], as done by the North-west University in collaboration with the Department of Water Affairs South Africa.

## 2.6 Data analysis

The Microsoft (MS) Excel 2013 was used to compute the mean and standard deviation of the replicates, differences between the three fish feeds (commercial feed, raw pigeon pea feed formula and roasted pigeon pea formula) were analysed using one-way ANOVA at level of significance at  $p < 0.05$ .

Cyanobacteria and phytoplankton presence, absence and abundance were used to determine the diversity of phytoplankton. The Czekanowski coefficient was used to measure the similarities and dissimilarities in abundance of the enumerated species between the three tanks used as different sampling points. Species abundance in Tank 1 was compared to Tank 2. Species abundance in Tank 1 was compared with Tank 3 species abundance. Species abundance in Tank 2 was compared to abundance in Tank 3.

The Czekanowski coefficient (SC) was calculated as follows:

$$SC = \frac{2 \sum \min(X_i, Y_i)}{\sum X_i + \sum Y_i},$$

where  $X_i$  and  $Y_i$  are the abundance of species in two tanks and  $\sum \min(X_i, Y_i)$  = the sum of lesser scores of species where it occurs in both quadrants.

## 3 RESULTS AND DISCUSSION

### 3.1 The physical-chemical quality of the water in the fish tanks

The average pH values from Tanks 1, 2 and 3 were alkaline; none of the values were acidic (Figure 2a). There was no significant difference among mean pH values  $p > 0.05$ . This may indicate that the pigeon pea formula was comparable with the commercial fish feed as fish survival is dependent on the water's pH as fish and most beneficial phytoplankton cannot survive in acidic environments. According to DWAF [10] aquaculture guidelines basic-alkaline waters are most suitable for fish growth. The pH ranges agree with study of Yada and Ito [11] who said that *O. mossambicus* tolerate alkaline conditions for their optimum growth. The alkaline conditions also promote the growth of soft algae such as (*Bacillariophyta*, *Anabaena* and *Chlorophyta*), the production of these soft algae led to clogging in the water filters which were used for water circulation. *Cymatopleura W. Smith* is one of the phytoplankton that strives/prefers alkaline still waters and it was found in abundance in the three tanks as through the sampling period the water was alkaline in the tanks. Chlorophyta is beneficial algae which may be a source of nutrition for *O. mossambicus* [12]. The deep in pH on week 5 was associated with a heat wave that was taking place in South Africa during that time [13].

The total dissolved solids (TDS) and electrical conductivity (EC) in all the fish tanks was below DWAF [10] aquaculture guidelines basic of 1,200 ppm (Figure 2b–c). There was no significant difference among mean TDS and EC values  $p > 0.05$ . The highest TDS concentration (279.3 mg/L) was recorded in Tank 2 which contained raw feed during the 11th week. This phenomenon showed that the feed contained a lot of dissolved ions hindering/impeding the growth of *Bacillariophyta* and promoting the growth of *Microcystis*. However, when the

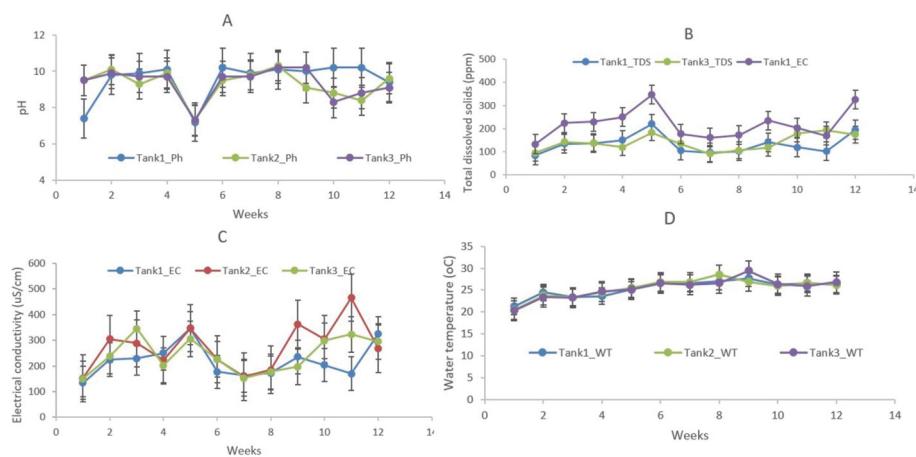


Figure 2: The variation of physical quality of the water: (a) pH, (b) total dissolved solids, (c) electrical conductivity and (d) water temperature in the fish tanks. Whiskers indicate the error bars of the mean.

TDS values went up in week 5 (in all tanks) due to the massive heat wave that was experienced in South Africa [13], algae which are intolerant to alkaline water and high TDS levels such as (*Cryptophyta*) disappeared in all the tanks this indicated that conditions were getting intolerable for fish survival.

Temperature is an essential factor when it comes to fish and phytoplankton survival. Phytoplankton can tolerate temperatures ranging from 16°C to 27°C and start to disappear at 28°C [14] whereas tilapia fish species can tolerate temperatures ranging from 15°C to 30°C [10]. There was no significant difference among mean water temperature values  $p > 0.05$ . The water temperature was below 30°C and compiled with the DWAF [10] aquaculture guidelines (Figure 2d). These temperature conditions are ideal for the growth for both fish and phytoplankton. However, during week 9 some algae and phytoplankton started to disappear as the temperatures were getting intolerable for some of the species, species such as *Craticula grunow* and *Sphaerodinium woloszynska* are heat intolerant. However, with the high-temperature readings, growth of *Anabaena*, was associated with death of fish in the tanks (10 fish died in tank 1, 7 fish died in tank 2, 13 fish died in tank 3) during week 5.

The nutrients, ammonium, nitrates and soluble phosphates, were available in the fish tanks in variable concentrations. The level of nitrate in the tanks were within the desired nitrate range of 0.1–4.5 ppm and the DWAF target value is 300 ppm [11], except for one extreme value of 5.3 in tank 2, week 9 (Figure 3a). This was due to a technical error (failed water pump) so the water was not circulated to the biofilters to remove excess nitrates. There was no significant difference among mean nitrate values  $p > 0.05$ . The availability of nitrates is due to bacteria nitrification of ammonia [10] and the fish feed itself [3]. The fish excrete ammonia as a waste, and this is converted to nitrates. The presence of nitrates is not harmful to fish [10]. However, the excess nitrates stimulate the growth of algae, as shown by bad odour and watercolour changing to dark green in the fish tank, showing growth of algae (*Chlorophyta*, *Cyanophyta*, *Euglenophyta*, *Dinophyta* and *Bacillariophyta*) causing decrease in dissolved oxygen, during the first weeks there was rapid growth of phytoplankton.

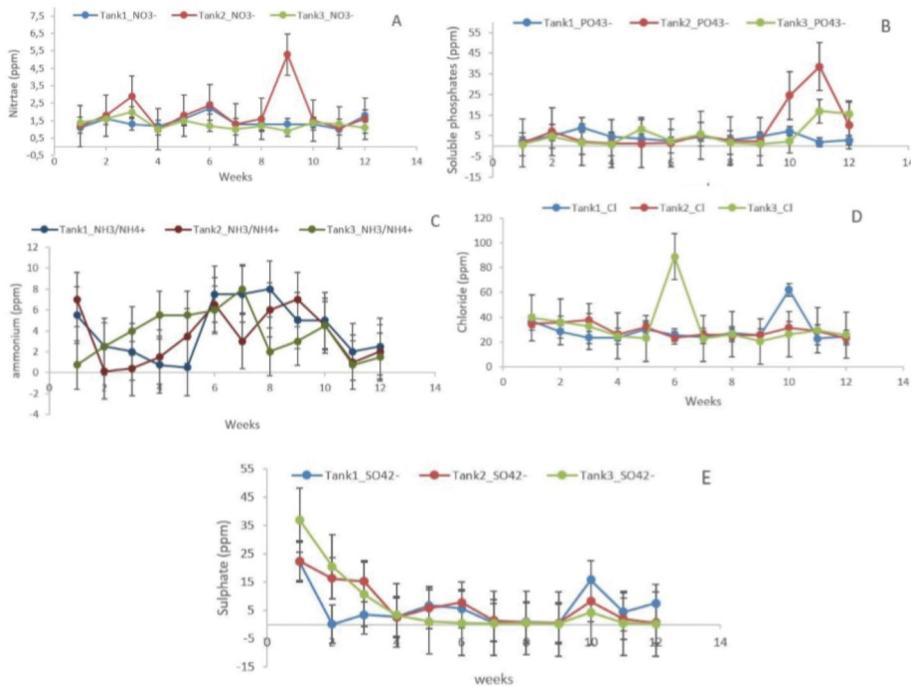


Figure 3: The variation of chemical quality of the water: (a) nitrate, (b) soluble phosphates, (c) ammonia/ammonium, (d) chloride and (e) sulphate in the fish tanks. Whiskers indicate the error bars of the mean.

The 0.6 ppm phosphates are the maximum desired level set by DWAF [10]. The phosphates levels in this study were variable, mean range was from 0.01 to 3 ppm, some recorded values were above the desired level (Figure 3b). There was no significant difference among mean phosphates values  $p > 0.05$ . In Tanks 2 and 3, probably due to weak circulation of the water pumps, there were high levels of phosphates that were recorded on week 11. The water pumps were clogged by *Closterium Nitzsch ex Ralfs*. The pump was used to circulate the water through the biofilters which were designed to remove excess phosphates. The source of phosphates is fish feed and animal excreta. The presence of phosphates is not harmful to fish. But the excess phosphates stimulate the growth of phytoplankton and algae (species such as *Anabaena*, *Cocconeis Ehrenberg*, *Peridinium Ehrenberg*, *Euglena Ehrenberg* and *Dityosphaerium Nageli*).

Ammonium is an important substance in every ecosystem as it is needed to balance the nutrient cycle in the aquatic system, ammonium is unavoidable in aquatic systems especially fish aquariums/tanks and ponds as fish feeds are introduced into the systems frequently; ammonium can be converted to nitrates or ammonia provided that there is enough oxygen (O) for the conversion to occur [15]. All the three tanks had levels of ammonium levels (Figure 3c) which were within the fish survival rate ( $< 8$  ppm) [10]. There was no significant difference among mean ammonium values  $p > 0.05$ . The highest recording was captured in Tank 2 in week 9 (0.5 ppm), this was due to scums forming in the tanks which prevented light penetration, in turn, there was not enough oxygen in the tank to convert the ammonium

to nitrates. In all the tanks where there was a decrease in ammonium, reading there was an increase in nitrates reading, in all the three tanks during week 10, there was a decrease in ammonium reading as there was water that was added and the water contained a lot of oxygen. The ammonium values did not show any sign of extremity as there was no external anthropogenic or natural pollutants which had high ammonia content being introduced into the system. Furthermore, since every aquatic ecosystem has nitrosamines bacteria which can convert ammonium, there was a lot of conversion on the little ammonium that was available to nitrates. The levels of ammonium did not favour the growth of phytoplankton such as *Cryptomonas Ehrenberg* which cannot withstand high ammonium levels, the levels were also suitable for tilapia farming as they had no direct negative effect on the health of the fish.

The chloride levels were variable in the fish tanks but within the DWAF [10] of 600 ppm (Figure 3d). There was no significant difference among mean chloride values  $p > 0.05$ . High levels of chloride in this study did not affect the health of *O. mossambicus*. As stated by Barnabe [16], most species cannot withstand high chloride levels as chloride is known to destroy the membranes and cells of most microspecies. However, it was observed that *Microcystis*, *Dinophyta* and *Euglenophyta* species disappeared in tank 1 during week 10 and tank 3 during week 6.

The sulphate levels were variable in the fish tanks (Figure 3e). There is no DWAF guideline value [10], since sulphate is considered as non-toxic in comparison with hydrogen sulphide. There was no significant difference among mean sulphate values  $p > 0.05$ . Conversely, sulphate does not easily affect the health of fish as it can change during oxidization. The sulphate values in all the tanks were below 35 ppm and this influenced the normal growth of phytoplankton (i.e. all of the recorded 6 phyla: *Cyanophyta*, *Chrysophyta*, *Bacillariophyta*, *Dinophyta*, *Euglenophyta* and *Chlorophyta*).

The metals, zinc, lead, cadmium and mercury levels were variable in the fish tanks (Figure 4). According to Britz and Hecht [17], zinc (Zn) is an essential element responsible for fish growth and metabolism in the required concentrations. The zinc levels were variable in the fish tanks but within the DWAF [10] salmonids guideline value of 0.5 ppm (Figure 4a). There was no significant difference among mean zinc values  $p > 0.05$ . There was a slight increase in Zn in tank 3 during week 7 (where there was a heat wave). The zinc levels in the three tanks did not have a negative effect on the survival of fish as the levels were within the recommended levels. However, the zinc levels did have a negative impact on the growth of *Pinnularia Ehrenberg* which is a beneficial alga to fish as they can feed on it when food is scarce. The other recorded species (Table 1) under the six phyla remained in high abundance as Zn did not influence them.

The lead levels were variable in the fish tanks but within the DWAF [10] guideline value of 10 ppb (Figure 4b). There was no significant difference among mean lead values  $p > 0.05$ . Lead is not an essential element, is a pollutant and does not contribute to fish physiology. The lead values were low and did not have a negative effect on fish survival in the three tanks. The growth of most phytoplankton that was recorded cannot be affected by Lead (Pb) thus lead has no known effect on the abundance of phytoplankton species as stated in Carignan [18].

The cadmium levels were variable in the fish tanks but within the DWAF [10] guideline value of 0.2 ppm (Figure 4b). There was no significant difference among mean cadmium values  $p > 0.05$ . Cadmium is not an essential element and does not contribute to fish physiology. The cadmium values were low and did not have a negative effect on fish survival in the three tanks. Most of the cadmium values were below the detection point <0 ppb and as such cadmium did not have a detrimental effect on the limnology. Even though cadmium was

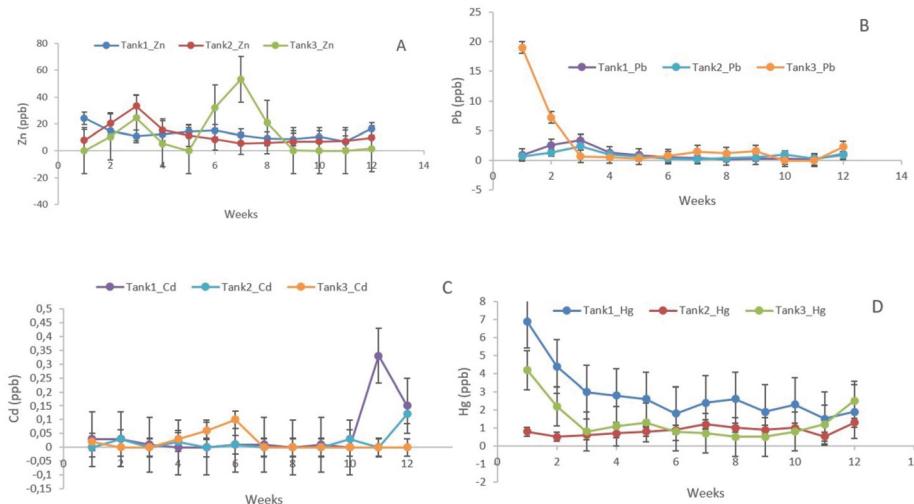


Figure 4: The variation of metals in the water: (a) zinc, (b) lead, (c) cadmium, and (d) mercury in the fish tanks. Whiskers indicate the error bars of the mean.

present, it was in very low levels and in such levels, it did not have any effect on the phytoplankton abundance and the fish health. The same results were found by Edwards et al. [19], when assessing the health of the delta stream using the fish index in polluted streams, it was noted that, cadmium did not have effects on the aquatic life of the delta.

The mercury levels were variable in the fish tanks but within the DWAF [10] guideline value of 1 ppm (Figure 4d). There was no significant difference among mean mercury values  $p > 0.05$ . Mercury is not an essential element and does not contribute to fish physiology. Mercury is a metal which is usually not found in high values in normal environmental circumstances, for mercury values to be found in high toxic values, there must be a lot of organic and inorganic pollution occurrence [20]. The levels of mercury did not also favour the growth of most phytoplankton as mercury destroys most phytoplankton cells, but since the levels were low, mercury could not completely destroy the phytoplankton that were growing there as the other physio-chemical parameters were favouring the growth of the phytoplankton as such the six phyla that were recorded were striving throughout the experiment as mercury did not destroy them.

### 3.2 Phytoplankton biodiversity in the three tanks over the sampling period

The presence of physio-chemical parameters influenced the growth of algae/phytoplankton as shown in Table 1. The identified genera were quantified, with (0) meaning that the genera were not present in that tank and any number above zero ( $>0$ ) meaning that the genera were identified in that particular tank.

The three tanks had high phytoplankton/algae abundance recorded with tank 2 having the highest number of species being recorded (630) under 22 genera and 5 phyla, followed by tank 3 with (601) species under 22 genera and 5 phyla and lastly tank 1 with the least species recorded (446) under 21 genera and 5 phyla.

Table 1: Phyla/genera (absence, presence, and abundance), for all sample weeks.

Phyla/genera	Fish tanks			Total per genera/spp.
	1	2	3	
<b>1. Cyanophyta</b>				
i. <i>Anabaena</i>	<b>42</b>	<b>48</b>	<b>57</b>	<b>147</b>
ii. <i>Cylindrospermopsis</i>	<b>17</b>	<b>34</b>	<b>28</b>	<b>79</b>
iii. <i>Microcystis</i>	<b>85</b>	<b>102</b>	<b>71</b>	<b>258</b>
iv. <i>Oscillatoria</i>	<b>27</b>	<b>18</b>	<b>30</b>	<b>75</b>
<b>2. Chrysophyta</b>				
i. <i>Dinobryon Ehrenberg</i>	<b>20</b>	<b>14</b>	<b>11</b>	<b>45</b>
ii. <i>Mallomonaspty</i>	<b>7</b>	<b>5</b>	<b>9</b>	<b>21</b>
iii. <i>Cryptomonas Ehrenbeg</i>	<b>6</b>	<b>13</b>	<b>18</b>	<b>37</b>
<b>3. Bacillariophyta</b>				
i. <i>Asterionella Hassall</i>	<b>0</b>	<b>3</b>	<b>8</b>	<b>11</b>
ii. <i>Aulacoseira Thwaites</i>	<b>5</b>	<b>27</b>	<b>17</b>	<b>49</b>
iii. <i>Cocconeis Ehrenberg</i>	<b>14</b>	<b>35</b>	<b>12</b>	<b>61</b>
iv. <i>Craticula Grunow</i>	<b>12</b>	<b>7</b>	<b>15</b>	<b>34</b>
v. <i>Cymatopleura W. Smith</i>	<b>16</b>	<b>32</b>	<b>21</b>	<b>69</b>
<b>4. Dinophyta</b>				
i. <i>Peridinium Ehrenberg</i>	<b>20</b>	<b>25</b>	<b>37</b>	<b>82</b>
ii. <i>Sphaerodinium Woloszynska</i>	<b>5</b>	<b>17</b>	<b>22</b>	<b>44</b>
<b>5. Euglenophyta</b>				
i. <i>Euglena Ehrenberg</i>	<b>62</b>	<b>73</b>	<b>51</b>	<b>186</b>
<b>6. Chlorophyta</b>				
i. <i>Ankyra fott</i>	<b>12</b>	<b>17</b>	<b>9</b>	<b>38</b>
ii. <i>Chlamydomonas Ehrenberg</i>	<b>34</b>	<b>57</b>	<b>79</b>	<b>170</b>
iii. <i>Closterium Nitzsch exralfs</i>	<b>14</b>	<b>9</b>	<b>22</b>	<b>45</b>
iv. <i>Crucigeniella Lemmermann</i>	<b>7</b>	<b>13</b>	<b>10</b>	<b>30</b>
v. <i>Dityosphaerium Nageli</i>	<b>25</b>	<b>44</b>	<b>32</b>	<b>101</b>
vi. <i>Golenkinia Chodat</i>	<b>4</b>	<b>22</b>	<b>18</b>	<b>44</b>
vii. <i>Stigeoclonium Kutzning</i>	<b>12</b>	<b>15</b>	<b>24</b>	<b>51</b>
<b>Total</b>	<b>446</b>	<b>630</b>	<b>601</b>	<b>1,677</b>

The high number of species in tank 2 shows that there was nutrient overload from the feed used in tank 2 (raw feed), the high nutritional value of the feed led to high nitrates values being recorded in tank 2 with the average of 1.9 ppm, the feed was not bounded therefore it broke apart and settled easily. The high nutrient content of the feed also led to over nitrifications in the tank. According to Bruisma [21], when there is nutrients' overload, the abundance

of zooplankton decreases in aquatic systems, which is in contradiction with the results from this study where there is high abundance when there is nutrient overload. However, a study by Kugrens [22], conducted in North America concluded that there was an increase in algae/phytoplankton when there is an increase in pollution, however, not all phytoplankton can tolerate high nutrient levels which explains the nonexistence of *Asterionella Hassall* in tank 1 (Table 1).

All the six (6) identified phyla were present in all the tanks (Tanks 1, 2 and 3). Phylum cyanophyta was the most identified phylum in Tank 2 with 202 species/genera being identified, under this phylum genera such as *Microcystis*, *Cylindrospermopsis*, *Anabaena* and *Oscillatoria*, were identified. These identified genera are known to produce cyanotoxins such as *microcystin*, *cylindrospermopsin*, *anatoxin-a*, and *microcystin-LR* respectively. *Microcystis* was the most abundant genera under phylum Cyanophyta. Under the phylum Cyanophyta, *Microcystis* were identified in high abundance in all the three tanks, the *Microcystis* are not easy to get rid of, as such the fish were the source of the *Microcystis*, the Mokgopong farm water (where the fish were bought) tested positive for *Microcystis* presence, swabs from the fish's mouth and gills also showed the presence of *Microcystis* which explains the high abundance of the *Microcystis*. Phylum Chlorophyta was the most identified phylum in tank 3 with 194 species from seven genera, *Dinobryon Ehrenberg* was the most abundant species under phylum Chrysophyta. Similarly, Chlorophyta was the most abundant phylum in tank 2 with the species *Chlamydomonas Ehrenberg* being the most abundant one.

The Dinophyta phylum was most abundant in tank 3 with *Peridium Ehrenberg* being the most abundant species identified (Table 1). Phylum Euglenophyta was in abundance in tank 2 as the production of phosphate was high in that tank, *Euglena Ehrenberg* species was the only species identified under this phylum with the most species identified in tank 2. The Chlorophyta phylum was the phylum with the greatest number of species/genera identified, under Chlorophyta, *Chlamydomonas Ehrenberg* was the most identified species. Out of all the identified phyla, it was only the *Asterionella Hassall* species which was not identified in tank 1 during the entire sampling period, the rest of the species were identified in all the three tanks.

Phytoplankton/algae have different nutritional requirements with some species requiring a high amount of nutrients and some requiring minimum nutrient levels [23]. There were a lot of fluctuations in the physio-chemical parameters because of different reasons hence the fluctuations in those parameters together with the continual introduction of pollutants (fish feeds) caused nutrient overload in the systems. Starting from the first week of sampling there were phytoplankton identified in the system, nutrient overload intolerant phylum such as Bacillariophyta were recorded during the first weeks of sampling and disappeared when the system got polluted/stressed. However, since groundwater was being used to dilute the over nitrified systems the phylum Bacillariophyta did not completely disappear however, during week 3 and week 12 when the three systems were stressed, the abundance of that phylum was low/minimal as the phylum is not tolerant to pollution, the phylum Bacillariophyta was found in all the three tanks as the phylum requires turbid and slow moving water and such conditions were recorded in all the tanks. The abundance of this phylum seem to be high when compared to the others phylum, e.g. Chrysophyta because there was a lot of water dilution (which brought down the levels of physio-chemical parameters favouring Bacillariophyta) during the sampling period and there were also times where the systems were flushed completely when cleaning the clogged filters, e.g. during week 9. *Euglenophyta* is intolerant to high nutrient content and as such the phylum is not found in abundance and was absent in tank 2 where the phosphate average was 8.2 ppm.

Unlike Dinoflagellates, Diatoms cannot move on their own, however, they depend on water currents for distribution [14] the distribution of the diatoms in the system was a result of the bio-filters used for water circulation which shows why the species were distributed almost equally in the system. The bio monitoring results and Czekanowski calculation results showed that there was high similarity between the three tested feed. The similarity between the commercial fed and the roasted feed was at 78%, it was 66% between the commercial feed and the raw feed, it was at 81% between the raw feed and roasted feed.

#### 4 CONCLUSION

The physio-chemical parameters (pH, total dissolved solids, temperature, turbidity, chloride, nitrate, phosphate, sulphate, ammonium, zinc, lead, mercury and cadmium) were assessed and were found to be within the prescribed aquaculture guideline limits. This may show that the local fish feed is not harmful to tilapia fish (*O. mossambicus*) and is comparable to the commercial fish feed. The pigeon pea fish feed formula like any animal feed influenced the assemblage of cyanobacteria and phytoplankton as such there were micro-species that grew in the freshwater system. However, the assemblage of cyanobacteria and phytoplankton was not extreme as compared to the commercial fish feed. There were phytoplankton phyla such as Cyanophyta and Bacillariophyta that are known to produce cyanotoxins when all the conditions are favourable, however, the conditions in this study were not stable enough for cyanotoxins production.

#### ACKNOWLEDGEMENTS

We acknowledge National research foundation (NRF), NRF/DAFF project UID 98686, “Cyanobacteria and their cyanotoxins Impact on Inland Aquaculture” for funding this project. SH is a recipient of the grant-linked scholarship.

#### REFERENCES

- [1] FAO, *The State of World Fisheries and Aquaculture 2018 – Meeting the sustainable development goals*. Rome. Licence: CC BY-NC-SA 3.0 IGO. <http://www.fao.org/3/i9540en/i9540en.pdf>, 2018 (accessed 22 January 2018).
- [2] Tshifura, R. A., *An Assessment of Algae and cyanotoxins in small-holder Aquaculture farms in Vhembe, South Africa*. Unpublished master dissertation, University of Venda. 2018.
- [3] Gumbo, J. R., & Tshifura, A. R., The Dominance of *Microcystis* Species and Microcystin Congeners in a Small Holder Fish Farm, a Case Study of Vhembe District, South Africa. Page 5–8. Proceedings 10th International Conference on Advances in Science, Engineering, Technology and Healthcare (ASETH-18). <https://www.eares.org/siteadmin/upload/7495EAP1118202.pdf> 2018.
- [4] Gabriel, U. U., Akinrotimi, O. A., Bekibele, D. O., Onunkwo, D. N. & Anyanwu, P. E., Locally produced fish feed: potentials for aquaculture development in subsaharan Africa. *African Journal of Agricultural Research*, 2(7), 287–295, 2007.
- [5] Mohammed, R. M. & Ali, M. M., *Effects of Soaked Pigeon Peas Seed Using graded Levels on the Growth performance and Food Utilization of Mon-sex Nile Tilapia, (Oreochromis niloticus) Fingerlings Diets*. Unpublished master dissertation, Sudan University of Science and Technology. 2018.
- [6] Khakhathi, M. R., Pigeon peas (*Cajanus cajan*) seed meal as a dietary protein source for Mozambique tilapia (*Oreochromis mossambicus*) fish. Work in progress. 2020.

- [7] Sehnitzler, A.I, Eglin, F, Robaeh, K.M. and Tremolieres, M., Response of aquatic macrophytes communities to levels of P and N in an old swamp of the upper Rhine plain (Eastern France). *Ecology*. 27: 51–61. Smithsonian Environmental Research Centre, (2012), 2006.
- [8] Smithsonian Environmental Research Centre, [www.serc.svzdu](http://www.serc.svzdu) Accessed on 22 January 2018, 2012.
- [9] Janse van Vuuren S, Taylor J, Gerber A, van Ginkel C., Easy identification of the most common freshwater algae. A guide for the identification of microscopic algae in South African freshwaters. 2006.
- [10] DWAF., DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). South African Water Quality Guidelines – Agricultural Water Use: Aquaculture Volume 6. Pretoria, South Africa. 1996.
- [11] Yada, T. & Ito, F., Differences in tolerance to acidic environments between two species of Tilapia, *Oreochromis niloticus* and *Oreochromis mossambicus*. Bulletin of the Institute of Fisheries Sciences 9: 26–29, 1997.
- [12] Ajuzie, C. C., Microalgae, zooplankton and macroinvertebrate food components of *Oreochromis niloticus* (Piscis: Cichlidae) in liberty reservoir, Jos, Nigeria. International Journal of Fisheries and Aquatic Studies 2017; 5(3): 14–20, 2017.
- [13] SAWS., South African weather service (SAWS). Available at: <http://www.weathersa.co.za/>. Accessed on 22 January 2018. 2018.
- [14] Ronald, W.H., Optimum temperatures and temperature ranges for growth of snow algae. Arctic and Alpine Research 7(1), 13–24, 2007.
- [15] Drikas, M., In: Toxic Cyanobacteria: Current Status of Research and Management. Australian Centre for Water quality Research. Salisbury, Australia: Pdp (Pty) Ltd. 1994.
- [16] Barnabe, G., Aquaculture biology and ecology of cultured species. New York: Ellis Horward Ltd. 1994.
- [17] Britz, P.J. and Hecht, T., Aquaculture in South Africa: History, status and prospects. South Africa: NMB printers (Pty) Ltd. 1990.
- [18] Carignan, V. and Villard, M.A., Selecting indicator species to monitor ecological integrity: a review. Environmental Monitoring and Assessment. 78: 45–61, 2002.
- [19] Edwards, C., Beattie, K.A., Scrimbeour, C.M. and Codd, G.A., Identification of ana-toxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Scotland. Journal of Toxicology. 10: 1165–1175, 2008.
- [20] Bukhari, S.S, Chaudhary, A.A. and Salam, A., Studies on Biodiversity and water Quality Parameters of Indus River (Abstract). Punjab, Pakistan. Zoology, 18(2): 25–65, 2008.
- [21] Bruinsma, J., (2003). World Agriculture towards 2015/2030: A FAO Study. Earthscan: London
- [22] Kugrens, P. and Clay, B.L., Cryptomonads. In: Freshwater Algae of North America. 715755. San Diego: Academic Press. 2002.
- [23] Guiry, M.D. and Guiry, G.M., Algae Base. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; Accessed on 23 March 2018. 2018.