Impact of Tea Processing Water Wastes on Phytoplankton Composition of Tshinane River, Limpopo Province

Sinthumule H, Mokgoebo MJ and Gumbo JR

***Abstract*—** The discharge of industrial waste water on freshwater resources is on the increase worldwide, including in South Africa. The study aimed at assessing the response of phytoplankton upon exposure to high levels of nutrients along the Tshinane River Limpopo Province. The study showed different phytoplankton assemblages with different changes in physico-chemical levels. Environmental factors do have a noticeable effect on phytoplankton abundance as it was shown by statistical analysis. Results computed by the Czekanowski coefficient showed that various environmental factors components contributed to the different composition and types of phytoplankton abundance ($p<0.05$). When environmental factors showed fluctuation (Increase or decrease) a different type of plankton was found to be tolerant to those levels. A total of 64 species were identified upstream and 103 species identified downstream. Phytoplankton spectrums were recorded from six taxonomic groups namely *Chrysophyta*, *Dinophyta*, *Chlorophyta*, *Bacillariophyta*, *Cyanophyta* and *Dinophyta*. The dominant taxonomic group was *Chlorophyta (Downstream)* and *Bacillariophyta* was the dominant phytoplankton upstream. The results supports the assumption that an increase in nutrients lead to a diverse phytoplankton species even if all the other parameters are within the South African Water Quality Range for Aquatic ecosystems. This shows that tea processing waste has a minimal impact on the ecosystem health of Tshinane River and the river is able to recover from the nutrient enrichment.

Keywords*—Phytoplankton, Water quality, Physico-chemical parameters, Tshinane river system*

I. Introduction

Black tea (*Camellia sinensis*) is a drink that is enjoyed worldwide whether hot or c old (ice tea). Black tea production in Vhembe district of Limpopo province occurs at Tshivhase and Mukumbani Sapekoe Estates with estimated 1081 ha under cultivation producing 2 million kg of black tea [1]. The picked tea is processed at the Tshivhase tea estate and after that the production area cleaned with water. This wastewater flows into Tshinane River. The tea processing waste is rich in antioxidant and antimicrobial phenolic compounds [2], lignocellulose [3] and catechins and caffeine, lignin, condensed tannins, structural proteins [4]. Thus the discharge of the black tea wastewater to the environment is a major challenge.

Aquatic ecosystems in rural South Africa have been impacted over many years by both commercial and subsistence anthropogenic activities [5]. These impacts consist of subsistence and commercial farming, natural pollution and domestic use (washing clothes, cars, washing dishes and for bathing) and recreational use such as swimming. Most of South Africa water use is dominated by the agricultural sector followed by domestic and urban use, industrial use and power generation and forestry [6].

Eutrophication due to the increase of anthropogenic nutrients loading, mainly nitrogen and phosphorus, from watersheds has been a central environmental issue along many marine coastal areas over a decade [7]. It causes an increase of phytoplankton biomass and turbidity and decrease of submerged grasses, thus inducing bottom-water hypoxia due to deterioration of excess organic matter [8].

The direct extraction of water from rivers, as well as the point and non-point pollution by agricultural herbicides, pesticides and fertilizers has been compounding, damaging effect on water ecosystems reducing water quality and quantity and changing the natural habitat of aquatic biota in the river as they contribute largely to high nutrient level, these high nutrient levels promote the abundance of phytoplankton whilst decreasing water quality of the Tshinane river system.

In many aquatic systems phytoplankton communities have been mostly used to investigate the level of nutrient loading. Microorganisms such as phytoplankton respond quickly to nutrient content changes which mean these species can be used to monitor water quality. The Tshinane River forms a fundamental part of the local community as it offers goods and services such as provision of water to wash clothes, for bathing, abstraction for domestic use and for religious purposes (Baptism), other services include the use of sand /soil for house construction. The main objective of the study was to determine the phytoplankton assemblage/composition upstream and downstream of the tea proceeding waste discharge into Tshinane River.

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II. MATERIALS AND METHODS

A. Location of the Study Area

The study was done at various communities such as Maranzhe, Mukumbani, Tshivhungululu, Lunungwi and Gondeni Limpopo Province South Africa, along the Tshinane River (Figure 1). The Tshinane River runs through these communities and it was important to get both upstream and downstream samples of the industrial tea processing estate.

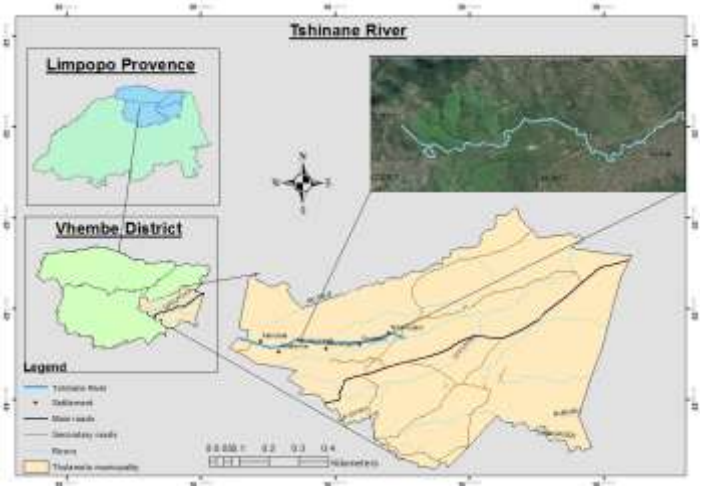

Fig 1: The location of sample sites in the Tshinane river, Vhembe district, Limpopo province, South Africa

B. Sample Collection and Onsite Physical Measurements

A total 20 water samples were collected with a total of 10 water samples being from upstream and 10 samples from downstream during the months of May 2015. Each point was identified by marking every ten meter-interval in a 50 meter line placed parallel to the Tshinane River flow. The 90ml plastic non-transparent water bottles were carried in a cooler bag with ice. Physical water quality parameters: pH, temperature and total dissolved solids were measured at each point with a multimeter instrument.

C. Laboratory Analysis

The chemical water quality parameter: nitrates and phosphates were measured in the laboratory using an Ion chromatography instrument.

D. Phytoplankton Collection

Phytoplankton samples were collected during the month of May 2015. The samples were collected from the 1st of May to the 31st of May (31 days) because the month of May. Water samples were collected once between 12h00 and 14h00 on the same day. The phytoplankton samples were collected at the depth of 5 to 20 cm (so that bottom dwelling specimen and top dwelling specimen could be collected for analysis as different phytoplankton species dwell in different depth of a water system), using a handheld phytoplankton net with a 10 cm radius with a mesh size of 25 µm net placed inside the river and moved in a zig-zag motion within the river system (moved from the middle of the river to the left river bank, then moved to the right river bank via the middle of the river, in order to collect specimen that dwell at the river banks and the middle of the river). Bottom samples were also collected as the river discharge varied at each sampling point, this was done by placing the net at very close proximity to the bottom of the river system but without placing the net directly on the river bed. Rocks, leaves and other debris were taken out of the collected samples. Lugols solution and an additional 1ml of formalin was added to the collected sample for preservation of the specimen and were stored in a dark bottle at a temperature of 4° C (to prevent rapid decay) for 4 h.

E. 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 at 500x magnification. The samples were enumerated by using 10ml. A sample of 10ml was extracted from each of the 90ml collected sample without using a syringe filter, the sample was allowed to settle for 24hours before analysis. All of the 20 samples were analyzed.

For hard algae analyses, 10ml from each of the collected samples were poured into an enumeration chamber and allowed to settle for 24hours and enumerated using a compound microscope at 1250x magnification. The abundance of the specimen was weighed by counting the number of identified species. 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 a sample, the specimen were identified by using a chart adopted from [9] accessible on www.serc.si.edu. Phytoplankton presence, absence and abundance was used to determine the diversity of phytoplankton, the Czekanowski coefficient was used to measure the similarities and dissimilarities in abundance of the enumerated species at different sampling points.

F. Data Analysis

Microsoft excel 2013 was used for data analysis, differences in nutrients between upstream and downstream were analysed using one way ANOVA at level of significance at $p<0.05$ and the preparation of graphs.

The Czekanowski coefficient was calculated as:

$$S_C = \frac{2\,\Sigma \min (Xi, Yi)}{\Sigma Xi + \Sigma Yi}$$

Where: Xi and Yi = the abundance of spp in both quadrats and
Σ min (Xi, Yi) = the sum of lesser scores of spp where it occurs in both quadrats.

III. RESULTS AND DISCUSSION

A. Nutrients Concentrations Comparison of Upstream and Downstream

The level of phosphates were above 3 ppm and nitrates were above 30 ppm in the upstream and downstream section of the Tshinane river (Figure 2) and are within the guideline of the [10], which is 25ppm for phosphates and 50 ppm for nitrates. The single factor ANOVA show that the upstream and

downstream phosphate levels are similar and not significantly different (p=0.79). The downstream nitrate level are excess of the South African quality guideline value of 50 ppm. Also the single factor ANOVA show that the upstream and downstream nitrate levels are dissimilar and are significantly different (p=0.00).

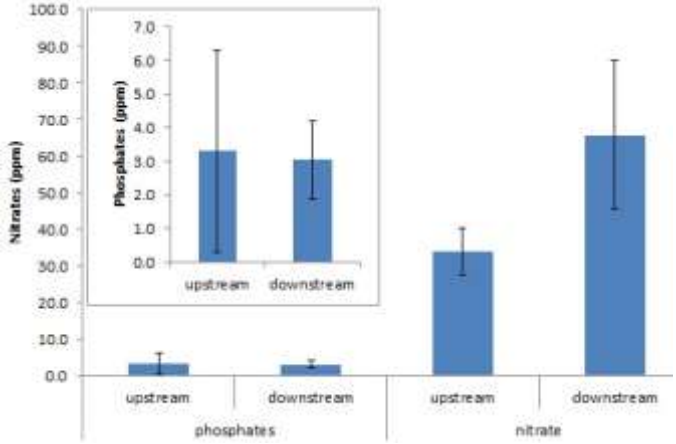

Fig 2: The variation in phosphate and nitrate levels in the Tshinane River

The phosphate and nitrate levels in the Tshinane River probable originate from anthropogenic and not from geological sources (Figure 3). The anthropogenic sources are probable from the discharge of black tea processing wastewater and discharge from sewage plant with regards to the nitrate levels and from commercial and subsistence farming operations. This may be true since the nitrate levels seem to increase downstream of Tshinane River. In separate study in the Luvuvhu River catchment it was shown that the nutrient enrichment was partly due to sewage discharge [11]. This study is in agreement with the study of Han et al. [12] in China that nitrate from a tea plantation was the source of nitrate in their environment.

Fig 3: The location of the sewage plant and tea processing plant and Tshinane River

B. Phytoplankton Biodiversity Along Segment of the Tshinane River, During May 2015

Thus, the presence of nutrients is likely to promote the growth of algae and phytoplankton. Phytoplankton are classified into 3 growth stages namely survival, bloom and generalist [13]. The level of phosphate show that the species are still in the generalist stage, which support as to why there is no visible algal bloom, however they can still be collected and analyzed. The concentration of phosphates in water bodies is also an indicator of eutrophication, Singare et al.[14], argues that, even at low level, phosphate pollutants accumulate in the form of sediments and settle down at the bottom. According to [15], there is a need to minimize the phosphate originating from commercial farming so as to protect aquatic ecosystem.

Table 1 illustrates the identified phyla for all the areas that were sampled in May 2015. Phyla richness was shown by (absence, presence and abundance), for all sampled points in May of 2015, each species was identified using the absence, presence and abundance method, at different points either upstream or downstream. Some species were recorded at both upstream sites and downstream sites. The table shows where each species was identified, with (0) meaning that the species was not present and (>0) meaning the species was present (identified) and quantified.

TABLE 1: THE BIODIVERSITY OF PHYTOPLANKTON RICHNESS (ABSENCE, PRESENCE AND ABUNDANCE), IN THE STUDY AREA

PHYLUM/ PHYLA	Upstream	Downstream
1.Bacillariophyta		
I. Cylindrical	**0**	**5**
II. Single-celled	**0**	**2**
III. Aphideae	**9**	**7**
IV. Monoraphideae	**2**	**0**
V. Raphidioideae	**10**	**0**
VI. Monoraphideae	**5**	**0**
VII. Biraphideae	**3**	**5**
2. Cyanophyta		
I. Oscillatoria	**0**	**5**
II. Microcystis	**0**	**2**
III. Merismopedia	**0**	**3**
IV. Cylindrospermopsis	**11**	**2**
V. Anabaena	**0**	**10**
VI. Cylindrospermopsis	**0**	**8**
3. Chrysophyta		
I. Synura	**3**	**0**
4. Dinophyta		
I. Peridinium	**2**	**8**
5. Euglenophyta		
I. Strombomonas	**8**	**0**
6. Chlorophyta		
I. Ankistrodesus	**4**	**10**
II. Closterium	**0**	**12**
III. Dictyosphaerium	**5**	**8**
IV. Cladophora	**0**	**3**
V. Chlorella	**2**	**5**
VII. Spirogyra	**0**	**7**
TOTAL	**64**	**103**

The observation made was that, the downstream points had the most identified phyla (103) which were the highest. Similar results were found by [16] when they found that phytoplankton biodiversity was linked to nutrient availability.

The upstream points had the least identified phyla (64) and upstream having (103) recorded phyla during the analysis period. Seven species were found at both upstream and downstream, this is because the phytoplankton species identified were tolerant to both polluted and not polluted environments. The Tshinane River is not heavily polluted,

which explained as to why their present upstream and downstream i:e *Peridinium* species are tolerant to pollution which is why there in abundance downstream as compared to upstream where there is low nutrient content. Whereas the species *Cylindrospermopsis* species were nutrient intolerant, which is why their found in high abundance upstream and low abundance downstream.

The downstream points had high phyla, this goes to show that when there is introduction of pollutants then phytoplankton phyla increases. This study is similar to the study of [17] in China, where nutrients availability contributed to increase in phytoplankton biodiversity. However, [18] conducted a study in Nigeria wherein it was revealed that the increase in nutrients caused an increase in phytoplankton phyla being recorded; this is similar to the results of this study.

As stated by [19] showed that the movements of organisms in stream was strongly influenced by the movements of overlying waters and well mixing which can create spatial homogeneity in diversity. This statement explains as to why most phytoplankton phyla that were identified upstream were also recorded downstream, this was so because the river is a perennial river therefore there is well mixing of the water and even diatoms can move downstream.

The results of phytoplankton diversity to increase when there is high levels of nutrients is different with a study done by [20], where no relationship was found between water quality along commercial agricultural farms and fish diversity. The following Czekanowski coefficient of 0.29 shows the similarity in abundance of species recorded upstream and downstream. The low similarity percentage showed that more phytoplankton species prefer areas with high nutrient level as compared to where there is low nutrient concentration. Overall a significant difference ($p>0.05$) in phyla richness was observed between upstream and downstream sites of the commercial farm as shown by the Czekanowski coefficient small similarity and the high percentage difference (71% dissimilarity). The dissimilarity show that most species were pollution intolerant however they can withstand little nutrient pollution. However the presence of available nutrients is likely to lead to harmful algal blooms in the long term and the productions of cyanotoxins which are hazardous to the environment and affect human health [21-22].

IV. Conclusion

There was no significant difference was experienced in phytoplankton diversity between upstream and downstream using species absence, presence and abundance, the difference between the numbers of phyla that were recorded showed that even though there is a difference however the difference is not significant. The difference in the phyla composition was a result of agricultural and anthropogenic activities occurring in the river system. According to Roux (2005), in ecological terms species phyla can act as indicators in the event of pollutants entering a system. The disappearance of most *Bacillariophyta* species (which is pollution intolerant) downstream and high abundance of *Chlorophyta* species (which is nutrient tolerant) downstream support the statement that even micro-organisms can be used as pollution indicators, the absence of two most pollution intolerant families i:e *Chrysophyta* and *Euglenophyta* show that indeed microorganisms such as phytoplankton can be used to monitor water pollution. The study shows that the tea processing wastewater is contributing to the deterioration of water quality within the Tshinane River. Thus more research is required in order to reduce the black tea processing waste in order to protect the environment.

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