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THE UNUSUAL REDDISH-BLOOM APPEARANCE IN A FRESHWATER FISHPOND AT KINGOLWIRA NATIONAL FISH FARMING CENTER, MOROGORO, TANZANIA

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Abstract

The study aimed to examines a case of what constituted the uncommonly reddish-bloom appearance in the fishponds during the dry season (September 2018) at Kingolwira National Fish Farming Center located in Morogoro, Tanzania. The study used a benchtop FlowCAM® to investigate species' morphology. One-time assessment of physico-chemical characteristics during the event was performed from the reddish and non-reddish fishponds. Images were compared with the available literature, but also t-test statistics were performed to examine the difference between the fishponds. The results show that the fishponds were significantly ($p<0.05$) different from each other in terms of physico-chemical parameters except for water temperatures. Furthermore, *Microcystis* species dominated the non-reddish fishpond whereas *Euglenophytes* species were pervasive in the reddish fishpond. The two species have the potential to produce secondary metabolites (toxins) or to produce a hypoxia condition that is harmful to the fishery, aquatic ecology, and human. To confirm toxicity nature and dynamics further, future studies should consider extensive and regular diurnal and long-term monitoring.

Keywords: Harmful algae; *Microcystis*; Reddish-bloom; Euglenophytes; FlowCAM

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1. Introduction

Harmful algal blooms (HABs) are now a concern to the global community, and that is predicted to compromise climate change adaptation as well as mitigation across sectors (GEOHAB, 2015). In the aquatic environment, three main things are considered regarding HABs, namely, toxin production, food web altering, and hypoxia generation (Paerl et al., 2011). In Tanzania, about 1119 species of phytoplankton have been reported, which includes diatoms (54.7%), green macroalgae (25.5%), blue-green algae (14.5%), and euglenoids (5.5%) (The United Republic of Tanzania, 2001). It is further estimated that there are close to 14,000 freshwater fishponds in the country, and their distribution depends on water availability, suitable land for farming, and awareness and motivation (Mushi, 2006). The dwindling of the available surface water resources and the need for diversification of livelihoods (Paavola, 2008) has stirred the movement into fish farming for both small and large scale farmers even with limited resources (Kaliba, 2006). A recent report also noted that the fish farming industry as a fast-growing social-economic activity in Tanzania (Rukanda & Sigurgeirsson, 2018). Besides, small-scale fish farmers lack extension services to support the sector (Kangalawe & Liwenga, 2005; Mdegela et al., 2011; Niang et al., 2014). A study by Chenyambuga et al. (2014) assessed the productivity and marketing of Nile Tilapia in Mbarali and Mvomero districts in Mbeya and Morogoro regions, respectively noted several constraints including irregular water supply, drought cases, and poor management practices. Furthermore, the occurrence of harmful algal blooms in the chosen area is scant in the literature (Kimambo et al., 2019). Some other observation, for example, Miraji et al. (2016), noted that standards and guidelines for nuisance algal blooms (example, cyanotoxins) are yet to be developed.

Due to the impacts associated with nuisance algal blooms on the fishery, water quality, and human, rapid assessment is inevitable. Red algae are a widespread group of uni-to-multicellular aquatic photoautotrophs, of which about 98% are marine, and 2% are freshwater (Deluquei & Lopez-Bailtista, 2007). As noted earlier, red algae as harmful algal blooms have the potential to deplete oxygen hence effect to fish (Stone & Daniels, 2006). Red algae have been reported in fishponds, and their implication on water quality and fish are also vivid (Zuccarello et al., 1999; Stone & Daniels, 2006; Kim & Kim, 2014; Mandal et al., 2017; Mandal et al., 2018).

In Tanzania, studies on red algae have been reported interchangeably (seaweeds and algae) in marine or coastal ecology (Msuya & Neori, 2002; Buriyo et al., 2004; Msuya, Kyewalyanga, & Salum, 2006; Troell et al., 2011). The social-economic potentials of the industry have led Tanzania to be among the group of leading carrageenan-producing (food derived from red algae or seaweeds) in the tropical area along with the Philippines and Indonesia (Deluquei & Lopez-Bailtista, 2007).

The impacts of red algae on aquaculture, ecology, and human health are trace or non-existing in Tanzania. The only available work is that of Hahn (2009) who isolated algal strains from freshwater ponds located at the University of Dar es Salaam and Lake Victoria. He then developed candidate species (due to lack of pure culture) with the phylum *Actinobacteria* species to represents planktonic freshwater bacteria (cyanobacteria inclusive). Respectively, Hahn (2009) placed the strains under "*Candidatus* Aquiluna rubra" [*Aqua* water; *Luna* the moon and *rubra* the red] and "*Candidature* Rhodoluna planktonica" both referred as aerobic red-pigmentation strains. This overview demonstrates the need for investigation on algal blooms species and their dynamics in freshwaters in the region.

In studying algal blooms, several techniques are available, including conventional ones such as manual microscopes, molecular, analytical techniques, and the use of remote sensing. The traditional technologies are laborious and time-consuming but are still useful. Advanced technologies such as flow imaging camera (FlowCAM) have been reported to be efficient in screening, determination, and or assessing the morphological characteristics of individual species in a variety of liquid samples. The techniques have been used before for algal species identification and enumeration in both field and laboratory. The present study aimed to identify species responsible for the reddish blooming which happened at Kingolwira National Fish Farm Centre, Morogoro, during the dry season (July-September 2018) and consequently, to provide feedback to farmers during the site visit.

2. Materials and methods

The Kingolwira National Fish Farming Center is located at 6° 45' 20.46'' S latitude and 37° 45' 15.56'' E longitude, Morogoro, Tanzania (Figure 1). The center is government-owned, mainly for farmers' demonstrations, hatching, and production of fingerlings to the nearby fish farmers. The infrastructures are well maintained, and the source of raw non-treated water for the center is from the Bigwa River, which is a tributary of the Ngerengere River.

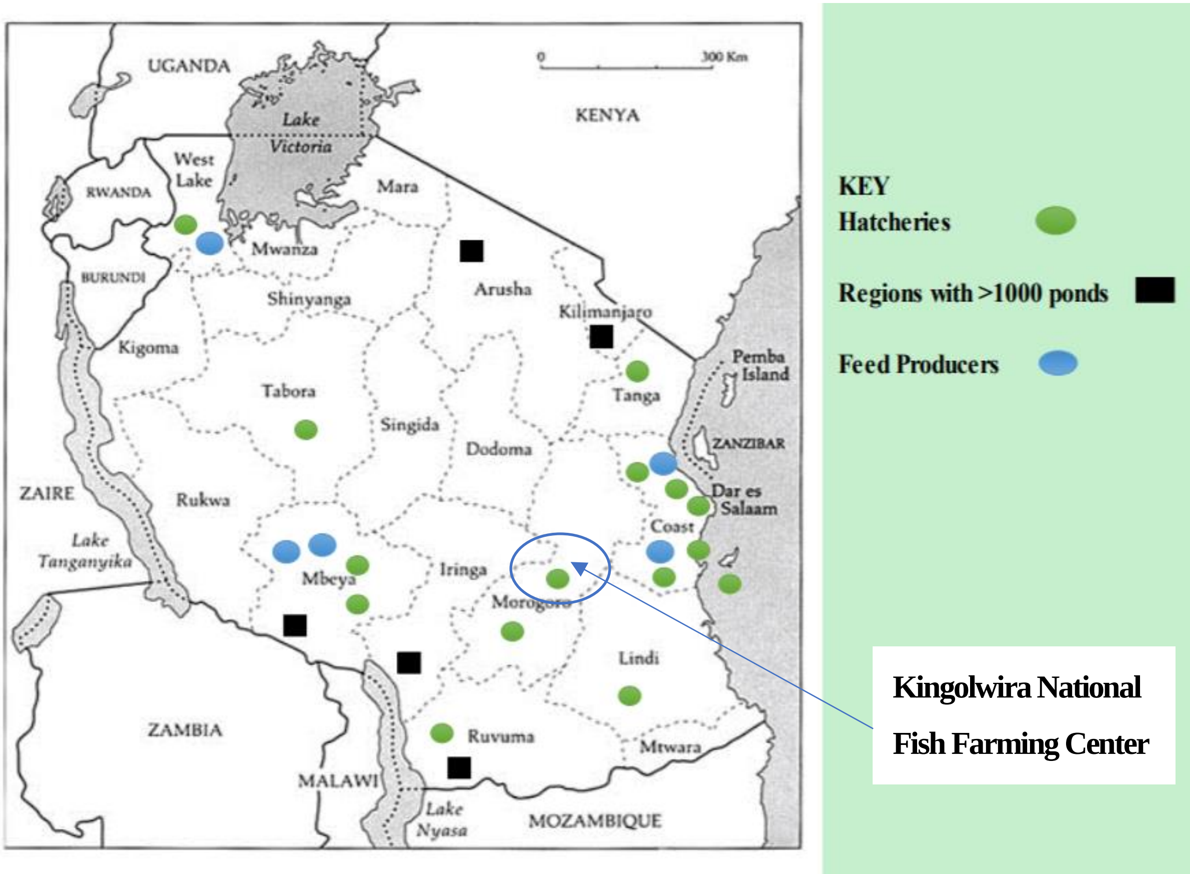

Figure 1: Map of Tanzania with the distribution of areas including >1000 fishponds, hatcheries, and feed-produce centers. Source: (Rukanda & Sigurgeirsson, 2018).

One-time composite sample (1 L) was collected from the two fishponds (i.e., reddish-green and the other with greenish coloration) at the time of observation for the main project and during the dry season (September 6, 2018). Polypropylene bottles were used for collecting water samples for the algal identification and nutrients analysis. Subsamples of 50 mL in an amber glass bottle preserved with 1% Lugol's solution as in Shan et al. (2019) were transported in a cooler box to the University of Venda, South Africa for analysis.

A benchtop FlowCAM® (Model VS4 serial Number 5049 copyright 2003-2012 Fluid Imaging Technologies, Inc., 65 Forest Falls Drive, Yarmouth, Maine 04096, USA) particle analyzer was used in the identification of individual cells in the specimens. The FlowCAM® was configured at 10X objective, flow cell (FC) 100 µm, and under auto image mode. Parallel to sampling observations field photos/images (Figure 2) and physico-chemical parameters (Table 1) (i.e., pH, Oxygen-Redox Potential, dissolved oxygen, total dissolved solids, electrical conductivity, and water temperature) were measured in triplicate using the calibrated HANNA HI98194 multiparameter meter. The total phosphorus was determined in the laboratory using a Spectrophotometric analysis - phosphomolybdenum test, analogous to a method of EPA 365.2+3 as per the manufacturer's directives (Merk KGaA, Darmstadt, Germany). The images from the FlowCAM® were compared (mainly the morphometric features) with the field guidelines (Kannan & Lenca, 2012).

Descriptive statistics and distribution of the particles were handled with the Visual Spreadsheet® 3.0, which comes with the FlowCAM. Anecdotal observation (photos from the field) were also taken for recognition. The descriptive and t-test statistics for the physico-chemical characteristics between the two fishponds were performed using XlSTAT 2019.1.3 (Addinsoft, 2019).

3. Results and discussion

Table 1 shows a comparison between the physico-chemical characteristics of the water in both reddish bloom and non-reddish bloom fishponds, as measured in situ at the time of sampling.

Table 1: Selected water quality parameters for both non-reddish bloom and reddish bloom fishponds at Kingolwira National Fish Farming Center, Morogoro Tanzania.

	Non-reddish fishpond			Reddish fishpond			
Parameter	**Mean (triplicate)**	**SD**	**CV (%)**	**Mean (triplicate)**	**SD**	**CV (%)**	**T-test statistics (P value at α = 0.05)**
pH	9.07	0.02	0.25	7.68	0.15	1.90	0.003*
Oxygen Redox Potential (mV)	100.37	1.63	1.62	132.80	8.71	6.56	0.032*
Dissolved Oxygen (mg L^{-1})	4.34	0.05	1.06	3.11	0.22	6.92	0.006*
Electrical Conductivity (µS cm^{-1})	103.33	2.31	2.23	65.67	2.08	3.17	<0.0001*
Total Dissolved Solids (mg L^{-1})	51.67	1.15	2.23	32.67	1.15	3.53	<0.0001*
Temperature (°C)	27.27	0.23	0.85	27.85	0.35	1.27	0.441 (NS)
Total Phosphorous (mg L^{-1})	BDL			1.13	0.057	5.04	NA

CV: Coefficient of variation; N: Not Applicable; *Statistically Significant; BDL: below the detection limit (<0.5 mg L^{-1}); NS: Statistically Not Significant; SD: Standard Deviation

Water temperature showed no significant differences (p>0.05), while other investigated parameters (pH, ORP, DO, EC, and TDS) showed a significant difference between the two fishponds. The pond with reddish coloration gauged relatively lower pH, with higher oxygen redox potential and lower electrical conductivity but also relatively lower dissolved oxygen. Total phosphorus (TP) for the non-reddish bloom pond was below the detection limit (<0.5 mg L^{-1}), while the reddish bloom pond recorded a mean TP of 1.13 mg L^{-1}. Despite the differences, all the measured parameters were within the desirable ranges compares well with the ranges in fishponds reported by Bhatnagar and Devi (2013). The dissolved oxygen values were on the threshold for the fish to survive. Field images of both non-reddish and reddish blooming are presented in Figures 2A and 2B for recognition.

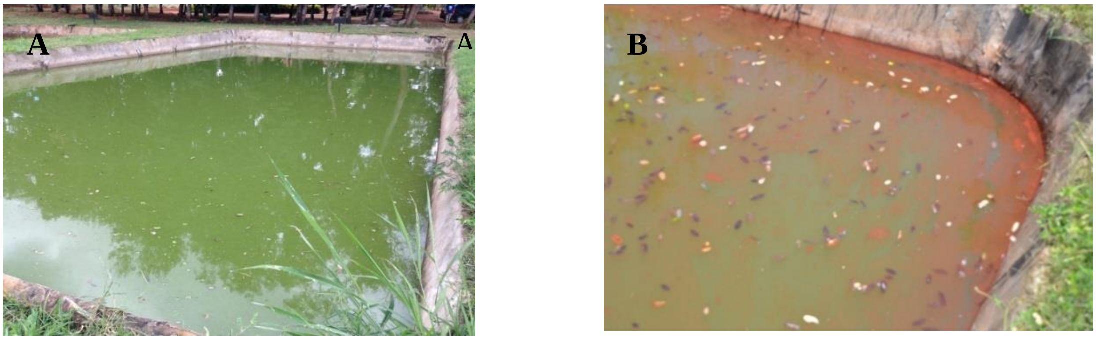

Figure 2: Plates for the non-reddish fishpond (A) and reddish fishpond blooming (B) as observed during the dry season (September 6, 2018) at Kingolwira National Fish Farming Center, Morogoro, Tanzania (Kimambo et al., 2018).

Cyanobacteria vary in colour from blue-green, grey-green, violet, brown, purplish to red when examined under the microscope. The colours depend on the relative proportion of their photosynthetic pigment, for example, chlorophyll (green), phycocyanin (blue), and phycoerythrin (red) (van Vuuren et al., 2006). The field images, for example, Figure 2B, show a visible mixture of both green and reddish blooming. The red algae in the photo look like the one reported by Stone and Daniels (2006). According to Stone and Daniels (2006), euglenoids (e.g., *Euglena Sanguinea* and *E. granulata*) are associated with the red coloration of water but also can produce toxins. During sampling (anecdotal observation and or filed experiences), the bloom was smelling earthy/musty, which is sometimes associated with nuisance algal blooms, as noted by Watson et al. (2015).

Morphological identification

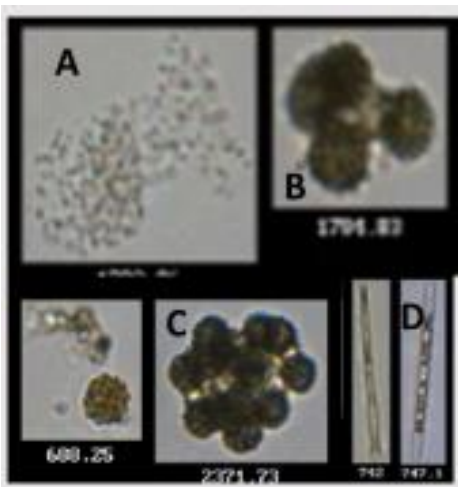

Figure 3: Species identified at the non-reddish blooming pond are *Microcystis* (A, B & C) and filamentous species of *Nodularia (D).*

Unlike the reddish fishpond, the non-reddish (as in Figure 3A to 3D) fishpond was dominated by defined *Microcystis* cells than the reddish pond (Figure 4A to 4C), that is, there are distinct features (by virtualization) between the species from the two ponds.

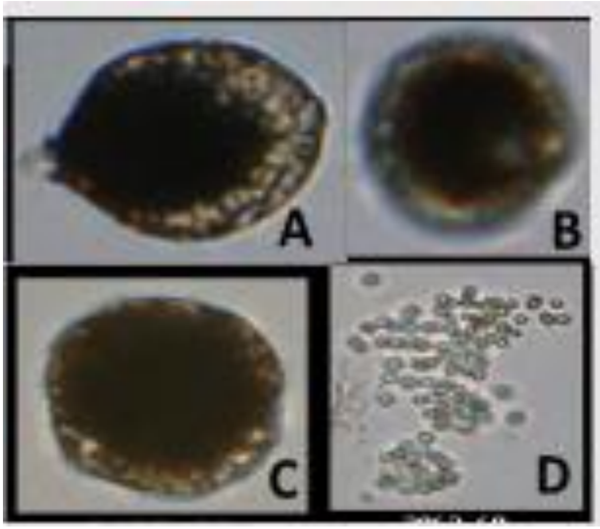

Figure 4: Species identified at the reddish-blooming ponds were *Phacus limnophilus (A) (*resembles species under *Euglena*), *Haematococcus* (B & C), and *Microcystis* (D).

Species identified at the reddish-blooming ponds, for example, *Phacus limnophilus* (A), resembles species under *Euglena,* which is described by is movement during growth stages (http://algaevision.myspecies.info/taxonomy/term/1798). Other species were *Microcystis* (D) and *Haematococcus* (B), as previously reported by Bellinger and Sigee (2010). FlowCAM® uses equivalent spherical diameter (ESD) and area-based diameter (ABD) algorithm to estimate particle/image bio-volume (Buskey & Hyatt, 2006; Edler & Elbrächter, 2010; Davis, 2015; Ma et al., 2014; Wang et al., 2015). Summary results from the FlowCAM of both non-reddish and reddish blooming fishponds are well depicted in Table 2 by their area-based diameter (ABD) and equivalent spherical diameter (ESD).

Table 2: Summary statistics as per the FlowCAM® results for non-reddish and reddish ponds.

Summary statistics	Non-reddish pond		Reddish-fishpond	
	Area-Based Diameter (ABD) µm	Equivalent Spherical Diameter (ESD) µm	Area-Based Diameter (ABD) µm	Equivalent Spherical Diameter (ESD) µm
Mean	31.08	44.34	26.18	41.16
Minimum	18	18.91	18	18.75
Maximum	89.72	209.11	72.17	89.94
Standard Deviation	13	22.49	10.25	14.84
Coefficient of variation (%)	41.84	50.73	39.13	36.05

The results suggest that ESD for non-reddish fishpond registered significant high values (209.49 µm) while the reddish fishpond registered a maximum value of 89.94 µm. This implies that the non-reddish fishpond had high biovolume than that of the reddish blooming fishpond. During the FlowCAM® runs, particles with less than 18 um Area-based diameter were filtered out, therefore similar minimum values for both fishponds. Form the descriptive results (Figure 4) show a dominance of two species, namely *Microcystis* and *Euglena* species. The anecdotal observation as well shows the differences in the color of the water, which is the interest of the study and worrisome to farmers.

According to Stone and Daniels (2006), most of the red algae in freshwaters are *Euglena* species, such as *Euglena sanguinea*, *E. granulate*, and *Planktothrix* species and mostly "*Haematococcus*." Searching on different algal databases, the study findings compare well with that in algaebase (http://www.algaebase.org/search/species/detail/?species_id=aace9d12a12d42e45) and the algaevision (http://algaevision.myspecies.info/taxonomy/term/1840 which is closely related to *Haematococcus* species. Other species, for example, Figure 4A, resembles *Phacus limnophilus (*http://algaevision.myspecies.info/taxonomy/term/1798), which falls under *Euglena* species (Guiry in Guiry & Guiry, 2019), and that may change in shapes (sometimes appearing oval/round) as they develop (Kannan & Lenca, 2012). *E. sanguinea*, as well, has been reported to be able to form reddish bloom and toxins (Kannan & Lenca, 2012). According to Zimba et al. (2010), *E. sanguinea* has been associated with many fish kills which were difficult to believe, which means that a considerable gap in the literature that need quick excursion. Greenish-reddish (from euglenophytes) blooming might be challenging because "euglenophytes do not have unique pigment biomarkers which can be used to locate using remote sensing and or other analytical procedures" Zimba et al. (2010).

During an interview with an officer in charge of the Kingolwira National Fish Farming Center regarding the performance of the pond, he replied that;

> "the fishpond which showed a reddish coloration is not so productive as compared to other ponds and fish kept in sometimes their skins look like fungal-infected" (Kajitanus O.O, personal communication, September 06, 2018).

With that concern, one might speculate and link it with the levels of dissolved oxygen (lower values) and total phosphorus (high values), which differed significantly between the two fishponds (Table 1). This uncommon observation of green-reddish bloom and which happened during the dry season in one of the freshwater fishpond at Kingolwira National Fish Farming Center provokes more scientific questions. Some other studies elsewhere right away consider them as invasive species (Zohdi & Abbaspour, 2019). Since climate change has been linked with blooms proliferation, future studies should assess the linkage between cases of extreme algal blooms and extreme weather events and or climate variability. There are still schools of thought that can be depicted from the case study, such as a competition between red-pigmented, green-pigmented species in freshwater resources, and small-scale farmers might be exposed to harmful algal blooms. The use of palm hand, as reported by Rukanda and Sigurgeirsson (2018), which is commonly for assessing water quality, is unhealthy if exposed to HABs events. The study acknowledges that several limitations are thought to impact the quality of our findings, for example, according to Manoylov, (2014), common preservatives such as formaldehyde and Lugol's solution can discolor cells which alter the morphology of colonial and filamentous forms. The use of Lugol's solution in the present study could have distorted the cells, although previous studies have used the same with the positive results. In future studies of the same can be repeatedly and compared with a standard microscope but also comparing the stained versus destained samples.

4. Conclusion

The study employed a case study approach to morphologically identify algae species responsible for reddish-green bloom in freshwater fishponds at Kingolwira National Fish Farming Center, Morogoro, Tanzania during the 2018 dry season. The present study adds to the body of knowledge about potential harmful algal blooms in the region with a paucity of data. The study found that *Microcystis* species dominated the non-reddish fishpond was while the reddish pond registered more of *Euglena* species, which could be the one responsible for the reddish coloration. *Microcystis* is popularly known for its ability to produce secondary metabolites (cyanotoxins) while *Euglena* is in rare cases but have the potential to harm fish, ecology, and human if exposed. The anecdotal observation noted that blooms were from noon hours towards the evening but also could smell an earthy/musty smell, which is also associated with HABs. Likewise, the fishponds attest to a significant difference between the two fishponds for almost all the water quality parameters between the reddish and non-reddish blooming fishponds, except for the water temperature. The present study is the first or among the very few reports on reddish blooming in freshwater fishponds in Tanzania, although it has been reported in some other parts of the world. The study serves as the basis for future studies on reddish bloom in freshwater reservoirs/aquaculture systems in Tanzania. Future studies could fruitfully explore this issue further by considering a potential health risk assessment and toxicological effects of nuisance blooms on the fishery industry and humans health in Tanzania. It is also a question of future research to conduct monitoring, especially the diurnal variation of blooms and molecular-level analysis.

Conflict of interest

Authors declare that there is no known conflict of interest.

Authors contributions

KON idea conceptualization, design, data analysis, and manuscript writing; **JRG & HC** funding acquisition, review, and editing; **TNM** review and editing.

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