Microalgae (*Selenastrum Capricornutum*) Growth Inhibition Following Exposure to Pond Water: Monitoring Pond Toxicity

Victor Eshu Okpashi*

Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria.

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Bio-indicators are organisms, that can be used to determine the state of health and biogeographic changes in the environment. Microalgae, are also used as bioindicators to assess the health of natural ecosystems such as pond water and are utilized to evaluate the media in which a biological system operates, as well as the molecules within it. They serve as a symbol of the high quality of their surroundings. Microalgae respond quickly to changes in the environment, making them an excellent biomarker for evaluating the quality of ponds and water pollutants. This work was designed to monitor the onset of pond water toxicity using Selanastrum capricornutum. During the present study, fish were grown in a pond, with old water being replaced every five days or more. The hazardous metabolites in the pond water were examined for the beginning or build-up of harmful metabolites. The Algaltoxkit approach was used for five days. Selanastrum capricornutum, a microalga, was grown as a bio-indicator of pond toxicity. The pond water was collected without dilution from day one to 5th day. The microalgae test was conducted for 72 hours, with minor deviations from ISO guideline 8692. Microalgae growth inhibition varied among the days monitored, according to the results, findings imply that pond water poisoning begins on 2nd to 5th day. Findings shows that hazardous compounds in the pond water at 5th day may be affecting the fish's ability to grow and develop normally. The Algaltoxkit kit could also be a useful, quick test instrument for detecting the beginning of hazardous metabolites in a fish pond.

Keywords: Algae Growth-Inhibition; Bioindicator; Catfish; Pond-water; toxicity.

Aquaculture refers to the practice of cultivating populations of aquatic plants and animals in a variety of water conditions, including freshwater, brackish, and marine water[1,]. Fish is believed to offer around one-sixth of the world's protein, making aquatic foods a major source of protein and other nutrients[3]. The feeding habits, development rate, illnesses, and survival value of pond fish can all be influenced by the water quality[4,5]. Several elements, including dissolved oxygen (DO), temperature, pH, organic load, and ammonia, as well as meteorological conditions, influence pond quality[6]. Phytoplankton production and respiration are linked to dissolved oxygen[7]; pH and ammonia are linked to temperature and the number of organic matter inputs, as well as ammonia excretion by fish[8]. Nutrients such as nitrogen and phosphorus are supplied by the fish diet, with

*Corresponding author E-mail: Vic2reshu@gmail.com

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roughly 35% of these nutrients partitioned in fish biomass[9]. The excretion of metabolic waste is caused by the uptake and metabolism of nutrients[9]. Sediment can hold 100–1000 times more nutrients than water and accumulates unused nitrogen and phosphorus[10]. The concentration of stored nutrients, organic matter, and bacterial density in the sediments may rise and then gradually be released for uptake[11]. Under aerobic conditions, mineralization of stored organic debris results in the development of harmful compounds, which degrades pond water and health[7]. The quality of the fish pond is determined by the amounts of dissolved oxygen, ammonia, and nitrite, which are regulated by the feeding rate. Microorganisms such as microalgae are employed to monitor aquatic and terrestrial environments as indicators. It is simple to test them and they are readily available. When microorganisms are exposed to toxins in the environment, such as heavy metals like cadmium and polycyclic aromatic hydrocarbons, they produce new proteins called stress proteins, which are employed as early warning indicators[12]. Microorganisms make up a large portion of marine biomass and are responsible for the nutrient cycle in the ocean[13]. Environmental contaminants in water can be detected using microbial indicators[14]. The presence of toxins in water can easily be detected by observing changes in bacteria' digestive systems, which are rejected or affected by toxins that may restrict their growth[15]. This test is speedy in comparison to other procedures. This test is quick to monitor when compared to other test procedures. However, the ability to only detect changes in organisms caused by poisons is a significant shortcoming[12]. The bacterium Vogesella Indigofera, for example, reacts quantitatively with heavy metals[16,14]. Since various studies have shown that baseline toxicity often demands chemical activities of 0.01-0.1,[22] whereas chemical activity measures the energy level of an organic molecule relative to its liquid purity of (0–1)[22]. This baseline toxicity test was intended to monitor the build-up of toxicity in pond water for five days. The microalgae *Selenastrum capricornutum* were used as test organisms in this study to. Therefore, This work was designed to monitor the onset of pond water toxicity using *Selanastrum capricornutum.*

MATERIALS AND METHODS

Collection of Pond water samples

Five days of test samples were taken from a fish pond with a diameter of 10 x 8 x 7 m, 500 liters of water, and 200 fingerlings. The sampling began the day the pond's old water was replaced with fresh water, and samples were taken every 24 hours. After collection, the pH of the samples was calculated and they were stored in the refrigerator.

Toxkit

The kit used for the study was AlgalToxkit which was having all of the necessary ingredients, including test organisms, to conduct sensitive and repeatable toxicity experiments. The AlgalToxkit is designed to test the toxicity of fresh and waste water released from both aquatic and terrestrial habitats. Toxkits offer a significant advantage over traditional bioassays in that the test organisms are included in the kits in a "dormant" or "immobilized" state, ready to be activated before the test[17.]

Principle of algaltoxkit F^{tm}

This bioassay kit is based on a 72-hour growth inhibition test using the green microalgae *Selenastrum capricornutum* (formerly *Raphidocelis subcapitata* and *Pseudokrichneriella subcapitata*). After de-immobilization and transfer into an appropriate algal culture media, the green microalgae were immobilized in a specific matrix, where they persist for several months without losing viability and resume normal growth[17].

Experimental design

The water samples were collected from the fish pond and were tested for their toxic effects on the microalgae as outlined below:

Sample 1: collected on day 1
Sample 2: collected on day 2
Sample 3: collected on day 3
Sample 4: collected on day 4
Samples 5 collected on day 5
Control – algal culturing medium for the algal test obtained from the Algaltoxkit; reference sediment. The algal test was performed for 72 hours (with 3 replicates).

Toxicity tests

A battery of Algaltoxkit microalgae - *Selenastrum capricornutum* in speciation of the primary consumer was used for this study.

Testing with microalga

The growth inhibitory effects of pond water on the microalgae *Selenastrum capricornutum* were determined after a 72-hour test using the microalgae *Selenastrum capricornutum*. The tests were carried out in accordance with ISO (International Organization for Standardization) guideline 8692, with minor adjustments. The inhibitory effects of poorly soluble organic and inorganic materials, volatile chemicals, heavy metals, and waste water can be tested using this method as described in ISO 14442 and ISO 5667-16. This was attributable to the preference of cells with a path length of 9 cm over cells with a path length of 10 cm. The Algaltoxkit technology entailed using a spectrophotometer to measure the optical density of algal cells at 670 nm[17].

Preparation of algal culture medium

A 1000 ml volumetric flask was filled with 800 ml deionized water. One of the two Nutrient Stock "A" vials was uncapped, and 10 ml of its content was placed into the flask. The caps of Nutrient Stocks B, C, and D were removed, and 1 mL of each was put into the flask. Deionized water was used to fill the flask to the 1-liter mark. To homogenize the algal culturing media, it was corked and shaken. The solution was allowed to equilibrate in the air overnight. The pH of the solution was adjusted to 8.3 with 1 mol/l sodium hydroxide ISO[17].

De-immobilization of the algae

One of the two tubes containing algal beads had its liquid content drained out, and 5 ml of matrix-dissolving solvent was placed into the tube, sealed, and rapidly shaken. Using a vortex shaker, the shaking was repeated every two minutes for 10 minutes until the algae were completely dissolved. The supernatant was decanted after centrifuging the tube containing the dissolved algal solution for 10 minutes at 3000 rpm. It was replaced with 10 mL of deionized water, capped, and violently shaken to homogeneously re-suspend the algae. After 10th minute centrifugation at 3000 rpm, the supernatant was decanted and the algae were re-suspended in 10 mL algal culturing media[17]

Preparation of concentrated algal inoculum

The algal suspension was poured into a 25 mL flask, which was then filled to the 25 ml mark with an algal culture medium. To homogenize the algal suspension, the flask was corked and shaken. Calibration and Algal Stock cells were labeled on two cuvette cells. The calibration cell was sealed with tape and filled with 2.5 mL algal growing medium. For zero-calibration, the cell was placed into the instrument. The algal suspension was then transferred to the algal stock cell in 2.5 mL increments and tightly taped. To properly disperse the algal suspension, the cell was violently agitated. After that, the Algal Stock was placed in the spectrophotometer, and the optical density (OD 1) was measured after 10 seconds ISO 8692[17].

Preparation of the fish pond water samples

The bottles containing the five-day-old fish pond water samples were shaken, then 10 mL from each bottle was placed into 5 separate centrifuge bottles and centrifuged for 5 minutes. The supernatants were collected and poured into 5 separate flasks labeled C1, C2, C3, C4, and C5, with each flask containing 100 mL of the algal culturing medium. A control sample was made by pouring 110 mL of algal culturing media into a 6th flask. The algal suspension was taken and poured into each of the six flasks, 1 mL at a time. The flasks were corked and thoroughly shaken to equally disperse the algae ISO 8692[17].

Transfer of the algal-sample solutions into the test vials

The test vials were labeled in 3 sets: 3 (a, b, c) for each test sample collected for 5 days: C0, C1, C2, C3, C4, and C5. The control was the 6th set marked - C6. 2.5 mL of the algal-sample solutions were put into the three test vials ISO 8692 after the flasks were opened.

Incubation of the test vials

The cells were taped shut and placed in a holding tray at random. The tape was slightly punctured on one side of the cells to allow for gas exchange in the center. The cells were incubated in a locker with continual sideways lighting of 10000 lux provided by cool white fluorescent lamps at a temperature of 23 ^{0}C according to ISO[17].

Scoring the results

Every 24 hours, the amount of algal growth inhibition was measured in comparison to the control. They were measured as the OD (optical density at 670 nm) of algal suspensions in test cells after exposure to the toxicant for 24 hours, 48 hours, and 72 hours, respectively. After

each measurement, the cells were returned to the holding tray and retained at random (ISO 8692). (2012).

Computation of data

The mean values of the daily – optical density (OD) for the three replicates, the control cells, and the fish pond water (test samples) were calculated[17].

Validating the test result

The International Standard for Organization (ISO Standard 8692 – Section 8) requires that the average growth rate in the control to be at least 1.4 per day, which corresponds to an increase in cell density by a factor - 67 in 72 hours ISO[17].

Statistical Analysis

Data are expressed as mean ± SD and, a test of statistical significance was carried out using a two-way analysis of variance (ANOVA). Mean values with $p < 0.05$ were considered significant.

RESULTS

Table 1 shows that the pH of the pond water samples increased from day to day for 5th days interval following the replacement of the old water with fresh water. The increments in pH levels from day one to 5th day, were significant at $p < 0.05$.

Table 2 shows the extent to which pond water inhibited the growth of microalgae, S. capricornutum. Significant ($p < 0.05$) decreases in OD were observed when the pond water cultures were compared with the control culture.

The initial nominal density = 0.002±0.001; Data were expressed as mean ± SD; Mean values with $p < 0.05$ were considered significant. Day 6: (Control – algal culturing medium for the algal test in the Algaltoxkit).

Table 1. The pH values of the fish pond water samples

Days	Number of tests	pH values
Day 1	3	6.60 ± 0.010
Day 2	3	6.66 ± 0.015
Day 3	3	6.95 ± 0.010
Day 4	3	7.26 ± 0.021
Day 5	3	7.34 ± 0.020

DISCUSSION

Fish consume nitrogen and phosphorus from their food and produce ammonia as a waste product of metabolism[18,19]. The elevation in pH observed from day one to 5th day was most likely due to the release of ammonia into the pond water by fish and the decomposition of organic materials as shown in Table 1. Fish feed and excrement provide nutrients to the fish pond[2]. Absorption and metabolism of nutrients result in the production of metabolic products, which cause pond water to deteriorate and discolor. The oxidation process, as well as the degradation of organic particles by bacteria, definitely contributed to the decline of pond water quality over time[7,21] The explanation for this can be traced back to pond water inhibiting the growth of the microalgae *S. carpricornutum*. Due to their presence in the first level of the trophic chain, algae are sensitive and crucial in elutriate toxicity testing to check the impacts of dangerous compounds in water[7]. They created oxygen, which is required in the marine ecosystem's food chain.

Table 2. Algal growth inhibition results

Day s	N	Mean ±SD of OD at 670 nm after an exposure time 24 hours	48 hours	72 hours
Day 1	3	0.012 ± 0.001[4]	0.011 ± 0.001[4]	0.090 ± 0.001[4]
Day 2	3	0.010 ± 0.001[3]	0.009 ± 0.001[3]	0.009 ± 0.001[3]
Day 3	3	0.007 ± 0.003[2]	0.005 ± 0.001[2]	0.005 ± 0.001[2]
Day 4	3	0.004 ± 0.001[1]	0.004 ± 0.001[1]	0.004 ± 0.001[1]
Day 5	3	0.004 ± 0.001[1]	0.003 ± 0.001[1]	0.003 ± 0.001[1]
Day 6 (Control)	3	0.017 ± 0.001[5]	0.019 ± 0.002[5]	0.013 ± 0.003[5]

Algal growth in the cultures caused significant increases in algal density in both the tests (days 1, 2, 3, 4, and 5) and the control group (day 6) after 24 hours. Table 2 summarizes the outcome. The maximum algal growth was seen on 6th day (control), compared to the original nominal algal density at zero hours. This was expected because it didn't include any toxicants that may have stopped microalgae from growing[10]. The rate of inhibition rose from day 1 to days 2, 3, 4, and 5, according to the measured algal densities. This may be as a result of degradation of organic waste such as feeds, toxicant production such as ammonia, and pond water deterioration over time [9,8].

The accumulation of pollutants may have contributed to the decrease in algal growth in the fish cultures after 48 hours, compared to an increase in algal growth in the control culture after 24 hours. The toxicological effects of pond water on microalgae development. *S. capricornutum* had been severely harmed. Considering that microalgae are a type of algae, because microalgae are photoautotrophs, their viability is dependent on nitrogen and phosphorus availability[19,20]. The pollutants in the test cultures interfered with the algae's normal photosynthetic activity, causing growth suppression. The reductions in the recorded algal densities of the test samples - days (1, 2, 3, 4, and 5) were linked to the growth inhibition of the microalgae, *S. capricornutum*, in the fish pond water cultures, and therefore implicated the pond water in the light of toxicity. At the end of 72 hours, growth inhibition of microalgae was found in both the control and test cultures. In the test cultures, this was due to the accumulation of a toxicant from excretion and decomposition, but in the control cultures, it was due to a shortage of nutrients, gas depletion, insufficient irradiation, and a natural fall in algae growth, among other things. The maximum average microalgae growth in the control was 1.1 percent per day (measured after 24 hours), which fell short of the validity criteria of at least 1.4 percent per day[17]. The inconsistencies and contradictions in this study's elutriate toxicity test could have been caused by minor changes to ISO guideline 8692. Using the results of this investigation, one may estimate the pond's poisonous level and possibly anticipate the fish's perceived risk and survival value.

CONCLUSION

The use of microalgae toxicity as a bioindicator was used to examine the in-situ assessment of pond water. The microalgae growth rate was used as an indicator of pond water toxicity without measuring or defining the nature of the contaminants in the pond. This experiment was beneficial simple, and repeatable. This technique revealed information about how well aquatic life was doing in respect to its surroundings. As a result of this finding, it's reasonable to conclude that the toxicity of pond water was significantly increased on 5th day.

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Conflict of Interest

No conflict of interest was reported as the authors is the sole contributor.

REFERENCES

1. F. A. O. (2014) http://www.fac.org/fi/satist/fisoft/fishplus.asp download, access 12 February, 2022.
2. Trishala KP, Deepak R, & Agrawal YK. (2016) Bioindicators: the natural indicator of environmental pollution, Frontiers in Life Science, 9:2, 110-118, DOI: 10.1080/21553769.2016.1162753.
3. Helfman GS (2007). Fish Conservation: A Guide to Understanding and Restoring Global Aquatic Biodiversity and Fishery Resources. Washington, D. C.: Island Press. p. 11.
4. Chainark S, Boyd CE (2010) Water and sediment quality, phytoplankton communities and channel catfish production in sodium nitrate-treated ponds. *Journal of Applied Aquaculture*, 22: 171-185.
5. Hosmani SP. (2013). Freshwater algae as indicators of water quality. Univers J Environ Res Technol. 3(4):473–482.
6. Ahmed Z, Hisham A, Rahman A. (2011) Eco-monitoring of climate impact on earthen pond water quality in El-Fayoum, Egypt. *International Research Journal of Microbiology*, 2: 442-454.

7. Hari B, Madhusoodana-Kurup B, Varghese JT, Schrama JW, Verdegem MCJ (2006) The Effect of Carbohydrate Addition on Water Quality and the Nitrogen Budget in Extensive Shrimp Culture Systems. *Aquaculture*, 252 (2-4): 248-263.
8. Sipaúba-Tavare LH, Millan RN & Do-Amaral AA. (2013). Influence of management on the water quality and sediment in a Tropical fish farm. *Journal of Water Resource and Protection*, 5: 495-501.
9. Rahman MM, Jo Q, Gong YG, Miller SA & Hossain MY. (2008). A comparative study of common Carp (*Cyprinus Carpio*.) and Halbasu (*Labeocalbasu hamilton)* on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated Rohu (*Labeorohita hamilton*) ponds. *Aquaculture,* 285 (1-4): 78-83.
10. Avnimelech Y (2000) Activated Suspension Ponds: A New Concept in Water Treatment. *Hatchery Magazine*, 1 (2): 24-30.
11. Jain A, Singh BN, Singh SP, Singh HB & Singh S. (2010). Exploring biodiversity as bioindicators for water pollution. National Conference on Biodiversity, Development and Poverty Alleviation; 2010 May 22, Uttar Pradesh. Lucknow (India): Uttar Pradesh State Biodiversity Board.
12. Khatri N, & Tyagi S. (2015). Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. Front Life Sci. 8(1):23–39.
13. Hosmani S (2014). Freshwater plankton ecology: a review. J Res Manage Technol. 3:1–10.
14. Malik DS & Bharti U. (2012) Status of plankton diversity and biological productivity of Sahastradhara stream at Uttarak hand, India. J Appl Natural Sci. 4(1):96–103.
15. Uttah EC, Uttah C, Akpan PA, Ikpeme EM, Ogbeche J, Usip JO. (2008). Bio-survey of plankton as indicators of water quality for recreational activities in Calabar River, Nigeria. J Appl Sci Environ Manage. 12(2):35–42.
16. Aslam M, Verma DK, Dhakerya R, Rais S, Alam M, Ansari FA. (2012) Bioindicator: a comparative study on uptake and accumulation of heavy metals in some plant leaves of M.G. Road, Agra City, India. Res J Environ Earth Sci. 4(12):1060–1070.
17. ISO 8692 (2012). Water Quality – Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae. https://www.iso.org/standard/54150.html. Accessed on June 16, 2017.
18. Boyd CE, Tucker CS (1998) Pond aquaculture water quality management. Kluwer Academic Publishers, Boston. p. 50.
19. Randall DJ & Tsui TKN. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin*, 45: 17-23.
20. Sadiq M, Pakrashi S, Chandrasekaran N, Mukherjee A. (2011). Studies on the toxicity of aluminum oxide (Al_2O_3) nanoparticles to microalgae species: *Scenedesmus* sp. and *Chlorella* sp. *Journal of Nanoparticle Research*, 13 (8): 3287-3299.
21. Blanken W, Postma PR, Winter L, Wijffels RH, Janssen M (2016) Predicting microalgae growth. *Algal Research*, 14: 28-38.
22. Stine NS, James MA, Jon AA, Donald, M, Philipp M. (2018). Linking algal growth inhibition to chemical activity: Excess toxicity below 0.1% of saturation. *Chemosphere*, 208, pp: 880-886. doi.org/10.1016/j.chemosphere.2018.05.168