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Evaluation of Microalgae: Isolation and Characterization and the Physicochemical Properties of the Osun Fish Pond

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Abstract
Africa represents a relatively underexplored region concerning microalgal diversity and biotechnological applications. Therefore, isolation and characterization of algae from this region serve as initial steps towards prospecting high-value products. The present study aimed to isolate and characterize microalgae associated with a fish pond in Osun state, Nigeria. Water samples were aseptically collected from the Osun fish pond and cultured with Beneck's medium supplemented with growth factors using the pour plate technique. The cultures were incubated for 7 to 14 days at room temperature (28±2 ^{0}C) under natural illumination. Pure colonies were obtained using the streak technique. The microalgae were morphologically identified microscopically. To understand the adaptation of microalgae to their environment, physicochemical parameters of the water samples were analyzed using standard methods. Algae identified include *Spirulina* sp., *Microcystis aeruginosa*, *Phacus* sp., *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Pediastrum* sp., *Scenedesmus quadricauda*, *Euglena* sp., *Pinnularia* sp., *Closterium* sp., *Oscillatoria* sp., *Navicula* sp. and *Spirogyra* sp. The presence of *Microcystis aeruginosa*, as well as low dissolved oxygen (3.40 ppm), indicates eutrophication of the pond, which may eventually affect fish yield. In addition, the water quality parameters such as nitrate (0.610 mg/l) and phosphate (40.2 mg/l) are favorable for the growth of microalgae. The microalgae isolated has a long history of biotechnological applications, which means the next phase of prospecting will establish its potential to produce high-value products and possibly remediation of nutrient-rich effluents.

Keywords: *Chlorella vulgaris*, Biotechnology, Phytoplankton Community

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Introduction
A large collection of photosynthetic eukaryotic creatures is referred to as algae. They come in a variety of shapes and sizes. The organisms range from diatoms, which are unicellular, to giant kelps, which are multicellular. They lack the specific features found in terrestrial plants, including leaves, roots and rhizoids. Algae species range from 30 000 to over 1 million, with an estimated 72,500 recognized species globally (Guiry, 2012). These organisms, as well as the high-value products they biosynthesize, are being utilized in a variety of industries. Algae, for example, might be a great source of vitamin and mineral-rich food (Morton and Steve, 2008; Rani *et al.*, 2018), excellent saturated fatty acid compositions for biofuel production (Wijffels *et al.*, 2010), biofertilizers (McHugh and Dennis, 2003), and agar manufacturing (McHugh and Dennis, 2003; Lewis *et al.*, 1988). They produce pigments like

chlorophyll and carotenoids that could be used as chemical dyes and coloring agents, as well as carbon sequestration to delay or stop global climate change (Arad *et al.,* 1998; Paul *et al*., 2020).

Despite the amazing prospects presented by microalgae, little is known about the variety and uses of the strains and species found in most tropical areas of Africa and several other unexplored regions of the world (Chia *et al*., 2013). Exploring and bioprospecting algae from hitherto undiscovered locations may hold the key to unlocking algal potential for wider industrial deployment than is currently possible. Furthermore, high temperatures define locations in Africa such as Nigeria, implying that species flourishing in this part of the world are preadapted to high temperatures (Aquila *et al*., 2009). Because of this adaptation, they offer good replacements and additions to current collections of algae cultured for high-value products for human consumption.

One of the critical processes in obtaining well-adapted species is the choice of isolation sites and species. At present, it is important to screen the indigenous lipid producing microalgae for biofuel (the microalgal biofuels are biodegradable, renewable, non-toxic fuel and do not compete with food crops) application and cultivate native microalgal strains that adapt to their local environmental conditions.

Therefore, the present study aimed to describe and isolate microalgae species from the Osun fish pond and assess the bioprospecting and bioapplication of the microalgae. To understand the ecology of isolated species, the physicochemical parameters of the water sample were assessed.

Materials and Methods

Site of Collection and Experimentation

The study samples were taken from a fish pond in Osogbo, Osun State, which is located in the southwestern region of Nigeria. Osogbo is about 88 kilometres by road, northeast of Ibadan. Osogbo is located at latitude 7º46ºN and longitude 4º34ºE. The experiment was conducted in the Biotechnology Laboratory, Department of Biotechnology, School of Science, Federal University of Technology, Akure, Nigeria. Sampling was done at monthly intervals from February 2021 to July 2021. Water samples were collected from the pond using two different sample bottles; one for the analysis of physicochemical parameters and another for qualitative and quantitative analysis of phytoplankton. The sample bottles were labeled with date. 500ml white-colored sterilized bottles were used to collect water sample containing algal species from Osun pond. The algal samples were taken in the morning and immediately preserved in opaque sample bottles containing 4% formalin solution.

Microalgal Analysis and Identification Preparation of Culture Media: Beneck's Media

Algal isolation was done using solidified and liquid Beneck's medium. Before the use of the medium, it was autoclaved at 121^0C for 15 minutes and allowed to cool (Aneja, 2007). One (1) ml of pond water was introduced into clean sterilized glass bottles containing Beneck's Medium. After that, they were covered with sterile cotton wool to facilitate gas exchange while preventing contamination. For two weeks, they were left near the window to have access to light energy (Aneja, 2007).

The streak plate technique was used to isolate pure algal strains from cultured plates. Individual colonies were chosen from mixed populations and subcultured until a pure isolate was obtained. To produce streaks of colonies on the growth medium, a sterile wire loop was utilized. The plate was thoroughly covered and sealed with paraffin, inverted, and incubated for 7–14 days at 25°C–30°C.

Sample Analysis

Microalgal composition of field and laboratory samples was identified and quantified microscopically using a binocular light microscope. Identification of microalgae was done with the use of standard taxonomic keys (Desikachary, 1959).

Temperature was estimated in-situ using a mercury-in-glass thermometer. Also, turbidity and water transparency were measured in situ with a Secchi disc. Other physicochemical parameters were analyzed in the laboratory. The pH and electrical conductivity were determined using a pH meter (Model H196107) and a Hanna conductivity meter, respectively. Dissolved oxygen, Biochemical Oxygen Demand, Chemical Oxygen Demand, Chloride Nitrate, Phosphate, Magnesium hardness, and Calcium hardness, were analyzed using standard methods for the examination of water and wastewater (APHA, 1998).

Results

Isolation of Microalgae Composition

Forty-one (41) taxa classified into 4 divisions namely Chlorophyta, Cyanophyta, Bacillariophyta, and Euglenophyta were observed in the pond. The Chlorophytes (222 organisms, 69.6%) dominated the algal flora with *Scenedesmus quadricauda* (38 organisms, 11.9%), *S. dimorphus* (34 organisms, 10.7%), and *Chlorella vulgaris* (38 organisms, 11.9%) forming the prominent organisms (Fig. 1). The Cyanophytes (48 organisms, 15.1%) were represented by *Microcystis aeruginosa* (38 organisms, 11.9%) and *Oscillatoria formosa* (4 organisms, 1.3%) Bacillariophytes (32 organisms, 10.03 %) were represented by genera such as *Navicula* sp. (8 organisms, 2.5%) and *Cymbella* sp. (6 organisms, 1.9 %). The euglenoids (17 organisms, 5.3%) were the least and represented by *Euglena viridis* (8 organisms, 2.5 %) and *Phacus acuminatus* (2 organisms, 0.6 %). Algae identified in the pond include *Spirulina* sp., *Microcystis aeruginosa*, *Phacus* sp., *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Pediastrum* sp., *Scenedesmus quadricauda*, *Euglena* sp., *Pinnularia* sp., *Closterium* sp., *Oscillatoria* sp., *Navicula* sp. and *Spirogyra* sp. (Plate 1). *Chlorella vulgaris* belonging to the division Chlorophyta was the only species successfully isolated from the pond (Plate 2). *Chlorella vulgaris* is a spherical unicellular organism with a thick cell wall (100-200 nm).

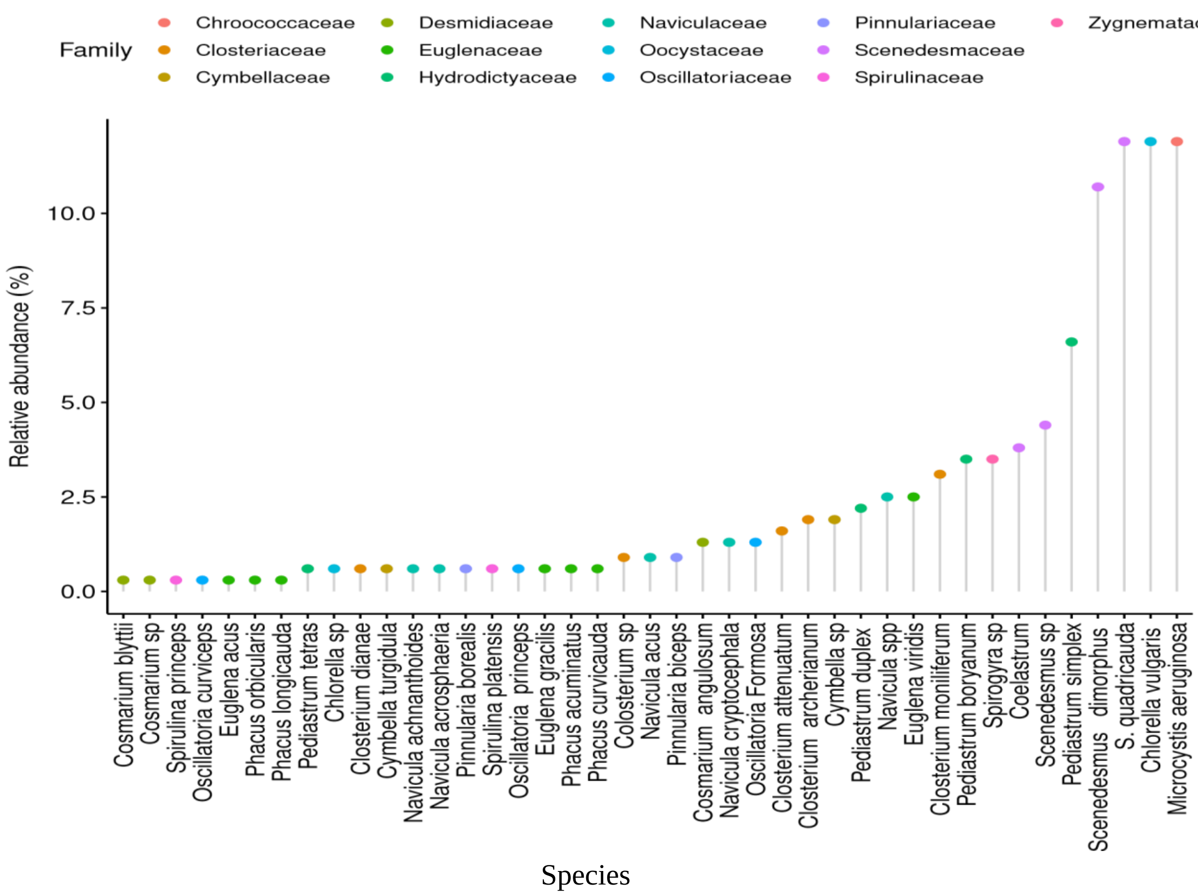

Figure 1 Phytoplankton composition of Osun Fish pond.
Values represent the relative abundance of algae and cyanobacteria

Plate 1: Microalgal species isolated from Osun fish pond. A. *Spirulina platensis* B. *Microcystis aeruginosa* C. *Phacus curvicauda* D. *Scenedesmus dimorphus* E. *Scenedesmus dimorphus* F. *Pediastrum simplex* G. *Scenedesmus quadricauda* H. *Phacus longicauda* I. *Chlorella vulgaris* J. *Microcystis* sp. K. *Pinnularia* sp. L. *Closterium* sp. M. *Oscillatoria limosa* N. *Navicula* sp. and O. *Spirogyra* sp.

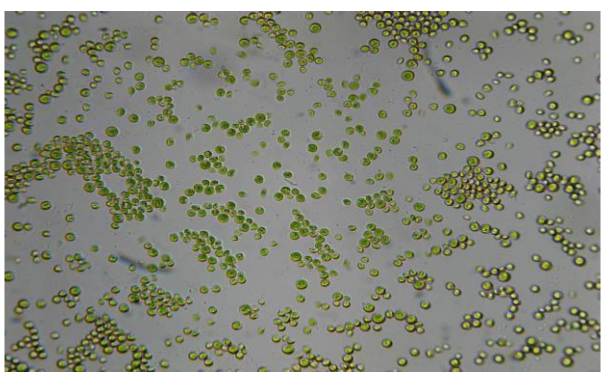

Plate 2: *Chlorella vulgaris* isolated from Osun fish pond in July 2021

Physicochemical Parameters of Osun Fish Pond

Table 1 shows the physical and chemical characteristics of the pond at the time of sample collection. The values of most physical and chemical parameters fall within levels reported of a typical eutrophied aquatic ecosystem in Nigeria. There was low nitrogen to phosphorus proportion, which was confirmed by the several folds higher phosphate concentrations compared to nitrate nitrogen levels. Specifically, phosphate phosphorus concentration was 40.2 mg/L, while nitrate nitrogen was 0.61 mg/L. Macronutrient and heavy metal levels were within acceptable levels for aquatic ecosystems

. **Table 1. Physicochemical characteristics of Osun Fish pond at the time of sample collection**

Physicochemical Parameters	Osun Fish Pond
pH	6.20
Turbidity (FTU)	263.00
Dissolved oxygen (ppm)	3.40
Temperature (°C)	28.20
Total Alkalinity (mg/l)	176.00
Total Hardness (mg/l)	288.00
Calcium Hardness (mg/l)	204.00
Magnesium Hardness (mg/l)	84.00
Chloride (mg/l)	15.50
Iron (mg/l)	0.62
Silica (mg/l)	1.41
Nitrate Nitrogen (mg/l)	0.61
Copper (mg/l)	3.50
Manganese (mg/l)	0.023
Aluminum (mg/l)	0.05
Chromium (mg/l)	0.54
Conductivity (µs/cm)	571.2
Potassium (mg/l)	4.80
Phosphate (mg/l)	40.20
Zinc (mg/l)	1.83
Carbonate (mg/l)	176.00
Bicarbonate (mg/l)	109.80
COD (mg/l)	634.00
BOD (mg/l)	1.30

Discussion

The only algae successfully isolated from the pond sample was *Chlorella vulgaris*. Asma *et al*., (2011) investigated the algal species makeup of different ponds in Malaysia and concluded that the microalga constitutes a major part of the phytoplankton community of ponds. *C. vulgaris* is a microalga with a size range of 2 to 10 mm and morphologically distinct from that of larger plants. The microalgae have a wide range of uses. It is a single-cell factory for proteins, lipids, carbohydrates, vitamins, minerals and colors that is powered by sunlight. It has garnered interest as a healthy food additive for human consumption (Lin, 2005; Li *et al*., 2002, Liang *et al*., 2004), as aquaculture animal feed (Fernandes *et al*., 2012), and as an environmental cleanup agent (Fulke *et al.*, 2010). It has attracted unprecedented interest as a feedstock for biofuels (Christi, 2008). Chlorella can also be used to control aquarium odor as well as improve the quality of the pond waste (Onianwah and Stanley, 2018).

Although *C. vulgaris* was the only isolated microalgae from Osun Fish pond, the aquatic ecosystem presents a rich diversity of phytoplankton and this conforms with the trend in tropical water bodies (Kadiri and Omozusi, 2002). The presence of organic pollution indicator species such as *Microcystis* aeruginosa, *Navicula* spp., *Oscillatoria* spp., Euglena *spp.* and *Phacus* spp. suggested that the pond was polluted. Physico-chemical characteristics of water regulate the biotic connection of organisms in water bodies, including their ability to endure pollution loads and offer nutritional balance. The pond's deteriorating state was reflected in the water analysis (oxygen, phosphate, and others). Only a few parameters (temperature, conductivity, total alkalinity, total hardness, and ions) fell within the optimum recommended range for survival (Tarique and Montahana, 2014).

The pond had relatively low levels of dissolved oxygen, chlorine, and BOD. However, the levels of phosphate, conductivity, alkalinity, and COD were relatively high compared to the recommendations by WHO (2011) and FEPA (1991). The fish pond has physicochemical properties such as nitrates and phosphates which positively support some species of microalgae but limit others because of the low nitrogen to phosphorus ratio. Certain physicochemical characteristics such as high electrical conductivity (571.2ųs/cm) and low dissolved Oxygen (3.40 mg/L) could adversely affect aquatic life.

Conclusion

It may be inferred that the Osun fish pond has a variety of microalgae species. However, further research studies would be carried out especially on techniques to isolate and cultivate microalgae successfully for use in biotechnology. The final isolate, *Chlorella* sp., has proven to be particularly useful in biofuel production and other high-value products.

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