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Study of Algal Species Isolated From River Ginzo in Katsina State, as a Potential Source for Biodiesel Production

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ABSTRACT: An ecological study of algae at River Ginzo in Katsina town was carried out for six weeks at three different sites. In this study, various strains of native microalgae were identified and isolated. The water's physicochemical properties were analyzed at Kofar Marusa, Kofar Sauri, and Kofar Durbi of River Ginzo in Katsina State. The physicochemical parameters studied showed temperature and pH range of 28^{O}C to 31^{O}C and 6.42 to 7.36. A total of eighteen **(18)** algal species were identified, out of which Ten (10) species belong to the Class *Chlorophyceae* with *Spirogyra* species having the highest cell counts, five (5) species belong to the Class *Cyanophyceae* with *Oscillatoria* species having the highest cell count and three (3) species belong to the Class *Bacillariophyceae* with *Nitzchia* spp having the highest cell counts. Among the isolates, *Chlorella* species showed an increased growth rate with higher biomass productivity of (88.67±2.57) X 10^{4}(cell/ml) after six days of incubation. The results showed that *Chlorella, Spirogyra, and Oocystis* species could be a possible candidate species for producing oils for sustainable biodiesel production, based on their high growth rate and presence in all the locations.

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The global energy demand is mostly fulfilled by formal energy resources, such as petroleum, coal, and natural gas. However, the negative impacts of these energy sources on the environment and human health have given way for research that is mostly geared towards the utilization of renewable energy resources (Elegbede *et al*., 2017). The search for non-edible feedstock in biodiesel production is necessary to provide environmentally sustainable and economically viable alternative green transport fuels. Algal derived biofuels are global emerging energy sources that can provide alternative fuel. However, globally, the most common way of producing energy worldwide is by utilizing fossil fuels, a finite resource that is diminishing rapidly. Biodiesel derived from microalgae is a potentially renewable and carbon-neutral alternative to petroleum fuels. One of the most critical decisions in obtaining oil from microalgae is the choice of algal species to use, based on their morphology and ease of cultivation. Microalgae are oxygen-evolving photosynthetic autotrophs commonly found growing in different aquatic environments, such as freshwater, marine water, and wastewater. A microalga converts carbon dioxide into biomass typically found in freshwater, and marine systems reproduce primarily by vegetative or asexual cell division. Biomass obtained from microalgae is considered to be a good source for the production of fuels when compared to other sources of renewable biomass; the microalgae oil yield is 15-300 times more than different traditional crops base on the area as well as the contents of the oil which ranges from 20-50% to other competitors. Furthermore, many microalgae species can double their biomass in a few hours and exhibit two doublings per day (Hannon *et al*., 2010). This decade has witnessed growth in microalgae use to produce third-generation biofuels, biogas, and bioethanol. The presence of high oil content of some microalgal strains makes them suitable for biodiesel production. For example, microalgae belonging to *Nannochloropsis, Neochloris Chlorella, Dunaliella, Nannochloris, Scenedesmus,* and Porphyridium, contains 20–50% of lipids by weight, making them desirable for biodiesel production (Mata *et al.*, 2010). Microalgae cultivation can be done in controlled closed-culture systems called photobioreactors (PBRs). A bioreactor means a system in which a biological conversion is achieved. Thus, a photobioreactor is a reactor in which phototrophs (microbial and algal) are grown or used to carry out photobiological reactions. PBRs are systems flexible to be optimized according to the algal species'

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biological and physiological characteristics, allowing one to cultivate algal species that cannot be grown in open ponds. The most popular photobioreactor configurations are tubular, vertical, column, flat plate, and annular reactors (Brennan and Owende, 2010). Depending on the type and design, PBRs are considered to have several advantages over open ponds. Different studies Arrendondo-Figueroa *et al*., (1998); Borowitzka, (1999); Chaichalerm *et al*., (2012) have shown environmental conditions, such as spectral characteristics, nutrient availability, and varying light intensities, and temperature have distinct impacts on community structure and microalgal cellular composition. Therefore, trying to identify the algal species that grow best under local environmental conditions and then using those species to assemble a microalgal community suitable for different growth environments is a good strategy. Nigeria (Katsina state) is located in the tropical zone and has abundant sunlight, land, and water. The unique geographic location of Katsina makes year-round high-yield algal cultivation possible. Katsina also has diverse microalgae species, implying that some of the native species could be excellent candidates for biofuel production. This research work aimed to isolate, identify, and study the influence of enriched culture media on biomass productivity of selected microalgae strains found in the natural habits of Katsina.

MATERIAL AND METHOD

Sampling: Water samples for microalgae isolation were collected aseptically from three (3) sampling sites that appeared to contain algal growth in River ginzo (R.G.). River ginzo is a river in Katsina State (Figure 1) located in Nigeria. It is situated on latitude 12", 60' and 120-75'N of the equator and between longitude 7^0-35' and 8^0-10'E of the Greenwich in Katsina metropolis. The samples were collected from R.G. along Kofar Sauri (K.S.), Kofar Durbi (K.D.), and Kofar Marusa (K.M.) Katsina metropolis. Six (6) different Physico-chemical parameters were determined from the River. The temperature was determined in situ using a thermometer as described by APHA (1992), pH was determined using dip-in mobile battery-operated, pH meter, and dissolved oxygen was determined using modified Wrinkler's titration method, biochemical oxygen demand was determined using five days incubation method, Electrical Conductivity was determined using E.C. meter and turbidity using turbidity meter.

Isolation and identification of microalgae: To identify the microalgae, samples were subjected to microscopic identification using a compound digital microscope (SWIFT M10). Bellinger and Sigee (2010) described a standard physiological key used for the determination and identification of species. A micropipette was used to pick up individual cells through repeated trial and error method. Ten drops of sterile media were placed in the groove of a glass slide. Then a reduction of the microalgal sample was added and observed under the microscope. Having confirmed the target organism, then it transferred into new conical flasks containing media.

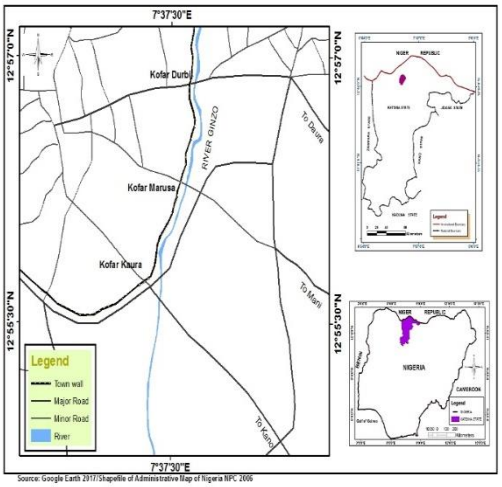

Fig 1: Map of Katsina metropolis showing the sampling areas (Source: Cartographic Unit, UMYU).

Cultivation and microalgal growth analyses: For the maintenance of algal cultured, commercially available fertilizer was used N:P:K 20:10:10+TE (provided from Zenith Energy enzymes® Inc.). The microalgae with the highest growth rate were inoculated into the media. These were kept for incubation at 25°C provided with 12:12 light/dark photoperiods. The culture was maintained for a further six days, and the cells were estimated.

Cell Count: Microalgae concentration was determined by direct counting of cells using a haemacytometer. A coverslip was used to cover the haemacytometer grids, and a pipette was used to fill its chamber. The pipette was placed at the haemacytometer's tip, and the sample flows into the room by capillary action. Cells were allowed to settle and checked under the microscope for the fair distribution of cells. The grid is divided into nine large squares, each large square is divided into 25 medium squares and each medium square is further divided into 16 small squares. For essential measurement, the large center square's average number of cells was counted; the procedure was repeated twice. The cell density was obtained by multiplying the average cell count for each species by Neubauer's conversion factor ($X10^4$). The X40 objective lens was used to count the cells, according to Schoen (1988).

Statistical Analysis: Raw data were subjected to ANOVA using General Linear Model (GLM) in SAS VERSION (9.3) means were separated using SNK (Student-Newman-Keuls Test) at 5%. The data was also subjected to Pearson's correlation coefficient using SAS.

RESULTS AND DISCUSSIONS

Sampling, Isolation, and Morphological Identification of Microalgae: The P.H. of the surface water for the different sampling sites was neutral to slightly basic, with the K.M. station having the highest pH at 7.36.

Table 1: Physicochemical properties of River ginzo at various locations.

	Kofar Marusa Stations			Kofar Durbi Stations			Kofar Sauri Stations		
Parameter	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Temperature	30	31	28	30	31	31	30	28	28
Ph	7.36	7.09	7.35	6.62	6.81	6.55	6.42	7.04	6.57
BOD (mg/L)	2.44	2.2	2.9	2.1	2.95	2	2	2.7	2.2
DO	4.31	4.91	5.1	4.5	4.89	3.9	3.65	4.72	4.17
Turbidity (mg/L)	30	40	31	50	30	34	35	34	50
EC (µs/cm)	50	43	34	115	124	145	150	135	115

The results in conformity with the study of Abba *et al*. (2017) they found that the mean P.H. of (7.90) was observed in the study areas, these result falls within the acceptable limit of 6.5-8.5 as recommended for drinking and inland water quality (WHO, 1996), except site KS with 6.42 reading. The low pH value in Site G (K.S.) might be attributed to irrigation activity concreted in the area. Since sampling was performed in the dry season, the K.M. site had the lowest temperature (28 °C), while the others had water temperatures between 28 to 32 °C, as listed in (Table 1). The result is related to the one reported by Abba *et al*. (2017). The values for dissolved oxygen and biochemical oxygen demand are shown in (Table 1). The D.O. values range between 3.65 to 5.1mg/l, with the lowest and highest values recorded at K.S. The Site and K.M., respectively. The recommended values for Dissolved Oxygen concentrations given by USEPA (1970) is (40-60mg/l), which is far below from the observed in the study; hence; this shows the high level of anthropogenic activities within the area, and also during the dry season, rivers witness slow or no movement these leads to low or non-uniform distribution of available dissolved oxygen (Deeker *et al*., 2010). The BOD range was 2.0 to 2.9mg/l; the lowest value was recorded at K.S., and the highest at K.M. Water turbidity and conductivity values varied considerably between the different sites studied (Table 1). These results fall within the FEPA (1991) regulated limit of <4mg/l. The available BOD can measure the pollutant in a river; the higher the values depict more pollutants in the River, from our result, the highest was obtain at K.M., this agrees with the findings of Abba *et al.* (2017). The high level of BOD observed in Site K.M. might be due to anthropogenic activity routinely taking place throughout the season. The turbidity value ranges from 30 to 50FAU, with the lowest value recorded at K.D. and the highest at K.S. The range of conductivity value was 34.00 to 150.00µs/cm, with the lowest value recorded at K.M. and the highest at K.S., respectively.

Table 2: Algal Species identified in the Samples of Katsina River (Six weeks) for sample A (KofarSauri Location

S/N	Species	Estimated (cells/ml)
Chlorophycaea		
1	*Email*	16 X 10^4
2	*Chlorella*	61 X 10^4
3	*Aphanochaete*	1 X 10^4
4	*Characium*	5 X 10^4
5	*Chaetophora*	1 X 10^4
6	*Enteromorpha*	1 X 10^4
7	*Trentepohlia*	6 X 10^4
8	*Spyrogyra*	14.8 X 10^5
9	*Stephanosphaera*	3 X 10^4
Cyanophyceae		
1	*Anabaena*	5.0 x 10^5
2	*Oscillatoria*	1 X 10^4
Xanthophyceae		
	Botrydium	5.0 x 10^5

Sample B (KofarDurbi Location)

S/N	Species	Estimated (cells/ml)
Chlorophyceae		
1	*Actinastrum*	7 X 10^4
2	*Chlorella*	73 X 10^4
3	*Enteromorpha*	1 X 10^4
4	*Characium*	4 X 10^4
5	*Chaetophora*	1 X 10^4
6	*Spyrogyra*	10.1 X 10^5
Cyanophyceae		
1	*Ochromonas*	4 X 10^4
2	*Nostoc*	1 X 10^4
Bacillariophyceae		
1	*Uroglena*	1 X 10^4
2	*Gyrosigma*	1 X 10^4

Sample C (KofarMarusa Location)

S/N	Species	Estimated (cells/ml)
Chlorophyceae		
1	*Oocysts*	21 X 10^4
2	*Chlorella*	92 X 10^4
3	*Prasiola*	15 X 10^4
4	*Chaetophora*	15 X 10^4
5	*Spyrogyra*	13.9 X 10^5
Cyanophyceae		
1	*Tolypothrix*	15 X 10^4
Bacillariophyceae		
1	*Nitzschia*	15 X 10^4

A significant variation (P<0.05) in the values among the sites. A previous study conducted by Abba *et al*. (2017) on physicochemical parameters from different areas agrees with our findings. Microalgae are virtually found in all types of earth ecosystems, not only restricted to aquatic but also found in terrestrial habitats. They are represented by various species present in a wide range of environmental conditions, including brackish, marine, freshwater, lacustrine, and hyper-saline sites (Abou-Shanab *et al*., 2011). The Algal species were identified from the River, and the total cell counts are presented (Table 2). Their classification into different classes is based on morphological characters. Furthermore, the name and type of each strain are summarized in (Table 3). A total of eighteen **(18)** algal species were identified, out of which Ten **(10)** species belong to the Class *Chlorophyceae* with Spirogyra species having the highest cell counts, five **(5)** species belong to the Class *Cyanophyceae* with *Oscillatoria* species having the highest cell count and three **(3)** species belong to the Class *Bacillariophyceae* with *Nitzchia* spp having the highest cell counts. The growth and biochemical composition of microalgae are affected by the environment's condition in which they are found. Temperature, light, and P.H. are considered primary environmental conditions affecting the microalgae's growth and biomass yield (Juneja *et al*., 2013).

Table 3: Summary of algal species identified at River ginzo at various locations

S/No.	Class	Species identified
1	Chlorophyceae	*Spyrogyra, Chlorella, Oocystis, Characium, Chaetophora, Enteromorpha, Trentepohlia, Aphanochaete,Actinastrum, and Prasiola.*
2	Cyanophyceae	*Oscillatoria, Anabaena, Ochromonas, Nostoc, and Tolypothrix*
3	Bacillariophyceae	*Nitzchia, Gyrosigma and Uroglena*

These ecological conditions favor the development of microalgae in the study area, and these corroborate our results in which different types of microalgae were isolated. The dominant class of microalgae isolated is *Chlorophyceae*; it is a diverse class of microalgae species that has been reported to have a high level of neutral lipids (Idenyi *et al*., 2021). The result of this study varies with some research in Nigeria. For example, the study of Ahmad and Indabawa (2015) found a total of fifty-six (56) algal species, out of which thirty-seven (37) species belong to the Class *Bacillariophyceae*, Seventeen (17) in *Chlorophyceae* were observed in Kano River in Tamburawa village.

The growth rate of microalgal strains: Under optimum conditions and sufficient nutrients, microalgae can grow profusely. Usually, they double their biomass within 3.5 h or 24 h during the exponential growth phase. The net growth rate differed among the examined microalgal species. The growth of algae is directly affected by the availability of light, temperature, nutrients, light, temperature, initial inoculum's density, and the stability of pH (Wang *et al*., 2010). The first and critical step in developing a commercial and viable algal plant (open pond) is a tremendous algal strain, most importantly a local strain adapted to the local climatic conditions area. Microalgal species (*Chlorella, Spirogyra* and *Oocystis*) that showed higher biomass yield were selected further to investigate their growth rates in a nutrient medium. Figure 2 shows a photo of representative isolated *microalgae* spp.

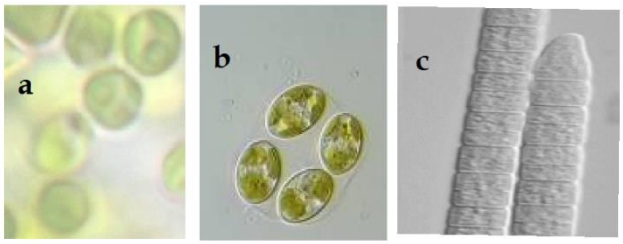

Fig 2: Photo of representative isolated microalgae spp; Chlorella (a), Oocystis (b), and Spyrogyra (c).

The growth cycles of the different strains in the prepared media are shown in Table 4. Cells in the media had approximately one day lag period and reached the exponential phase within 2–4 days. By about day 5 or 6, cells reached the stationary phase. The growth patterns of the three strains in the culture media were similar.

Table 4: Biomass composition of screened microalgal strains

ESTIMATED (CELLS/ML) S.D.

Days/Algal	*Chlorella*	*Spyrogyra*	*Oocysts*
Day 1	(38.33±2.58) x10^4	(2.00±0.23) x10^4	(2.00±0.00) x10^4
Day 2	(43.66±3.08) x10^4	(3.45±1.58) x10^4	(5.00±1.50) x10^4
Day 3	(55.44±1.57) x10^4	(11.34±2.16) x10^4	(10.33±1.18) x10^4
Day 4	(68.37±1.23) x10^4	(28.10±1.10) x10^4	(12.66±1.52) x10^4
Day 5	(73.43±2.16) x10^4	(38.33±3.45) x10^4	(19.00±0.20) x10^4
Day 6	(88.67±2.57) x10^4	(49.33±5.58) x10^4	(31.66±1.57) x10^4

Three (3) species identified and isolated for oil production were; *Chlorella, Spirogyra,* and *Oocystis*. *Chlorella* shows the highest growth and development with (38.33±2.58) x10^4at day one (1) and (88.67±2.57) x10^4 at day six (6), followed by *Spyrogyra* with (2.00±0.23) x10^4at day one (1) and (49.33±5.58) x10^4 at day six (6) and lastly *Oocystis* with cells density of (2.00±0.00) x10^4 at day one (1) and (31.66±1.57) x10^4 at day six (6). Microalgae usage in biofuel production, especially those with high-density lipid contents (*Chlorella, Nannochloropsis, Chlamydomonas*, and *Nitzschia*), are receiving much attention; in particular, the division chlorophyte has exhibited growth rate and ease of cultivation (Pereira *et al*., 2013). Different studies have reported the growth rate of *Chlorella* strains. It grows exponentially under optimum conditions; algal cells continued to rise from the first day to up to sixteen days (Ammar, 2016; Chia *et al*., 2017; Idenyi *et al*., 2021).

Conclusion: The study showed differences in physicochemical parameters and distribution of the species. The River Ginzo showed the highest levels of temperature, pH, and BoD measured at Kofar Marusa. A stable growth was observed using commercial N:P:K 20:10:10+TE as growth media, these conditions would thus offer the maximum productivity for an algal biomass. Furthermore, *Chlorella, Spyrogyra,* and *Oocystis* could be considered for oil production due to their abundance and high growth rate in all the locations.

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