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ORIGINAL RESEARCH ARTICLE

Distribution and Bioresource Potential of Duckweed (*Lemna minor* L.) in Maiduguri, Nigeria.

Ibrahim Musa Adamu[1]*, Ibrahim Musa[1], Abbator Ahmed[1], Auwal Sulaiman[1] and Saba Abdulwakil Olawale[2].

[1]Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria.
[2]School of Agriculture, Lagos State University, Epe Campus, Lagos, Nigeria.

ABSTRACT

Lemna minor L. (Arales: Lemnaceae) is a small, delicate, free-floating aquatic plant that grows mostly in a nutrient-enriched environment. It has wide applications in agriculture and biosciences. This study evaluated the physicochemical factors affecting the distribution, biomass, and proximate composition of *L. minor* in waterways within Maiduguri metropolis, northeastern Nigeria. Duckweeds were sampled with a quadrat (25 × 25 cm) along imaginary transect lines from different areas of the metropolis between the early hours of 9:00 and11:00 a.m. in December 2021. Some physicochemical parameters of the sites were measured. Proximate composition and biomass were determined. Principal component analysis (PCA) indicated that PC1, PC2, and PC3 explained 79.2% of the variation. The strongest positive correlations were between Mg and alkalinity, while K, Mg, alkalinity and TSS were negatively correlated. Proximate composition indicated 11.4-18.21% crude protein and 45.92-48.47% carbohydrate in the samples. Bray-Curti's index indicated that duckweeds in the Custom area and Bolori were the most similar (0.99). Samples collected from the Unimaid area recorded the highest wet biomass (5.0 ± 0.2 g/m^2), while a sample from Umarari was the lowest (2.24 ± 0.1 g/m^2). The physicochemical parameters of the site provided suitable factors for laboratory culture and large-scale production. The findings provide baseline data on the ecology and potential of duckweed as a cost-effective source of crude protein (CP) and other essential nutrients for aquaculture and animal feed, as well as a model plant for aquatic ecology, the environment and biotechnological research. Further studies to explore the applications of *L. minor* in different fields were recommended.

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Duckweed, Distribution, Bioresource, Model plant.

INTRODUCTION

Lemnaceae, which consists of 36 species classified into five genera: *Landoltia, Lemna, Spirodela, Wolffia, and Wolffiella*, and divided into the subfamilies Lemnoideae and Wolffioideae, which include the species of *Landoltia*, *Lemna*, and *Spirodela* and the rootless species of *Wolffia* and *Wolffiella*, respectively (Pagliuso *et al.,* 2020). All members of the Lemnaceae family are tiny, free-floating freshwater plants with global distributions; they are the most morphologically reduced higher plant species (Cheng and Stormp, 2009). Duckweed has lately attracted the attention of scientists and governments as a new feedstock with a high biomass production rate for bioenergy production (Cui and Cheng, 2015).

Duckweed (*Lemna minor*) has long been used to remediate municipal wastewater by recovering harmful nutrients through its growth, the nutrients needed to generate vast quantities of duckweed might be found in wastewater from livestock agriculture and towns (Cheng *et al.*, 2009). Duckweed can reclaim contaminated streams and transform wastewater into high-quality animal feed, given its efficient nutrient uptake and inclination to accumulate high protein levels (Demmig-Adams *et al.,* 2022). Particularly for the treatment of industrial and agricultural effluents, duckweed was incredibly successful at removing organic pollutants from aquatic ecosystems (Justin *et al.,* 2022). Humans can use duckweed as safe alternatives to conventional food and energy sources because of their distinctive physiological adaptations to their growth environments (Appenroth *et al.,* 2015).

In various nations of Southeast Asia, such as Laos, Thailand, and Myanmar, duckweed is frequently consumed by people as a vegetable known as "Khai-Nam" (De Beukelaar *et al.,* 2019). Duckweed is one of the major freshwater macrophyte models used in toxicology

Correspondence: Ibrahim Musa Adamu. Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria. ✉ moosaad8@gmail.com. Phone Number: +234 803 704 1623.

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(Appenroth *et al.,* 2015; Sońta *et al.,* 2019), ecology and environment (Cui and Cheng, 2015; Demmig-Adams *et al.,* 2022), agriculture (Cheng *et al.*, 2009; Sońta *et al.,* 2019; Demmig-Adams *et al.,* 2022), biotechnology & molecular biology (Khairina *et al.,* 2021), microbiology research. The effectiveness of the common duckweed plant in the remediation of organic pollutants, heavy metals, agrochemicals, and pharmaceuticals has been demonstrated through research, whereas its performance with radioactive waste, nanomaterials, petrochemicals, and other categories of pollutants is only moderate (Appenroth *et al.,* 2015). The majority of agrochemicals are tolerable by duckweed at low doses but harmful at larger concentrations (Zhou *et al.,* 2023). Heavy metals from aquatic environment contaminated by crude oil were only slightly remediated by duckweed (Ekperusi *et al.,* 2019). In wetlands contaminated with crude oil, *Lemna paucicostata* significantly biodegraded 97.74% of the petroleum hydrocarbons (Ekperusi *et al.,* 2020).

It is a good feedstock for the production of starch and, subsequently, biofuel (Cui and Cheng, 2015; Cheng *et al.,* 2009). It has been demonstrated that duckweed has high starch (maximum ~50%) (Khairina *et al.,* 2021), and low lignin (ca. 5%) (Pagliuso *et al.,* 2021), making it a beneficial fermentation substrate for the synthesis of both bioethanol and methane (Khairina *et al.,* 2021). Duckweed has a protein content of 15-45%, making it ideal for livestock, poultry, and fish feed (Cheng *et al.*, 2009; Aghoghovwia, 2018). The protein, ash matter, crude fiber, and ether extract contents in samples of blanched-sun-dried duckweed collected from Oghara, Delta State, Nigeria were 29.96%, 14.63%, 27.10%, and 2.83%, respectively. Ifie *et al.,* 2021).

Due to the large levels of vegetative storage protein that duckweeds accumulate in their fronds; the amount of edible protein produced by duckweed per hectare substantially exceeds that of soybean (Demmig-Adams *et al.,* 2022). The Kainji Lake region can produce 129 kg/ha of dry duckweed daily with an average standing crop of 309 kg dry mass (DM)/ha and a doubling period of 1.2 days, which may provide a daily supply of 59 kg of high-quality protein for poultry and fish feed formulation (Mbagwu and Adeniji, 1988). Incorporating 30% duckweed into practical meals in place of fishmeal enhanced fish growth and was economical. Fasakin *et al.,* 1999).

Members of the lemnaceae interact strongly with microbes, and duckweed's growth and photosynthesis rates can be influenced by the microbiome of plant (Demmig-Adams *et al.,* 2022). One useful technique is the inoculation of plant growth-promoting bacteria (PGPB) to speed up the growth of duckweed (Khairina *et al.,* 2021). The aim of this study therefore, was to evaluate the physicochemical factor that determines the distribution and abundance of duckweeds and explore its potential in diverse biosciences research as well as a model aquatic plant in the study area and beyond. The research questions are whether duckweeds in Maiduguri have better potential as bioresource, and as a model aquatic plant, this is primarily targeted to determine suitable conditions for laboratory or home (backyard) culture of duckweed that will enable its mass production, and biomass valorisation for research in the areas of ecology and environment, agriculture, and renewable energy in Maiduguri, Nigeria and beyond.

MATERIALS AND METHODS

Study area

Maiduguri (Long. 11° 49' 51.952" N and Lat. 13° 9' 3.4812" E, elevation; 324.735m) is one of the major metropolitan cities in Nigeria. The city is located along the seasonal River Ngadda, which flows into the *Firki* swamps in the Lake Chad region. Agriculture and commerce are the major activities of the populace.

Physicochemical parameters of the water

The physicochemical variables (temperature and pH) of the water samples were determined *in situ* and a glass bottle (1000 mL) was used to collect samples for *ex-situ* analyses of alkalinity, salinity, nitrogen (N), magnesium (Mg), dissolved oxygen (DO), potassium (K), phosphorous (P), calcium (Ca), total suspended solids (TSS) and total dissolved solids (TDS) along transect lines from different areas in the early hours (9:00 am – 11:00 a.m.) in December 2021.

Sampling of duckweed

Duckweeds were sampled systematically at an interval of 50 m with a quadrat (25 cm × 25 cm) (Roslan et al., 2021) along a transect line from 13 different areas (Sanda Kyarimi Zoo, Monday Market, Circular Road, Unimaid, Gamboru, Bolori, Umarari, Kalari. Custom, Gwange Bridge, Suleimanti, Damboa Road and CNB Quarters) within the metropolis, along with water samples, at the same time and month.

Biomass and proximate composition

The freshly collected duckweeds of each quadrat sampled were blotted with a paper towel to reduce excess water and oven-dried for 6 h at 100°C. Dry weight (g) was determined by placing the dried sample in a weighing boat on a balance scale (~ 0.01g). Proximate composition (carbohydrate, ash, crude fibre, fat, crude protein, moisture and dry matter) was determined using the methods of the Association of Official Analytical Chemists (AOAC, 2005).

Statistical analysis

Multivariate data analysis (MDA) was performed using PAST software (Ver. 1.4). Bar graphs were plotted using GraphPad Prism Software (ver. 8.0.2) for Windows. Normality test was conducted using D'Agostino and Pearson test ($p < 0.05$). Error bars present standard deviation (SD).

RESULTS

In the ordination biplots, PC1; Monday market, and Gamboru have the highest and lowest variations, respectively when compared to other sites. The fresh biomass and TSS, fresh biomass and TDS were negatively correlated. However, in PC2 salinity and TDS were not correlated. Salinity and TSS, Salinity and TDS were positively correlated. Duckweeds from Damboa Road have low variability among the sampling sites (Figure 1). The figure also shows the resulting ordination biplot.

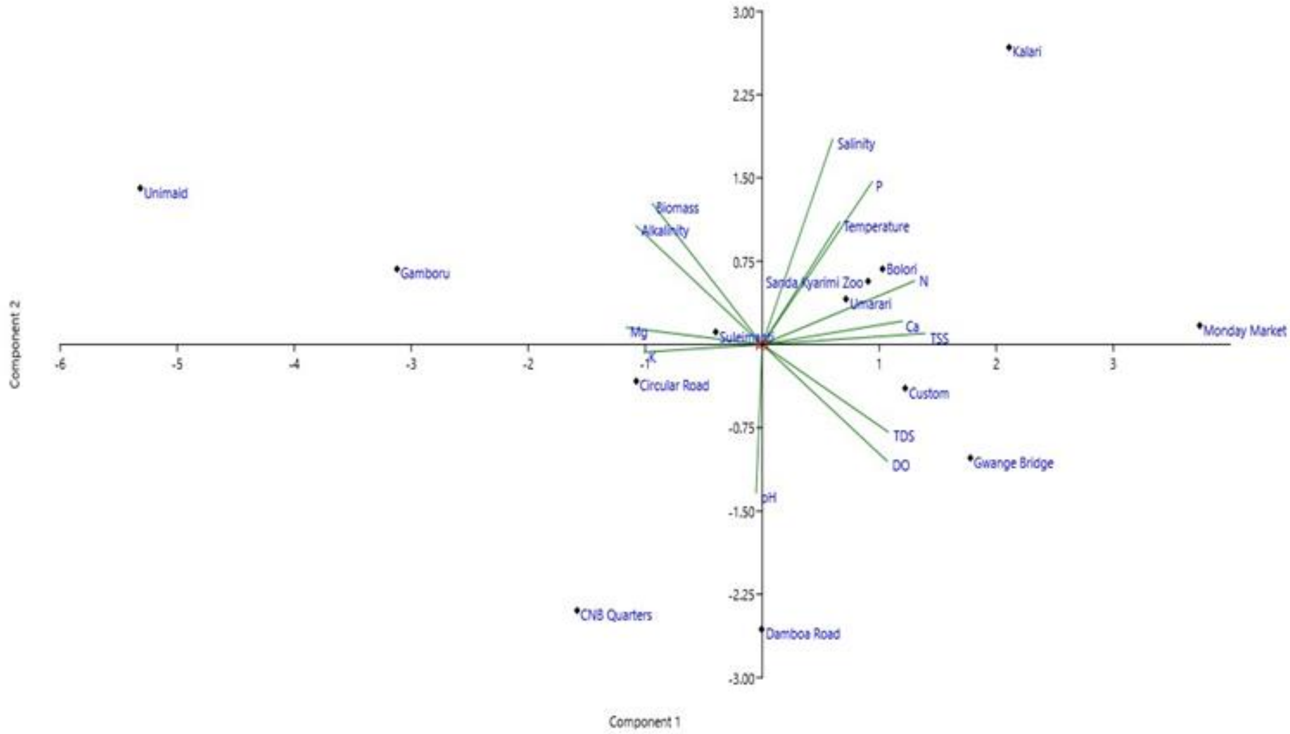

Figure 1: Ordination map of the principal components (PCs) biplots of duckweed (*Lemna minor* L.) in different wastewaters in Maiduguri, Nigeria. N = Nitrogen, pH = potential of hydrogen, Mg = Magnesium, DO = Dissolved oxygen, K = Potassium, P = Phosphate, Ca = Calcium, TSS = Total suspended solids, TDS = Total dissolved solids.

Table 1 shows the Principal Components (PCs) loadings of the extracted water quality parameters from the sampling sites. Based on the PCA, the first four PCs (PC1, PC2, PC3 and PC4) cumulatively explained 79.2% of the variation in the water quality parameters. The PCs in the four axes with eigenvalues ≥ 1 were considered significant and thus extracted. PC2 had strong loadings for salinity, PC3 had strong loadings for phosphate and pH, while PC4 has strong loadings for magnesium and temperature.

Table 1: Eigenvalue and the percentage variation of Principal Components (PC1 – PC4) of duckweed (*Lemna minor* L.) in different wastewaters in Maiduguri, Nigeria

	PC 1	**PC 2**	**PC 3**	**PC 4**
*Eigenvalue	5.607	2.019	1.426	1.247
% Variance	43.131	15.53	10.97	9.59
% Variance (cumulative)	43.131	58.661	69.628	79.219
Phosphate	0.256	0.399	**-0.409**	-0.039
Magnesium	-0.316	0.042	0.263	**0.469**
pH	-1.42E-02	-0.364	**-0.609**	0.187
Salinity	0.164	**0.504**	-0.132	0.367
Temperature	0.180	0.301	0.310	**-0.430**

*Extraction was based on eigenvalues > 1. Numbers in bold indicate the parameters with high loadings.

The correlation matrix of the physicochemical parameters of water from different sites shows a strong positive correlation between N and P, N and Ca, N and TSS, Mg and alkalinity, TDS and DO, TDS and TSS, DO and TSS. However, N and Mg, P and Mg, K and Ca, K and TSS, Mg and TSS, and alkalinity were significantly negatively correlated (Figure 2).

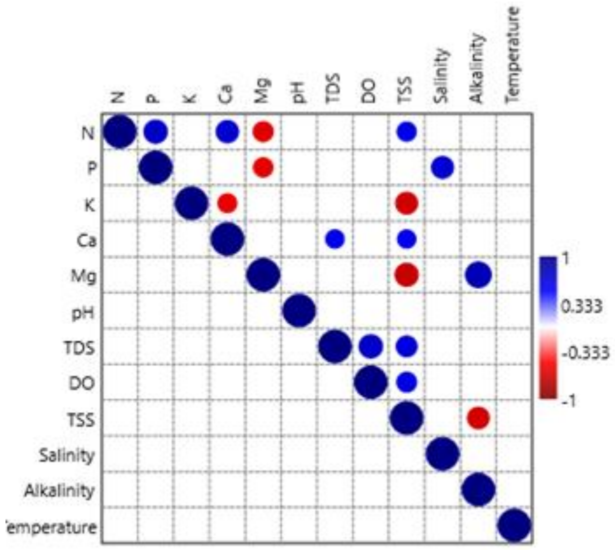

Figure 3: Correlation between physicochemical parameters of wastewaters inhabited by duckweed (*Lemna minor* L.) in Maiduguri, Nigeria, December 2021. N = Nitrogen, pH = potential of hydrogen ion, Mg = Magnesium, DO = Dissolved oxygen, K = Potassium, P = Phosphate, Ca = Calcium, TSS = Total suspended solids, TDS = Total dissolved solids.

The proximate composition of duckweeds indicated an average of 47.3% carbohydrate, 14.8% protein, and 39.6% crude fibre. Ash content and fat were 3.19% and 1.1%, respectively (Figure 3).

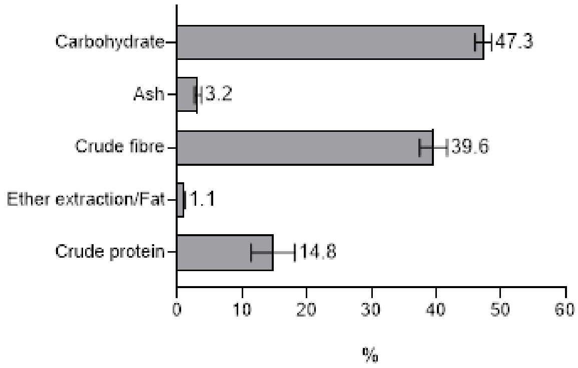

Figure 3: Proximate compositions of *Lemna minor* collected in December 2021 from wastewaters in Maiduguri, Nigeria.

The similarity index of the physicochemical parameters measured was generally high between most of the sampling locations. Custom area and Bolori have the highest index (0.99). The lowest index observed was CBN quarters and Unimaid with a score of 0.49 (Table 2). Likewise, the paired group two-way Euclidean similarity index of the physicochemical variables shows that pH and Mg, temperature and alkalinity, DO and K were the most similar parameters (Figure 4).

Table 2: Bray-Curtis's similarity index of *Lemna minor* collected in December 2021 from different sites in Maiduguri, Nigeria

Sampling site	Sanda Kyarimi Zoo	Monday Market	Circular Road	Unimaid	Gamboru	Bolori	Umarari	Kalari	Custom	Gwange Bridge	Suleimanti	Damboa Road	CNB Quarters
Sanda Kyarimi Zoo	1.00												
Monday Market	0.78	1.00											
Circular Road	0.93	0.75	1.00										
Unimaid	0.65	0.50	0.67	1.00									
Gamboru	0.76	0.56	0.77	0.86	1.00								
Bolori	0.85	0.83	0.89	0.64	0.71	1.00							
Umarari	0.90	0.74	0.93	0.59	0.72	0.87	1.00						
Kalari	0.82	0.82	0.84	0.56	0.64	0.91	0.83	1.00					
Custom	0.86	0.82	0.89	0.63	0.71	0.99	0.88	0.91	1.00				
Gwange Bridge	0.86	0.82	0.89	0.61	0.69	0.94	0.88	0.94	0.95	1.00			
Suleimanti	0.85	0.86	0.89	0.61	0.68	0.92	0.86	0.89	0.92	0.91	1.00		
Damboa Road	0.73	0.68	0.77	0.50	0.64	0.83	0.82	0.76	0.83	0.78	0.81	1.00	
CNB Quarters	0.67	0.67	0.72	0.49	0.63	0.78	0.76	0.72	0.78	0.73	0.79	0.94	1.00

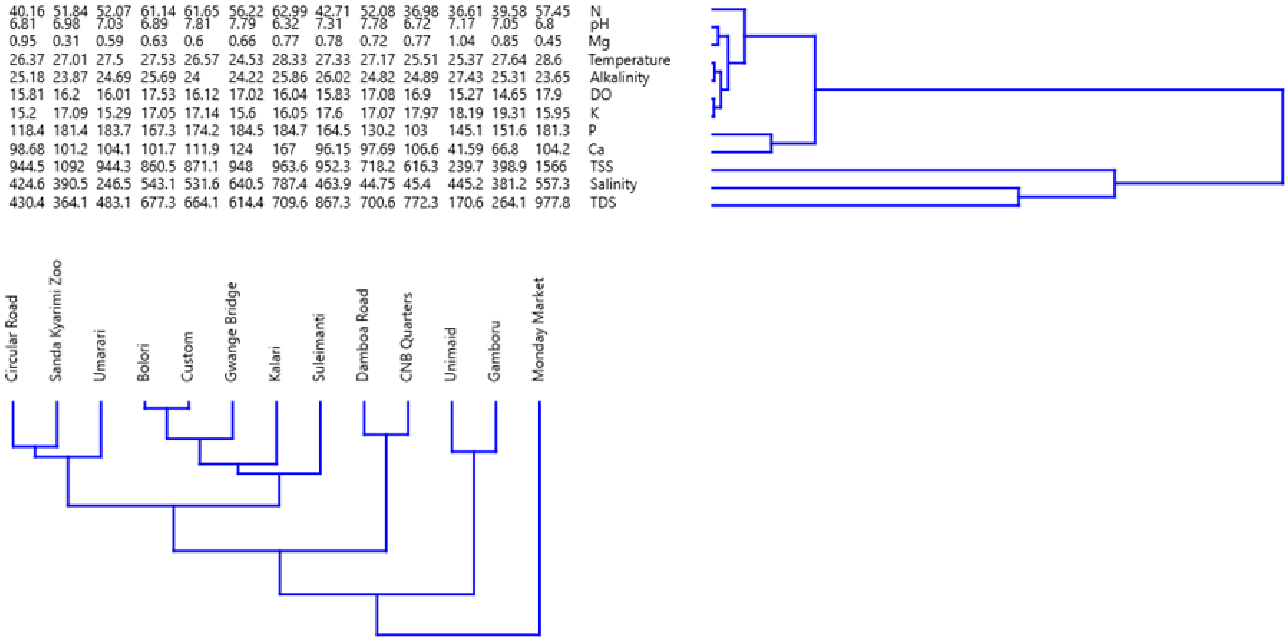

Figure 4: Paired group two-way Euclidean similarity index for physicochemical parameters of sites *Lemna minor* in different wastewaters in Maiduguri, December 2021. N = Nitrogen, pH = potential of hydrogen, Mg = Magnesium, DO = Dissolved oxygen, K = Potassium, P = Phosphate, Ca = Calcium, TSS = Total suspended solids, TDS = Total dissolved solids.

The duckweed sample collected from the Unimaid had the highest dried biomass (5.0 ± 0.2 g/m²), while the lowest biomass (2.24 ± 0.1 g/m²) was observed in samples from Umarari area (Figure 5).

Figure 5: Biomass of *Lemna minor* collected in December 2021 from different wastewater in Maiduguri, Nigeria. Error bar

DISCUSSION

Duckweeds of diverse species have been used in Nigeria and other parts of the world. The physiological and morphological features of this plant are some of the factors that make it make it special compared to other aquatic macrophytes. This study provides a preliminary survey of duckweed distribution, and proximate compositions in stagnant waters of Maiduguri metropolis, Nigeria. The sites showed that water quality parameters played a significant role in the ecology and proximate composition of this aquatic plant, this can be associated with the type waste discharges by the nearby residential, commercial and agricultural and other sources. These sources and similar human activities according to Kaaya *et al.* (2015) disturbed the water quality in some rivers in Tanzania. The temperature of duckweeds collected from the side with the highest biomass, Unimaid was similar to an earlier report by Culley *et al.* (1981), who observed that duckweed may grow to a maximum tolerance temperature of about 34°C. Typically, Devlamynck *et al.* (2021) has reported that the growth of *L. minor* ceases at water temperatures below 8°C. This indicated that the duckweeds in the study area fall within a suitable range of temperature for better mass production in a controlled environment to attain optimum biomass and other essential phyto-products. In the same way, in this study temperature has shown high loading, indicating its contribution as one of the key parameters determining the distribution of duckweed in the various sites sampled. When the pH is between 5 and 9, and the water temperature is between 6 and 33°C, duckweed can flourish. This corroborate with Sońta *et al.* (2019) who reported that pH levels between 6.5 and 7.5 and water temperatures between 20 and 28°C are the ideal growing conditions for excellent biomass output.

Water quality determines the physicochemical parameters that play a significant role in pant biomass, growth rate and mineral compositions of the macrophyte, *L. minor*.

This study shows that some essential macronutrients (N and P, N and Ca) are strongly positively correlated but N and Mg, P and Mg are negatively correlated. These nutrients are necessary for the building-up of vital nutrients including proteins, carbohydrates, and lipids that may be found in duckweed (Baek *et al.*, 2021).

The average carbohydrate content of the duckweed in this study was 47.3%, with this amount of carbohydrate in duckweed collected in Maiduguri has high potential to be used in biofuel (Cui and Cheng, 2015; Cheng *et al.*, 2009) generation, which is more than what was reported earlier by Baek *et al.* (2021) who found only 38% total carbohydrate content in *Lemna gibba*, and within the range of Cheng and Stomp (2009) who indicated a range of 3-75% carbohydrate. *L. gibba* was found to have a comparatively greater percentage of total carbohydrate content (38%) when compared to other *Lemna* species (Baek *et al.*, 2021).

Agriculturalist (especially animal scientist) rely on crude protein source to minimize cost and maximize profit in their farms. The average protein content of the sample duckweeds in this study was 14.8% and this falls with the range documented by Cheng and Stomp (2009) who observed that protein content ranges from 15-45% dry weight in a variety of duckweed species produced under different conditions. With this amount, the *Lemna minor* collected in Maiduguri can serve as a cheaper, easier to culture, fast growing source of crude protein. In a similar report, protein makes up 7–45% (typically 20–45%), fat at 2-9%, fibre at 12–28%, and carbohydrate at 14–44% (Sońta *et al.*, 2019). Although, in a study carried out in Ka'inji North-central Nigeria by Mbagwu and Adeniji (1988), two species of duckweed (*Spirodela polyrhiza* and *Landoltia punctata*) have crude protein content ranging from 29.3 to 37.9%. Similarly, a crude protein content of 14.96% in L. minor was reported in western Nigeria (Aderemi et al., 2018) which is almost the same (14.7%) as what was obtained in this study. However, this study indicates a higher crude fibre (39.6%) and less fat (1.1%) than what Sońta *et al.* (2019) reported. In a recent study by Baek *et al.* (2021), it was reported that duckweeds have total protein content per dry weight that ranges from 19.8% to 48.2% (Baek *et al.*, 2021). Duckweed is extremely productive and has a high protein content of up to 45% (Devlamynck *et al.*, 2021).

CONCLUSION

This study has revealed that the duckweed in the study area has the potential to be improved for optimization of their nutritional content for application as a bioresource and model to meet human needs in various sectors. The findings reveal the appropriate water parameters (nitrogen, pH, magnesium, dissolved oxygen, potassium, phosphate, calcium, total suspended solids, total dissolved solids) for better growth of the duckweed in Maiduguri metropolis, this will be used in a controlled environment for mass culture and subsequent utilization of duckweed as a model for research in different fields of agriculture (feed formulation for poultry fishmeal and other farm animals). For biological sciences and plant biotechnology; the duckweed can be used for research to understand some physiological, molecular processes. In environment sciences, native duckweed can be used to treat wastewater from commercial, residential and commercial sources, and aquatic toxicity studies. Further research to understand the biology and ecology of duckweed in the study area will give more insight on this beneficial macrophyte.

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