# Optimizing Nutritional Composition and Protein Productivity of *Lemna minor* Through Organic Manure Fertilization for Sustainable Livestock Feed Production

**Habtom Negussie**
Wolaita Soddo University
**Yisehake Kechero**
yisehak.kechero@amu.edu.et
Arba Minch University
**Yilkal Tadele**
Arba Minch University

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## Abstract

Poultry and livestock production in developing countries like Ethiopia is hindered by the high cost and scarcity of quality feed. Duckweed (*Lemna minor*) has emerged as a sustainable, protein-rich alternative, but optimizing its nutritional value through effective fertilization is crucial. Anaerobic digested poultry manure (PM), poultry-cattle mixed manure (PCM), and cattle manure (CM) were applied at three ammonium nitrogen ($NH_4$) levels (16, 24, and 32 mg N $L^{-1}$), alongside an inorganic fertilizer (IF 16 mg N $L^{-1}$) and no fertilizer as controls in mesocosm ponds according to completely randomized design. Treatments were applied initially and weekly for 56 days. Media water was sampled bi-weekly. Regularly harvested biomass was analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ash, and fat content. Manure type and nitrogen levels significantly influenced nutritional composition, protein yield, nitrogen uptake rate and efficiency of *L. minor*. CP content ranged from 35.4% to 41.6% DM. Increasing nitrogen levels increase CP while reducing CF. PM and PCM treatments outperformed CM, yielding up to 9.82 tons of protein per hectare annually. Nitrogen uptake rate increased with the concentration of N in the cultivation medium, while an opposite trend was observed for nitrogen uptake efficiency. Strong correlations between water quality parameters (pH, electrical conductivity, $NH_4$, and phosphate) and nutrient content and yield signified importance of optimizing growth conditions. Polynomial regression models further supported the predictive potential of these parameters. Weekly optimum application at 24 mg $NH_4$-N $L^{-1}$, supported high protein yield, nutrient uptake, and nutritional values of *L. minor* with desirable growth media water qualities. *Lemna minor* can enhance nutrient recycling while providing a sustainable, high-protein feed source. Further research is needed to validate its potential as a substitute for conventional protein sources and explore advanced technologies for broader application.


## 1. Introduction

Animal production, the fastest growing agricultural sector in most developing countries, is evolving in response to rapidly increasing demand for animal-source foods, driven by population growth, disposable income, consumer preference, and urbanization [1]. This has increased the demand for conventional feed ingredients such as soybean and cereals grains due to their high protein content and relatively well-balanced amino acid profile and energy density [2, 3]. However, limited availability of consistent quality, quantity, along with escalating price of ingredients and compound feeds, are the major challenges for poultry and livestock production in most developing countries, including Ethiopia [4, 5].

The contributing factors include climate change, land degradation, and water scarcities, which limit sustainable feed crop production. Increasing competition for these resources from human food and biofuel industries further exacerbates the problem [6, 7]. Furthermore, the environmental impacts of intensive animal agriculture, such as gas emissions, manure, and wastewater production, present sustainability challenges. Consequently, there is a constant need for the integration of environmentally friendly, cost-effective, high-quality feed ingredients in diets to enhance sustainability in animal production by embracing circular economy principles [8, 9].

Duckweed (*Lemna minor* L.), a small floating aquatic plant in the Lemnaceae family, holds immense potential for the sustainable production of protein-rich and nutritious biomass as a poultry and livestock feed resource that could reduce dependence on conventional protein ingredients [10, 11]. These attributes are due to its fast reproduction and growth rate, high biomass productivity, most efficient nutrient (nitrogen and phosphorus) uptake rate, and accumulation of protein rich in essential amino acid, vitamins and minerals. This is achieved through recycling of manure and wastewater nutrients and phytoremediation of water [12]. Biomass productivity and nutritional values of duckweed are influenced by nutrient sources and concentrations in the growth media, as well as environmental and water quality parameters [13–15]. Different inorganic fertilizers (e.g. Urea, DAP, Potash, and synthetic media) have been used in duckweed cultivation [16]. These sources are expensive, limited in availability—particularly for smallholder farmers—and have environmental impacts [17].

Animal manures and wastewater are rich sources of essential plant nutrients, although they are highly variable in composition, microbial, and pollutant loads. Proper treatment of organic manures before application could supply cheap and sustainable sources of nutrients for duckweed growth and water purification [18, 19]. Such systems address environmental and economic sustainability in agriculture and animal production, contributing to the circular economy [20]. Thus, optimization of growth media nutrient sources and concentrations through manure-based fertilization regimes could improve the nutritional values, nutrient yield and phytoremediation efficiency of duckweed system. However, there are limitations of long-term investigations on biomass productivity and nutritional values of duckweed adapted to prevailing environmental conditions of the area [21, 22]. This study aims to investigate the effects of different organic fertilization regimes—anaerobic digested poultry manure, poultry-cattle mixed manure, and cattle manure—applied at varying nitrogen levels on nutritional composition, protein yield and phytoremediation potential of *Lemna minor* in Ethiopian conditions. It seeks to evaluate the correlation among water quality characteristics, biomass yield, nutrient content and N uptake efficiencies. Additionally, the study aims to establish predictive equations to optimize duckweed growth and nutrient composition.

## 2. Materials and Methods

# 2.1. The study site

The experiment was conducted at the Kulfo Campus, College of Agricultural Sciences, Arba Minch University, Ethiopia. The site is located at an altitude of 1,280 meters above sea level, with geographical coordinates of 7°03'27.8" N latitude and 38°28'06.2" E longitude. The site lies within the Kulfo River watershed, situated between Lake Abaya and Lake Chamo in the southern part of the Great Rift Valley [23]. The area is characterized by a hot, humid, semi-arid climate, with annual rainfall ranging from 768 to 1,270 mm. The mean annual temperature is 22.9°C, with a minimum of 15°C and a maximum of 32°C. The rainy season extends from March to November, while the dry season spans from December to February [24].

## 2.2. Collection, Identification, and Pre-culture of Lemna minor

Common duckweed (*Lemna minor* L.) was selected for this study. Fresh plant materials were collected from natural wetlands near Lake Hawassa (7.05° N, 38.44° E), Lake Ziway (7.99° N, 38.82° E), and Lake Abaya (6.32° N, 37.87° E), Ethiopia, during July 2024. The reported coordinates represent the approximate locations of the collection sites. The collection of plant materials complied with applicable national and local regulations governing plant collection in Ethiopia, and all necessary permissions were obtained from the relevant authorities prior to sample collection.

Collected specimens were cleaned, sorted, and identified as *Lemna minor* L. by Dr. Temesgen Dingamo using standard taxonomic keys based on the morphological characteristics of the fronds and roots [27]. A voucher specimen was prepared and deposited at the Arba Minch University Herbarium, Arba Minch University, Ethiopia, under voucher specimen number AMUH-1436.

Following identification, the collected *L. minor* plants were acclimatized and propagated as inoculum for 14 days in a polyethylene sheet-lined pond fertilized with anaerobically digested poultry-cattle mixed manure (PCM) prior to the commencement of the experiment.

## 2.3. Preparation of diluted and anaerobic digested manures

Poultry manure (PM) and cattle manure (CM) was sourced from local animal farms. The PM was obtained from an egg-type chicken farm that used commercial layer rations. In contrast, CM was collected from a cattle farm where animals grazed on natural pasture with supplemental commercial concentrates and roughage. These organic manures were chosen based on availability, ease of collection, and cost-effectiveness. PM was sun-dried for two days, whereas CM was used fresh. Debris such as caked litter, sawdust, and grass was removed from the manure samples. Moisture content was determined, and the CM was mixed with water at CM: water dilution ratio of 1:4, while PM was mixed with water at PM: water dilution ratio of 1:9 ratios. The diluted CM and PM were then mixed at a 1:1 ratio to form poultry-cattle mixed manure (PCM). The manures were thoroughly blended to enhance solubility, and floating debris was removed. The diluted manures (PM, CM, and PCM) were anaerobic digested separately in 200-liter plastic containers for 21 days. Anaerobic digestion increase nutrient mineralization but reduce microbial load [18]. After fermentation, manure samples were mixed thoroughly and analyzed in triplicate for physicochemical qualities as outlined in Table 1. The river water used for the dilution of manures and preparation of growth media treatments had pH 7.4 ± 0.2, EC 217.3 ± 6.4 μS $cm^{-1}$, TDS 110.3 ± 58 mg $L^{-1}$.

Table 1
Physicochemical characteristics of Anaerobic Digested Manures (Mean ± SD)

| **Parameters** | **Unit** | **Poultry manure** | **Poultry-cattle manure** | **Cattle manure** | ***P* value** |
|---|---|---|---|---|---|
| Temperature | °C | 24.40 ± 0.80 | 24.30 ± 0.12 | 24.55 ± 0.03 | 0.470 |
| pH | | 4.50 ± 0.03[c] | 5.31 ± 0.03[b] | 6.16 ± 0.06[a] | 0.001 |
| Electrical conductivity (EC) | μS $cm^{-1}$ | 7675 ± 40[a] | 5300 ± 72[b] | 4140 ± 60[c] | 0.001 |
| Total dissolved Solid (TDS) | mg $L^{-1}$ | 3975 ± 160[a] | 2710 ± 36[b] | 2097 ± 51[c] | 0.011 |
| Dry matter (DM) | % | 5.92 ± 0.2 | 5.89 ± 0.3 | 5.93 ± 0.2 | 0.34 |
| Ammonium N ($NH_4$-N) | mg N $L^{-1}$ | 2364 ± 106[a] | 1870 ± 10[b] | 1403 ± 19[c] | 0.043 |
| Nitrate N ($NO_3$-N) | mg N $L^{-1}$ | 15.11 ± 0.4[c] | 18.94 ± 0.3[b] | 22.92 ± 0.4[a] | 0.001 |
| Total N (N) | mg N $L^{-1}$ | 2379 ± 106[a] | 1888 ± 10[b] | 1426 ± 20[c] | 0.043 |
| Soluble phosphate ($PO_4$) | mg P $L^{-1}$ | 167 ± 15.5[a] | 98.1 ± 4.0[b] | 66.7 ± 0.5[c] | 0.038 |
| Potassium (K) * | mg K $L^{-1}$ | 3130 ± 80[a] | 2325 ± 25[b] | 1935 ± 30[c] | 0.024 |

Means with different superscripts in the same row significantly different ($p < 0.05$) Tukey's HSD; Potassium* - concentration estimated according to IAEA [28] from EC and DM values

# 2.4. Experimental Design and Treatments

The experiment was conducted outdoor within a simple shaded structure constructed from timber and covered with perforated bamboo mats to protect *L. minor* from excessive solar radiation. Within the shaded area, 33 plastic-lined earthen mesocosm ponds were established, each measuring 0.5m*0.5m with a depth of 0.5 m. The bottom of each pond was covered with approximately 1 kg of dry topsoil [29], and the ponds were filled with river water to a mean depth of 0.32 m. The ponds were randomly assigned to 11 experimental treatments, each with three replications, following a Completely Randomized Design. The experiment lasted 56 days (2, November 2023 to 28, December 2023).

Treatments (T) include two control treatments, T0 media with no fertilizer only topsoil as negative control, and a positive control T1 using commercial inorganic fertilizer, involved the application of urea and di-ammonium phosphate, DAP at a rate of 1.5 g urea and 0.5 g DAP $m^{-2}$ $d^{-1}$ [16]. The calculated weekly nitrogen content of this fertilizer solution was 16 mg N $L^{-1}$. The organic fertilization treatments include anaerobic digested PM, PCM, and CM. The application rates for these organic manures were adjusted to provide 100%, 150%, and 200% of the recommended N levels for inorganic fertilizer, corresponding to ammonium nitrogen ($NH_4$-N) concentrations of 16, 24, and 32 mg N $L^{-1}$, respectively. The fertilization treatments were applied in varying amounts to match the targeted N concentrations, as detailed in Table 2.

Table 2
Types of manures and application rates in different fertilization treatments

| **Manure types** | **Fertilizer Application rate (g per L of water)** | **Treatments** |
|---|---|---|
| No fertilizer | - | T0-NF (Topsoil) |
| Urea-DAP Inorganic fertilizer | 0.045 | T1-IF-16 mg N $L^{-1}$ |
| Poultry Manure | 8.04 | T2-PM-16 mg N $L^{-1}$ |
| Poultry Manure | 12.0 | T3-PM-24 mg N $L^{-1}$ |
| Poultry Manure | 16.0 | T4-PM-32 mg N $L^{-1}$ |
| Poultry-cattle Manure | 10.8 | T5-PCM 16 mg N $L^{-1}$ |
| Poultry-cattle Manure | 16.2 | T6-PCM-24 mg N $L^{-1}$ |
| Poultry-cattle Manure | 21.5 | T7-PCM-32 mg N $L^{-1}$ |
| Cattle Manure | 14.0 | T8-CM-16 mg N $L^{-1}$ |
| Cattle Manure | 21.0 | T9-CM-24 mg N $L^{-1}$ |
| Cattle Manure | 28.0 | T10-CM-32 mg N $L^{-1}$ |

NF - no fertilizer; IF - inorganic fertilizer of Urea-DAP mix at 3:1 ratio; PM - Poultry manure; PCM - Poultry cattle mixed manure; CM - Cattle manure

The cultivation of *L. minor* was initiated after preparing the media in each mesocosm pond. A total of 60 g fresh weight (FW) of *L. minor* was inoculated into each pond, covering approximately 30% of the surface area. The duckweed was allowed to grow until it covered the entire surface of the ponds. The first harvest took place seven days after inoculation, and subsequently, duckweed was harvested every two to three days based on productivity. A harvesting rate of approximately 25–35% was maintained, ensuring an optimal fresh weight biomass density of 600 g FW $m^{-2}$ at each harvest [30].

The harvested biomass was collected using a metal sieve, rinsed with fresh water to remove any remaining nutrient solution, and allowed to drain for five minutes. The fresh biomass weight was then measured using a sensitive digital balance [13]. The growth media and duckweed were stirred daily in the morning with a rod to ensure uniform nutrient distribution. Water loss due to evapotranspiration was replenished with river water to maintain the volume of the culture media. Fertilization was applied weekly to sustain duckweed growth at the same rates as the initial treatments. Seven rounds of fertilization were used in total during the 56-day experimental period. At the end of the experiment, on day 56, all *L. minor* biomass was harvested and weighed for each replication.

# 2.5. Duckweed and physicochemical water quality sampling

Duckweed samples were collected before inoculation and after each harvest from each pond. At the beginning of the experiment, and every two weeks, duckweed samples from each pond were dried in the oven to determine DM contents. On other harvest days, fresh biomass yield was measured, and the samples were air-dried on paper sheets for 3–4 days. These air-dried samples were then bulked per treatment for further analysis. The dried duckweed samples were used to determine nutrient composition of *L. minor* on a per-treatment basis.

Climatic variables of ambient temperature, water temperature and relative humidity inside the shade was regularly monitored throughout the experiment using digital sensor. Moreover, physicochemical water quality sampling was carried out regularly throughout the study to assess the growth media conditions. Water quality measurements from each mesocosm pond were taken at the beginning, every two weeks, and at the end of the experiments. The water quality was measured *in situ* below the duckweed mat after gently agitating the media using digital devices (HANNA Instruments). Due to the variability of external conditions (light, temperature, humidity), a fixed measurement time was established, in the afternoon at 2:00 PM. The parameters and sampling devices were: dissolved oxygen (HD:40d), water pH and temperature (pH meter, HI:99121), electrical conductivity (Dual EC/TDS, HI:99301), and turbidity (HI:93703). Furthermore, visual evaluations of growth media characteristics such as color, amount of floating solids, gases, and bubble formations were also monitored. At the end of the experiment, water samples from each mesocosm pond were collected in polyethylene bottles, refrigerated at 4°C, and later analyzed for ammonium nitrogen ($NH_4$) and nitrate concentration to determine residual nitrogen levels. Water sampling and measurements were conducted following the *Standard Methods for Examining Water and Wastewater* [31].

# 2.6. Protein yield, Nitrogen Removal and Uptake

The treatments were compared based on nutrient compositions, protein accumulation rate (PAR), protein yield (PY), Nitrogen removal rate (NRR), Nitrogen uptake rate (NUR), Nitrogen uptake efficiency (NUE) each with three replications. Parameters were computed as described in previous studies [32.33,34], and Metabolizable Energy (ME) value of *L. minor* estimated based on ether extract (EE), crude fiber (CF), and Ash content on dry matter basis.

*Protein Accumulation Rate (PAR)* - protein accumulation rate of *L. minor* per unit area per day.

*Protein yield (PY)* is the cumulative protein productivity of *L. minor* during experimental period.

ME (kcal kgDM$^{-1}$) = 3951 + 54.4*EE% − 88.7*CF% − 40.80*Ash% (1)

$$\mathrm{PAR}\,(\mathrm{g\,m^{-2}\,d^{-1}}) = \frac{(\mathrm{BP,\ g\,DM})\ *\ \mathrm{CP\%\,DM}}{\mathrm{A} * \mathrm{days} * 100}$$

2

$$\mathrm{PY}\ \left(\mathrm{g\,m^{-2}}\right) = \frac{(\mathrm{BP,\ g\,DM})\ *\ \mathrm{CP\%\,DM}}{\mathrm{A} * 100}$$

3

$$NRR\ \left(mg\,N\ \ m^{-2}\ \ d^{-1}\right) = \frac{(N_A - N_R)\ *\ 1000}{A\ *\ days}$$

4

$$NUR\ \left(mg\,N\ \ m^{-2}\ \ d^{-1}\right) = \frac{\left(PY,\ g\,m^{-2}\right)\ *\ 1000}{days\ *\ 6.25\ *\ (N_A - N_R)}$$

5

$$NUE\ (\%\,) = \frac{\left(PY,\ g\,m^{-2}\right)\ *\ 100}{6.25\ *\ (N_A - N_R)}$$

6

Where: *BP*, biomass production on dry matter (g DM) basis; *A*, surface area of pond in m$^2$; CP% DM, crude protein percentage on DM,; *days*, duration of the experiment in days; $N_A$, total Nitrogen applied during the experiment (g N m$^{-2}$); $N_R$, Residual Nitrogen (g N m$^{-2}$) at the end of the experiment.

## 2.7. Chemical analysis of *Lemna minor* and growth media samples

Frequently harvested and partially dried *L. minor* samples were pooled per treatment, ground using a hammer mill, and homogenized through a 1 mm sieve for dry matter and nutrient analysis. The dry matter content was determined from a known weight by oven-drying at 105°C for 24 hours. For nutrient analysis, DM was determined by drying at 60°C to a constant weight. The dried duckweed samples were analyzed in duplicate per treatment for proximate composition, including crude protein (Kjeldahl method), crude fat, total ash, crude fiber, and nitrogen-free extract, calculated by difference, following the methods described by AOAC [35]. The anaerobic digested manures and growth media water were analyzed for $NO_3$, $NH_4$-N, and $PO_4$ following the methods outlined in [31]. The sample analysis was conducted in the chemistry laboratory of Arba Minch University, Ethiopia.

# 2.8. Statistical analysis

Statistical analyses were conducted using SPSS version 20.0 (IBM, SPSS, USA). Data were assessed for normality of residual distributions (Shapiro–Wilk test) and equality of error variances (Levene test). General Linear Model procedures were used to analyze the data. Significant differences among treatment means were determined at $p < 0.05$, and post-hoc comparisons were conducted using Tukey's HSD test. Contrast analysis compared means among manure types (N sources) and application rates (N levels). Correlation and regression analyses were also conducted to examine relationships among key variables.

## 3. Results and Discussion

# 3.1. Physicochemical Characteristics of Growth Media Water

The water quality parameters were regularly measured during the 56-days experiment and the results are summarized in Fig. 1. The average ambient temperature and relative humidity inside the shade was 27.91 ± 2.46°C (23.1–33.5°C) and 60.3 ± 10.2% (46.0–82.2%), respectively. The pond water temperature 25.4°C (22.0–28.2°C) was similar among the treatments ($p > 0.05$). However, the physicochemical characteristics of growth media were significantly influenced ($p < 0.001$) by fertilization treatments (manure types and application rates). Treatments with higher manure application rate had significantly higher EC, turbidity, $NH_4$, $NO_3$, and P concentrations compared to lower and no fertilized ponds. The $DO_2$ and pH values were significantly lower in PM fertilized ponds, while $DO_2$ concentration tend to decrease to levels of manure application rates. Among the manures, CM based fertilized ponds had higher turbidity compared to PM, IF and NF. Similarly, EC was higher for PCM and CM compared to PM and IF.

The mean water temperature in the ponds was within the optimum range of 20–30°C for efficient photosynthesis, rapid growth, nutrient uptake and high protein content of duckweed [15, 36]. Similarly, the pH values ranged from 6.5 to 7.4, which was within the optimal range (6.5–7.5) for *L. minor* growth and protein accumulation [18, 34, 37], indicating favorable growth conditions. The mean EC values in fertilized ponds ranged from 500 to 800 µS $cm^{-1}$, which was within the optimum range (400–1000 µS $cm^{-1}$) reflecting variations in ionic strength and nutrient concentrations in the media that influence nutrient bioavailability and uptake [38, 39].

The $NH_4$ concentration (15–33 mg N $L^{-1}$) was within the range for optimum growth and high protein content of duckweed [41, 42]. Similarly, the $PO_4$ concentration of the fertilized ponds was within suitable range for high biomass yield, protein synthesis, carbohydrate, and fat contents [36]. The high turbidity in the CM, and higher manure application rate interfere with the duckweed capacity to effectively uptake and utilize nutrients that may reduce growth and nutrient composition of the biomass [20].

The physicochemical characteristics and nutrient concentrations in growth media significantly influence growth and nutrient uptake efficiency of duckweed. The results in this study indicated that the pH, temperature, EC levels, N and P levels were within optimal ranges conducive to *L. minor* growth. However, excessive nutrient concentrations at the highest manure application rates (T4, T7, and T10) reduced nutrient uptake efficiency, emphasizing the importance of balanced nutrient management. These findings highlight the need for optimal nutrient inputs to maximize productivity and environmental benefits in manure-based duckweed cultivation systems.

# 3.2. Nutritional Compositions of *Lemna minor*

The effect of manure types (nitrogen sources) and application rates on the nutritive value of *L. minor* is summarized in Table 3. Dry matter (DM) content was significantly higher with PM and PCM compared to CM, but comparable to IF. DM increased linearly with $NH_4$-N concentration in the growth media. Likely due to greater nutrient and protein accumulation in biomass [19]. The fresh biomass had low DM (4.85–5.6%), posing handling and transport challenges [43]. To improve usability as livestock feed, moisture could be reduced by air-drying under shade for 3–4 days in tropical environment.

Crude protein (CP) content of *L. minor* is influenced by manure type and N concentrations. Highest CP contents ($p < 0.05$) were observed in T7, T6, and T4 compared to T8 and T9. CP increased linearly ($p < 0.01$) with nitrogen application rate, suggesting that higher N level in media were favorable for duckweed protein accumulation. At all N levels, PCM and PM showed higher ($p < 0.05$) protein content than CM.

Table 3

Nutritional Composition (% DM) and Metabolizable Energy (ME) value of *Lemna minor* cultivated with different manure types and application rates

| **Treatment** | **Nutritional composition (% DM)** | | | | | | **ME** |
|---|---|---|---|---|---|---|---|
| | **DM (%)** | **CP** | **Ash** | **EE** | **CF** | **NFE** | **kcal kgDM$^{-1}$** |
| T0-NF (Topsoil) | 4.85$^{c}$ | 26.49$^{d}$ | 23.18$^{a}$ | 1.95$^{d}$ | 26.22$^{a}$ | 21.92 | 786$^{d}$ |
| T1-IF-16 mg N L$^{-1}$ | 5.35$^{ab}$ | 38.74$^{ab}$ | 17.06$^{b}$ | 4.93$^{a}$ | 20.75$^{b}$ | 18.54 | 1683$^{b}$ |
| T2-PM-16 mg N L$^{-1}$ | 5.42$^{ab}$ | 36.88$^{ab}$ | 19.47$^{ab}$ | 3.50$^{bc}$ | 17.20$^{bc}$ | 21.95 | 1821$^{ab}$ |
| T3-PM-24 mg N L$^{-1}$ | 5.49$^{ab}$ | 38.70$^{ab}$ | 20.97$^{a}$ | 3.12$^{bc}$ | 15.94$^{bc}$ | 20.28 | 1851$^{ab}$ |
| T4-PM-32 mg N L$^{-1}$ | 5.59$^{a}$ | 41.31$^{a}$ | 22.68$^{a}$ | 3.16$^{bc}$ | 13.44$^{c}$ | 19.43 | 2005$^{ab}$ |
| T5-PCM 16 mg N L$^{-1}$ | 5.41$^{ab}$ | 39.03$^{ab}$ | 19.41$^{ab}$ | 3.65$^{b}$ | 16.48$^{bc}$ | 21.44 | 1896$^{ab}$ |
| T6-PCM-24 mg N L$^{-1}$ | 5.46$^{ab}$ | 40.48$^{a}$ | 20.79$^{ab}$ | 3.16$^{bc}$ | 12.12$^{c}$ | 23.45 | 2200$^{a}$ |
| T7-PCM-32 mg N L$^{-1}$ | 5.53$^{ab}$ | 41.60$^{a}$ | 21.41$^{ab}$ | 3.04$^{bc}$ | 14.65$^{bc}$ | 19.23 | 1943$^{ab}$ |
| T8-CM-16 mg N L$^{-1}$ | 5.22$^{b}$ | 33.20$^{c}$ | 20.75$^{ab}$ | 2.02$^{d}$ | 21.61$^{b}$ | 22.44 | 1297$^{c}$ |
| T9-CM-24 mg N L$^{-1}$ | 5.27$^{ab}$ | 35.36$^{b}$ | 21.87$^{a}$ | 2.67$^{cd}$ | 18.61$^{bc}$ | 21.51 | 1553$^{bc}$ |
| T10-CM-32 mg N L$^{-1}$ | 5.38$^{ab}$ | 38.79$^{ab}$ | 22.64$^{a}$ | 3.44$^{bc}$ | 16.06$^{bc}$ | 19.09 | 1790$^{ab}$ |
| SE | 0.11 | 1.405 | 1.101 | 0.215 | 1. 66 | 1.627 | 0.140 |
| **P-value** | **0.001** | **0.001** | **0.007** | **0.001** | **0.001** | **0.20** | **0.001** |
| **Manure Types** | | | | | | | |
| NF | 4.85$^{b}$ | 26.49$^{c}$ | 23.18$^{a}$ | 1.95$^{d}$ | 26.22$^{a}$ | 21.92 | 786$^{c}$ |
| IF | 5.35$^{a}$ | 38.74$^{ab}$ | 17.06$^{b}$ | 4.93$^{a}$ | 20.75$^{b}$ | 18.53 | 1683$^{ab}$ |
| PM | 5.50$^{a}$ | 38.96$^{a}$ | 20.87$^{a}$ | 3.26$^{b}$ | 15.51$^{d}$ | 21.40 | 1893$^{ab}$ |
| PCM | 5.47$^{a}$ | 40.04$^{a}$ | 20.54$^{a}$ | 3.28$^{b}$ | 14.42$^{d}$ | 21.40 | 2013$^{a}$ |
| CM | 5.29$^{a}$ | 35.78$^{b}$ | 21.75$^{a}$ | 2.71$^{c}$ | 18.76$^{c}$ | 21.01 | 1547$^{b}$ |
| **P-value** | **0.052** | **0.001** | **0.028** | **0.001** | **0.003** | **0.485** | **0.001** |
| **Application Rates** | | | | | | | |
| 0 mg N L$^{-1}$ | 4.85$^{b}$ | 26.49$^{c}$ | 23.18$^{a}$ | 1.95 | 26.22$^{a}$ | 22.17 | 786$^{b}$ |

| Treatment | Nutritional composition (% DM) | | | | | | ME |
|---|---|---|---|---|---|---|---|
| | DM (%) | CP | Ash | EE | CF | NFE | kcal kgDM$^{-1}$ |
| 16 mg N L$^{-1}$ | 5.35$^{a}$ | 36.96$^{b}$ | 19.17$^{b}$ | 3.52 | 19.01$^{b}$ | 21.34 | 1674$^{a}$ |
| 24 mg N L$^{-1}$ | 5.41$^{a}$ | 38.18$^{ab}$ | 21.21$^{ab}$ | 2.98 | 15.55$^{c}$ | 21.75 | 1868$^{a}$ |
| 32 mg N L$^{-1}$ | 5.50$^{a}$ | 40.56$^{a}$ | 22.07$^{a}$ | 3.21 | 14.70$^{c}$ | 19.45 | 1913$^{a}$ |
| **P-value** | **0.01** | **0.001** | **0.002** | **0.08** | **0.001** | **0.102** | **0.001** |

Means with different superscripts within the column are significantly different ($p < 0.05$); NF - No Fertilizer; IF - Inorganic fertilizer; PM - Poultry Manure; PCM - Poultry-Cattle mixed Manure; CM - Cattle Manure; DM - Dry Matter; CP - Crude Protein; EE - Ether Extract; CF - Crude Fiber; NFE - Nitrogen Free Extract; ME - Metabolizable energy.

Ammonium nitrogen ($NH_4^+$-N) constitutes the major N form in the anaerobic digested manures (Table 1), in which *L. minor* prefer for uptake and assimilation compared to Nitrate N [40]. The supply of $NH_4$, increased the availability of N for DNA and RNA synthesis, that increased synthesis of amino-acid and protein, chlorophyll, enhanced the accumulation of vegetative storage protein [44]. The result indicated that higher $NH_4^+$-N levels in the growth medium correlate with increased protein content in duckweed, as reported in previous studies [42]. The CP contents of *L. minor* in fertilized ponds in this study compared well to previous studies conducted with different media such as poultry manure, swine, aquaculture effluent, dairy and cattle slurry wastewater with CP ranged 30-44% DM [19, 34, 45].

Ether extract (EE) content varied significantly ($p < 0.05$) between treatments, with IF showing the highest values, followed by PM and PCM, and the lowest in CM. The EE content decreased at higher nitrogen levels (above 16 mg N L$^{-1}$), showing a quadratic response ($p < 0.001$) with $NH_4$-N levels. This reduction may be attributed to higher electrical conductivity (EC), which could influence lipid biosynthesis. The fat contents of *L. minor* in this study was comparable to *L. minor* grown in pig slurry [19], but lower than the value reported by Chakrabarti [16] for the same species cultivated using organic manures. The variations in the studies could be due to differences in clones, environments, nutrient concentrations and balanced ratio, EC and salinity level of the growth media. Despite its relatively low EE content, duckweed is rich in polyunsaturated fatty acids, particularly α-linolenic and linoleic acids accounting for 60–63% of total fatty acids with favorable omega-3 to omega-6 ratio, indicating its potential as a lipid source for livestock and humans [16, 43].

Crude fiber (CF) content was significantly lower ($p < 0.05$) in PM and PCM compared to CM and IF. The CF content decreased linearly ($p < 0.001$) with increasing nitrogen levels. Similar trend was also observed for the carbohydrate (CF and NFE) content of *L. minor* in the different fertilization treatments. Thus, the high nitrogen supply tends to reduce carbohydrate content, as the plant prioritizes protein synthesis over starch accumulation. Conversely, low nitrogen levels can increase storage carbohydrate (starch) and fiber content at the expense of protein quality [15, 32]. Therefore, higher application of N could improve the nutritional value by reducing the fiber content and hence digestibility. These findings signifies the role of N in determining nutritive value of plants. The CF content of *L. minor* in this study was lower than *L. minor* from collected from wetlands of Lake Hawassa, Ethiopia [25], but higher than *L. minor* grown in pig slurry [19]. The variations may result from differences in clones, media composition, and harvesting frequencies. Duckweed grown in CM had significantly lower ($p < 0.05$) CP and EE content but higher CF compared to PM and PCM, reinforcing the superior nutritional quality of PM and PCM fertilized duckweed. Given the sensitivity of poultry and fish to dietary fiber, PM and PCM at 24 mg N $L^{-1}$ application are recommended for producing high-protein, low-fiber biomass.

*Lemna minor* is known to effectively absorb and accumulate minerals from growth media, as indicated by its high ash content when grown in organic manures and higher application rates, which was in agreement with the findings of Chakrabarti et al. [16]. Increasing nitrogen levels linearly ($p < 0.01$) enhanced mineral uptake, suggesting its potential as a mineral supplement in poultry and livestock diets. The estimated ME value of *L. minor* was significantly different between the treatments as influenced by the types and application rate of manures. Among the treatments, T6 had the highest ME value, due to lower CF content, followed by T4, T7, and T5. The mean ME content in the PCM fertilized ponds was significantly higher than CM. Despite the numerical variations there was no statistical difference between the manure application rates. Duckweed is considered a low-to-medium energy source, making it suitable for poultry diets when combined with high-energy, low-fiber feed ingredients.

## 3.3. Protein Yield of *Lemna minor*

*Lemna minor* holds significant potential as a sustainable, protein-rich, and cost-effective livestock feed resource. To fully harness this potential, it is essential to cultivate and manage duckweed in suitable growing media to achieve optimal and sustainable production with predictable composition. This study evaluates the effects of anaerobic digested manure type (nitrogen sources) and application rates (nitrogen levels) on protein accumulation rate (PAR) and protein yield (PY) of *L. minor* under Ethiopian environmental conditions. The result indicated that protein productivity of *L. minor* were significantly different ($p < 0.01$) among fertilization treatments, as influenced by N sources (manure types) and levels in the growth media (Table 4).

Among the treatments T4 and T5 exhibited significantly higher yield ($p < 0.05$); while T1, T8, and T10 had the lowest yields, primarily due to lower biomass yields in these treatments. Fertilization increased protein yield by 663% compared to unfertilized pond. Organic manure-based growth media increased ($p < 0.05$) protein yield by 50.4% compered to the ponds fertilized with conventional inorganic fertilizer (Urea-DAP). Thus, the application of PM, PCM, and CM improved protein yield of *L. minor* by 64%, 64%, and 22%, respectively, relative to IF. Moreover, among manures, PM and PCM significantly increased protein yield by a similar value of 34.1% compared to CM. As manure application rate increased, protein yield linearly increased ($p < 0.01$) up to 24 mg N $L^{-1}$, but no significant difference was observed between the 24 and 32 mg N $L^{-1}$ application rates. Thus, source of nutrient (manure types) and application rates in the growth media were major factors affecting the protein yield of *L. minor*.

Table 4
Protein productivity of *Lemna minor* grown in different manure types and application rates

| Treatment | Protein accumulation rate | Protein yield | Annual protein yield |
|---|---|---|---|
| | g protein $m^{-2}$ $d^{-1}$ | g protein $m^{-2}$ | ton protein $ha^{-1}$ $y^{-1}$ |
| T0-NF (Topsoil) | $0.31^{f}$ | $17.5^{e}$ | $1.14^{e}$ |
| T1-IF-16 mg N $L^{-1}$ | $1.64^{e}$ | $91.9^{d}$ | $6.0^{d}$ |
| T2-PM-16 mg N $L^{-1}$ | $2.29^{cd}$ | $128.5^{bc}$ | $8.4^{bc}$ |
| T3-PM-24 mg N $L^{-1}$ | $2.71^{abc}$ | $151.7^{ab}$ | $9.9^{ab}$ |
| T4-PM-32 mg N $L^{-1}$ | $3.06^{a}$ | $171.2^{a}$ | $11.2^{a}$ |
| T5-PCM 16 mg N $L^{-1}$ | $2.83^{ab}$ | $158.5^{ab}$ | $10.3^{ab}$ |
| T6-PCM-24 mg N $L^{-1}$ | $2.51^{bcd}$ | $140.7^{b}$ | $9.2^{b}$ |
| T7-PCM-32 mg N $L^{-1}$ | $2.73^{abc}$ | $152.6^{ab}$ | $10.0^{ab}$ |
| T8-CM-16 mg N $L^{-1}$ | $1.77^{e}$ | $99.2^{d}$ | $6.5^{d}$ |
| T9-CM-24 mg N $L^{-1}$ | $2.13^{de}$ | $119.0^{c}$ | $7.8^{c}$ |
| T10-CM-32 mg N $L^{-1}$ | $2.12^{de}$ | $118.8^{cd}$ | $7.7^{cd}$ |
| SEM | **0.097** | **5.42** | **0.354** |
| **P-value** | **0.001** | **0.001** | **0.001** |
| **Manure Types (N sources)** | | | |
| NF | $0.31^{c}$ | $17.5^{c}$ | $1.14^{c}$ |
| IF | $1.64^{b}$ | $91.9^{b}$ | $6.00^{b}$ |
| PM | $2.69^{a}$ | $150.0^{a}$ | $9.83^{a}$ |
| PCM | $2.70^{a}$ | $150.9^{a}$ | $9.83^{a}$ |
| CM | $2.01^{b}$ | $112.3^{b}$ | $7.33^{b}$ |
| **P-value** | **0.001** | **0.001** | **0.001** |
| **Application rate (N level)** | | | |
| 0 mg N $L^{-1}$ | $0.31^{c}$ | $17.5^{c}$ | $1.14^{c}$ |
| 16 mg N $L^{-1}$ | $2.13^{b}$ | $119.2^{b}$ | $7.80^{b}$ |

| Treatment | Protein accumulation rate | Protein yield | Annual protein yield |
|---|---|---|---|
| | g protein $m^{-2}$ $d^{-1}$ | g protein $m^{-2}$ | ton protein $ha^{-1}$ $y^{-1}$ |
| 24 mg N $L^{-1}$ | 2.45[a] | 137.0[a] | 8.97[a] |
| 32 mg N $L^{-1}$ | 2.63[a] | 147.1[a] | 9.63[a] |
| **P-value** | **0.001** | **0.001** | **0.001** |

Means with different superscripts within the column are significantly different ($p < 0.05$) Tukey HSD; NF - No Fertilizer; IF - Inorganic fertilizer; PM - Poultry Manure; PCM - Poultry-Cattle mixed Manure; CM - Cattle Manure; TN - Total Nitrogen; FW - Fresh weight; DM - Dry Matter; Y-year

The superior protein yield observed with organic manures over IF may be attributed to higher biomass productivity, balanced nutrient availability, and slow mineralization from decomposing manures [46]. Moreover, the higher protein yield for PM and PCM compared to CM could be due to difference in nutrient balance and bioavailability as these manures contained higher nutrient concentrations (Table 1). As the manure application rate increased P level also increased, essential for energy metabolism, enhancing biomass yield, protein and lipid contents of duckweed [38].

To consider duckweed as potential livestock protein source, it must contain a high CP content and protein quality (i.e. high essential amino acid content and good digestibility); moreover, duckweed should ideally be a competitive alternative to conventional crops such as soybean in terms of productivity [42]. In this study, the CP content of *L. minor* was between 35.4 and 41.6% DM, which was comparable to SBM (39–44% DM) and rapeseed cake (37%); but higher than linseed meal (34%), noug-seed meal (33%), and grain legumes (22–35%) commonly used in intensive livestock diet [2]. Therefore *L. mino*r compete favorably with most other conventional plant protein sources in terms of protein content. Though amino acid composition was not analyzed, previous studies indicate that *L. minor* grown in optimally diluted manure media has high protein content with essential amino acid profile comparable to SBM [16, 19], and high intestinal digestibility compared to low CP containing duckweed species [47].

In terms of productivity, soybean grain on average produce 3 ton $ha^{-1}$ with CP 40% DM, equivalent to a protein yield of 1.2 ton $ha^{-1}$ [21]. In contrast, PM, PCM, and CM in this study produced 9.81, 9.82, and 7.32 ton CP $ha^{-1}$ $y^{-1}$, respectively. This indicates that diluted and anaerobic digested PM, PCM, and CM could produce 6 to 8 times more protein per hectare than soybean single crops. Previous studies reported annual protein productivity of duckweed system based on digested manure ranging 2.9 to 7.23 t $ha^{-1}$ $y^{-1}$ [33, 42, 48].

In developing countries like Ethiopia, limited feed availability, escalating price and poor-quality of feed ingredients constrain livestock productivity. The results of the present study suggest that nutritive values and protein yield of duckweed could be optimized through proper fertilization using diluted and anaerobic digested organic manures such as PM, CM, and PCM. The PM and PCM resulted in higher nutritional values and protein yield compared to CM. Furthermore, PCM could be considered the better growth medium for the sustained growth and optimum media qualities. Therefore, cultivation of *Lemna minor* and fertilization with ammonium nitrogen from anaerobic digested manure is an effective method to enhance local availability of high-quality, protein-rich feed ingredient, offering cost effective and a sustainable solution to livestock feed shortages and the environment.

# 3.4. Phytoremediation Potential of *Lemna minor*

*Lemna minor* holds significant potential for phytoremediation of nutrients, organic pollutants and heavy metals from eutrophic waters, contributing to sustainable environmental conservation [26, 36]. The effectiveness of this phytoremediation technology is evaluated based on nutrient recovery rates, biomass yield, water quality, and cost-effectiveness [8, 20]. While many studies have explored the use of *L. minor* for wastewater treatment limited study addressed potential of *L. minor* in livestock feed production and phytoremediation simultaneously based on anaerobic digested PM, PCM, and CM as growth media. The results indicated that N removal rate (NRR), N uptake Rate (NUR) and N uptake efficiency (NUE) were significantly ($p < 0.001$) influenced by the fertilization treatments (Fig. 2).

As shown in Fig. 2A, the NRR increased linearly ($p < 0.001$) with higher manure application rates, from 920 to 1892 mg N $m^{-2}$ $d^{-1}$ at 16 and 32 mg N $L^{-1}$, respectively. Similarly, NUR by *L. minor* increased linearly ($p < 0.01$) with higher manure application rates, reaching 422 mg N $m^{-2}$ $d^{-1}$ at 32 mg N $L^{-1}$ (Fig. 2B). The increase in nitrogen uptake corresponded with higher protein content and yield in the *L. minor* biomass as nitrogen levels in the media increased. However, NUE decreased linearly ($p < 0.001$) from 40% at 16 mg N $L^{-1}$ to 22.4% at 32 mg N $L^{-1}$ (Fig. 2C), with the lowest efficiency observed in treatment T10 (17.6%). The N removal and uptake mainly referrer to $NH_4$, the major N form, due to insignificant level of $NO_3$-N in the media (Fig. 1).

# 3.4.1 Factors Affecting Nitrogen Utilization

Nitrogen is primarily assimilated by algae and macrophyte as $NO_3$ and $NH_4$, and processes such as ammonification, nitrification, denitrification, volatilization, and uptake contribute to N removal [36, 41]. In this study, residual water contained only 4.1-11.3% of the N applied, indicating substantial portion was removed through these processes.

The high N removal and uptake rate and efficiency can be attributed to factors such as mixing of the growth media (once per day), anaerobic digestion of diluted manure before application, and optimal growth conditions, including a water temperature (25.4°C) and pH levels 6.5–7.4. These conditions likely enhanced N absorption by *L. minor* and additional N removal mechanisms, including nitrification and denitrification. Similarly, other studies also highlighted the significance of maintaining optimum environmental conditions and treatment of manures and wastewaters to maximize plant growth, nutrient removal and water purification [18, 46].

### 3.4.2 Optimal application rates and nitrogen uptake efficiency

Nutrient uptake efficiency in duckweed system is influenced by the species, initial nutrient concentration, growth rate, temperature, light, and pH [32, 33, 44]. *Lemna mino*r efficiently assimilated N in to biomass at lower N concentration; as N and other ions concentration increases the uptake of these nutrient is retarded due to accumulation of salt (high EC), nutrient imbalance, osmotic stress and ion toxicity [39]. High nitrogen uptake efficiency (40%) was observed at the lowest manure application rates (16 mg N $L^{-1}$), with treatment T5 achieving the highest (47.4%) followed by T2 (41.9%). This efficiency was associated with high growth rates, biomass yield, and optimum growth media quality in these treatments. In contrast, the lowest nitrogen uptake efficiency was recorded for T10 (17.6%), due to high turbidity (61.9 NTU) (Fig. 1E), result in high microbial fermentation, gas bubbles, and froth formation, which interfered with duckweed growth. This indicates that excessive manure application rate might result in undesirable fermentation processes, hence decreasing nitrogen uptake efficiency and biomass production. These findings align with previous studies indicating that *L. minor* is more efficient in utilizing nitrogen for growth at moderate nutrient concentrations [41, 42].

### 3.4.3 Impact of manure type on nitrogen uptake

Among the manure types, PM and PCM supported higher ($p < 0.05$) nitrogen uptake rates (430 and 431 mg N $m^{-2}$ $d^{-1}$, respectively), compared to CM (321 mg N $m^{-2}$ $d^{-1}$), likely due to more favorable nutrient release profiles. The rapid growth of *L. minor* in these media contributed to the differences in nitrogen uptake across treatments. Thus, mixing of PM and CM enhanced the potential of CM to supply adequate nutrients for plant growth. Additionally, nitrogen uptake rates increased with increasing N concentrations in the growth media, confirming that *L. minor* is effective in removing nutrients and accumulating them in its biomass. Results of the study indicated that anaerobic digested PM and PCM can significantly enhance the nutritional value and protein yield of *Lemna minor* when applied at optimal concentrations (16–24 mg $NH_4$-N $L^{-1}$). It boosts protein content and mineral uptake, making duckweed a more nutritious resource as livestock and fish feed. Thus, anaerobic digested manures represents a sustainable nutrient source for duckweed cultivation, aligning with circular economy principles.

## 3.5 Correlation analysis

The Pearson's correlation coefficients in Table 5 reveal significant relationships among physicochemical and nutrient-related parameters. These correlations provide insights into the interactions between water quality parameters, nutrient content, and system productivity.

Table 5
Pearson's correlation coefficients for selected parameters

| Parameters | pH | EC | Turbidity | $DO_2$ | $NH_4$ | $PO_4$ |
|---|---|---|---|---|---|---|
| **Physicochemical characteristics** | | | | | | |
| pH | 1 | 0.14 | 0.45** | 0.51** | -0.12 | -0.34 |
| Conductivity, EC µS $cm^{-1}$ | | 1 | 0.55** | -0.47** | 0.85** | 0.60** |
| Turbidity, NTU | | | 1 | -0.23 | 0.53** | 0.16 |
| Dissolved oxygen-$DO_2$, mg $L^{-1}$ | | | | 1 | -0.57** | -0.43* |
| Ammonium-$NH_4^+$, mg N $L^{-1}$ | | | | | 1 | 0.85** |
| Phosphate-$PO_4$, mg P $L^{-1}$ | | | | | | 1 |
| **Nutrient Content, %DM** | | | | | | |
| Dry Matter | -0.33 | 0.64** | -0.17 | -0.48** | 0.65** | 0.66** |
| Crude protein | -0.23 | 0.80** | 0.18 | -0.48** | 0.84** | 0.85** |
| Ether Extract | -0.05 | 0.26 | -0.18 | 0.15 | 0.23 | 0.50** |
| Crude fiber | 0.28 | -0.81** | -0.26 | 0.67** | -0.82** | -0.71** |
| ME, Mcal $kgDM^{-1}$ | -0.24 | 0.71** | 0.026 | -0.41* | .66** | .71** |
| **Nutrient Yield, g $m^{-2}$ $d^{-1}$** | | | | | | |
| Dry Matter | -0.40* | 0.71** | 0.205 | -0.72** | 0.71** | 0.59** |
| Crude protein | -0.41* | 0.74** | 0.17 | -0.70** | 0.76** | 0.68** |
| Ether extract | -0.34* | 0.61** | 0.004 | -0.46** | 0.59** | 0.65** |
| **Nitrogen Removals** | | | | | | |
| N removal rate (mg N $m^{-2}$ $d^{-1}$) | -0.09 | 0.82** | 0.17 | -0.58** | 0.96** | 0.80** |
| N uptake rate (mg N $m^{-2}$ $d^{-1}$) | − .41* | 0.74** | 0.62** | -0.70** | 0.76** | 0.68** |

**Correlation is significant at $p < 0.01$ (2-tailed); * - Correlation is significant at $p < 0.05$ (2-tailed); ME - Metabolizable energy; EC - Electrical conductivity; $NH_4$-N – Ammonium Nitrogen; $NO_3$ – Nitrate; $DO_2$ – Dissolved oxygen

| Parameters | pH | EC | Turbidity | $DO_2$ | $NH_4$ | $PO_4$ |
|---|---|---|---|---|---|---|
| **Physicochemical characteristics** | | | | | | |
| N uptake efficiency (%) | -0.01 | -0.75** | -0.61** | 0.42* | -0.82** | -0.71** |
| **Correlation is significant at p < 0.01 (2-tailed); * - Correlation is significant at p < 0.05 (2-tailed); ME - Metabolizable energy; EC - Electrical conductivity; $NH_4$-N – Ammonium Nitrogen; $NO_3$ – Nitrate; $DO_2$ – Dissolved oxygen | | | | | | |

## 3.5.1 Physico-chemical characteristics

The pH exhibited a strong positive correlation with $DO_2$, turbidity, and $NO_3$ ($r = 0.50$, $p < 0.01$) indicating that higher turbidity is associated with increase aerobic microbial activity and oxidation of $NH_4$ to $NO_3$ resulting the rise in $NO_3$ and pH levels. Similarly, high turbidity, increase algal and microbial activities, increase $DO_2$ and pH of growth media [18, 20]. However, pH showed a weak negative correlation with $PO_4$, suggesting that alkaline conditions may reduce $PO_4$ availability. Electrical conductivity (EC) demonstrated strong positive correlations with $NH_4$, $PO_4$, and $NO_3$, highlighting the role of EC as an indicator of nutrient concentration. Turbidity showed a positive correlation with $NH_4$, as manure application rate increase to increase $NH_4$ level it also increase turbidity.

## 3.5.2 Nutrient content and Nutrient yield

Dry matter and protein contents were strongly positively correlated with EC, $NH_4$, and $PO_4$, suggesting that nutrient availability mainly N and P enhance biomass accumulation and protein synthesis in duckweed, a finding consistent with previous works [41, 42]. In contrast, crude fiber content was negatively correlated with $NH_4$ and $PO_4$ concentrations, suggesting that N and P application improved nutritional values by increasing the protein content but reducing crude fiber content due to priority for protein synthesis [15, 33]. ME showed positive correlations with EC, $NH_4$, and $PO_4$ signifying the role of growth media nutrient concentrations on nutritive value of duckweed. The yield of biomass, protein and EE were positively correlated with EC, $NH_4$, and $PO_4$. This suggests that N and P availability drives biomass production, lipid accumulation, protein synthesis, and protein yield.

## 3.5.3 Nitrogen removals

Nitrogen removal rate and uptake rate were strongly positively correlated with EC, $NH_4$, and $PO_4$, and $NO_3$. These findings show that duckweed effectively removes high levels of pollutants, such as N and suspended solids from the growth medium. Nitrogen uptake efficiency, however, was negatively correlated with EC, $NH_4$, $PO_4$, and $NO_3$ but positively correlated with $DO_2$. This indicates that while nutrient-rich environments enhance nitrogen uptake, they may reduce efficiency, possibly due to nutrient saturation or imbalances. Generally, results of the present study indicated that, although $DO_2$ has a limited direct effect on duckweed growth and nutritional value, maintaining optimal $DO_2$ concentrations above 2 mg $L^{-1}$ creates a healthier growth environment for consistent duckweed production.

## 3.6 Regression analysis

The polynomial regression analysis revealed robust predictive equations for estimating the biomass yield (BY) and protein content (CP) of *L. minor* based on the physicochemical characteristics of the growth media (Table 6). The performance of prediction models are evaluated using significance level (p value), coefficient of determination ($R^2$) and Standard Error (SE) [14]. The models that demonstrated strong statistical significance ($p < 0.001$), with high $R^2$ values, and low SE were selected. Thus, for biomass yield, Eq. 7, incorporating $NH_4$, achieved an adjusted $R^2$ of 0.87, indicating 87% of the variability in biomass yield was explained by $NH_4$ concentration in the growth media. Similarly, Eq. 8, based on $PO_4$ concentration, showed strong influence of P on biomass yield. For protein content, Eq. 10, using $NH_4$, while Eq. 11, based on $PO_4$, had the highest $R^2$, indicating that P levels were the most influential factor for protein content. Eqs. 9 and 12, incorporating EC and pH, further emphasizing the role of these parameters in determining biomass yield and protein content of *L. minor*. The low standard errors and highly significant levels ($p < 0.001$) across all equations confirm the reliability and precision of the models. The results highlights the critical role of physicochemical parameters particularly EC, pH, $NH_4$ and $PO_4$ in influencing the growth and nutritional quality of *L. minor*.

Table 6
Regression equations for predicting biomass yield and protein content of *Lemna minor*

| Dependent variable | Equation | Eq. | Adj.$R^2$ | SE | F-value | P-value |
|---|---|---|---|---|---|---|
| Biomass yield (g DM $m^{-2}$ $d^{-1}$ | $\ln(BY, g\ DM\ m^{-2}\ d^{-1}) = -3.273*(1/NH_4) + 1.97$ | (7) | 0.87 | 0.186 | 206.6 | < 0.001 |
| | $\ln(BY, g\ DM\ m^{-2}\ d^{-1}) = -0.306*(1/PO_4) + 2.064$ | (8) | 0.84 | 0.203 | 168.7 | < 0.001 |
| | $BY (g\ DM\ m^{-2}\ d^{-1}) = 37.33 + 0.009(EC) - 5.02\ (pH)$ | (9) | 0.74 | 0.89 | 46.37 | 0.001 |
| CP (%DM) | $\ln(CP, \%DM) = 0.156*\ln(NH_4) + 24.028$ | (10) | 0.84 | 0.05 | 167.7 | < 0.001 |
| | $\ln(CP, \%DM) = 0.178*\ln(PO_4) + 36.95$ | (11) | 0.88 | 0.044 | 229.8 | < 0.001 |
| | $CP (\%\ DM) = 84.22 + 0.023\ (EC) - 8.277(pH)$ | (12) | 0.74 | 2.15 | 47.45 | 0.001 |

$NH_4$, Ammonium Nitrogen in mg N $L^{-1}$; $PO_4$ - phosphate in mg P $L^{-1}$; EC – Electrical Conductivity in µS $cm^{-1}$; SE, Standard Error of the estimate; $R^2$, coefficient of determination; Ln, natural logarithm to the base e; n = 33.

These findings have significant practical implications for optimizing the cultivation of *L. minor* for applications in aquaculture, biofuel production, and sustainable feed production. By monitoring and adjusting $NH_4$, $PO_4$, EC, and pH levels, growers can maximize yield and nutritional quality. Recent emerging technologies such as sensor-based monitoring systems [49], and machine learning algorithms [50] could further enhance these predictive models in real-world settings in supporting sustainable cultivation of *Lemna minor* as a resource-efficient crop. However, it is important to note that the models were developed under specific experimental conditions and may require validation across diverse environmental settings.

# 4. Conclusions

The physicochemical characteristics, N and P concentrations in the growth media supported optimal *L. minor* growth, with pH, temperature, and EC within ideal ranges. But excessive manure application reduced nutrient uptake efficiency, and growth media qualities, underlines the need for balanced nutrient management. Anaerobic digested PM, CM, and PCM significantly enhanced the nutritional composition and protein yield of *L. minor*. The PCM based media, particularly T5, exhibited high nitrogen uptake efficiency of 47.4%. Application of manure at 24 mg N $L^{-1}$ maximized nutritional value and protein yield with optimum media water quality. Manure-based duckweed systems offer environmental benefits, including nitrogen removal and water purification; with optimal application rates of 16–24 mg N $L^{-1}$, in addition to frequent harvesting and maintenance of water qualities. Strong correlations between EC, $NH_4$, and $PO_4$ suggest their interconnected roles in nutrient cycling. Polynomial regression models reliably predict *L. minor* biomass yield and protein content, emphasizing its potential as a sustainable biomass and protein source in resource-limited settings.

**Recommendations**

Based on the findings, maintaining nutrient concentrations within the optimal range of 16–24 mg N $L^{-1}$ is recommended to support healthy frond growth, high protein content and yield, and stable water quality. The PCM application at 16 mg N $L^{-1}$ ensures optimal nitrogen uptake and vigorous growth, while organic manures at 24 mg N $L^{-1}$ provide the best nutritional value, and protein yield in *L. minor* cultivation. Exploring large-scale, sustainable systems for *L. minor* production is vital for improving nutrient management and developing high-protein livestock feed.

**Further Study**

Further studies should explore the nutritional composition of *Lemna minor* under various organic fertilization regimes, focusing on amino acid content and nutrient digestibility. Research on mixed manure applications to optimize growth, and feeding trials with poultry could assess duckweed's potential as a soybean substitute. Additionally, evaluating the economic viability of scaling up duckweed cultivation for commercial livestock systems is essential.

# Declarations

### Author Contributions

**H.N**.: conceptualization, investigation, data collection, analysis, writing - original draft preparation, writing - review and editing, funding acquisition. **Y.K.**: writing - review and editing, methodology, supervision, validation. **Y.T.**: writing - review and editing, supervision, methodology, validation. All authors have read and agreed to the published version of the manuscript.

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### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Ethical Statement

The authors confirm that they have adhered to the ethical policies of the journal.

### Conflicts of Interest

The authors declare no conflict of interest.

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### Ethics and Consent to Participate declarations

not applicable

### Ethics and consent to participate

The collection of *Lemna minor* complied with all applicable national and local regulations governing plant collection in Ethiopia. Necessary permissions were obtained from the relevant authorities before sample collection. Fresh plant materials were collected from wetlands near Lake Hawassa (7.05° N, 38.44° E), Lake Ziway (7.99° N, 38.82° E), and Lake Abaya (6.32° N, 37.87° E). Plant specimens were identified by **Dr. Temesgen Dingamo** using standard taxonomic keys. A voucher specimen has been deposited in the **Arba Minch University Herbarium, Arba Minch University, Ethiopia**, under voucher specimen number **AMUH-1436**.

**Consent to publish declaration**

not applicable

**Clinical Trial Registration**

not applicable

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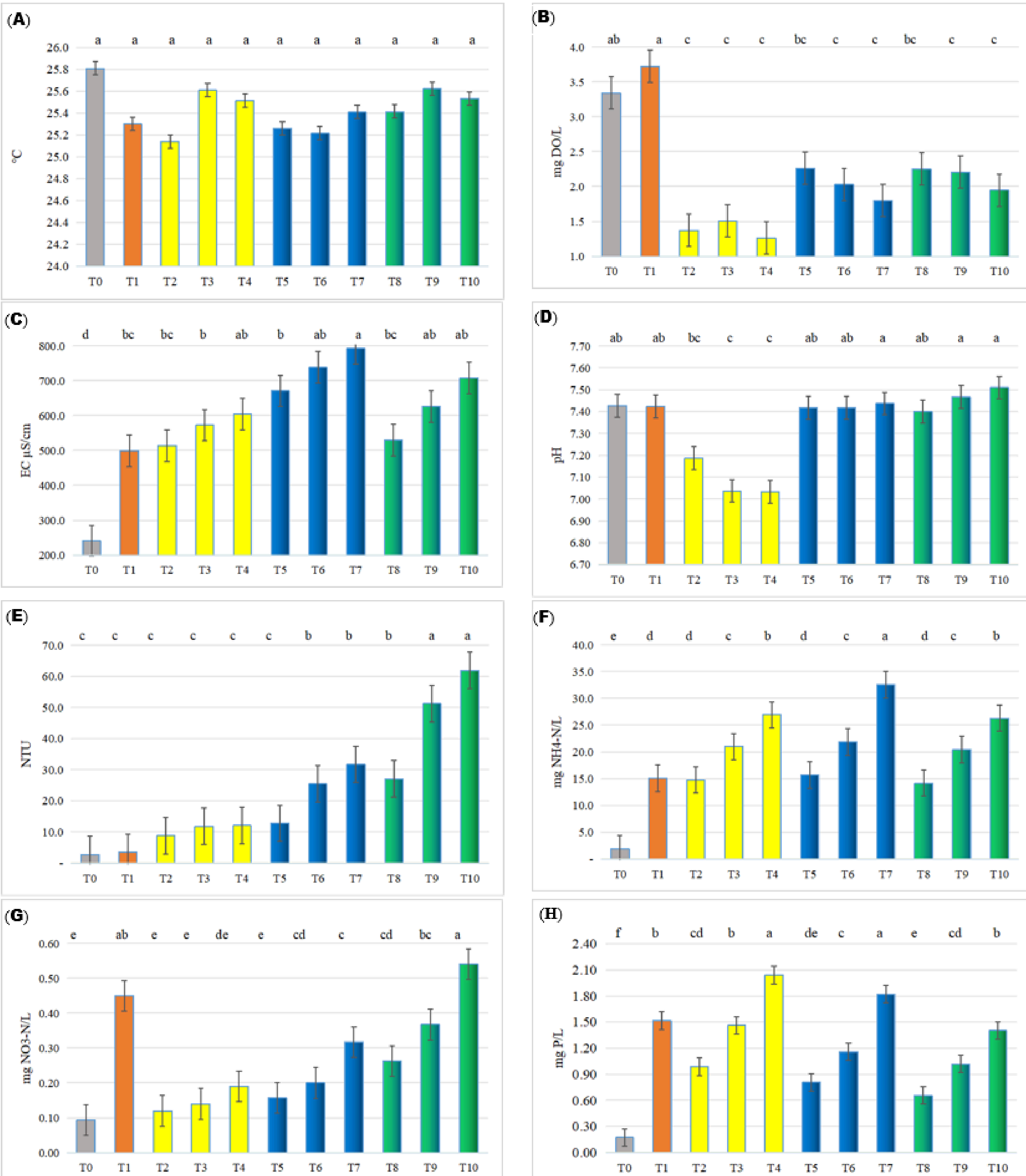
# Figures




## Figure 1

Physicochemical characteristics of growth media as influenced by fertilization treatments; (A) Water Temperature, (B) Dissolved Oxygen, (C) Electrical Conductivity, (D) water pH, (E) Turbidity, (F) Ammonium, (G) Nitrate, (H) Phosphate

**Legend:** T0, no fertilizer; T1 Urea-DAP inorganic fertilizer; T2, T3 and T4 represent PM at 16, 24 and 32 mg N $L^{-1}$, respectively; T5, T6, T7 represent PCM at 16, 24 and 32 mg N $L^{-1}$, respectively; T8, T9 and T10 represent CM at 16, 24 and 32 mg N $L^{-1}$, respectively; each value average of three replications; means with different superscript letters are significantly different $p<0.05$; EC, µS $cm^{-1}$, Micro Siemens per centimeter; NTU, Nitrophelic Turbidity Unit; bar, standard error.

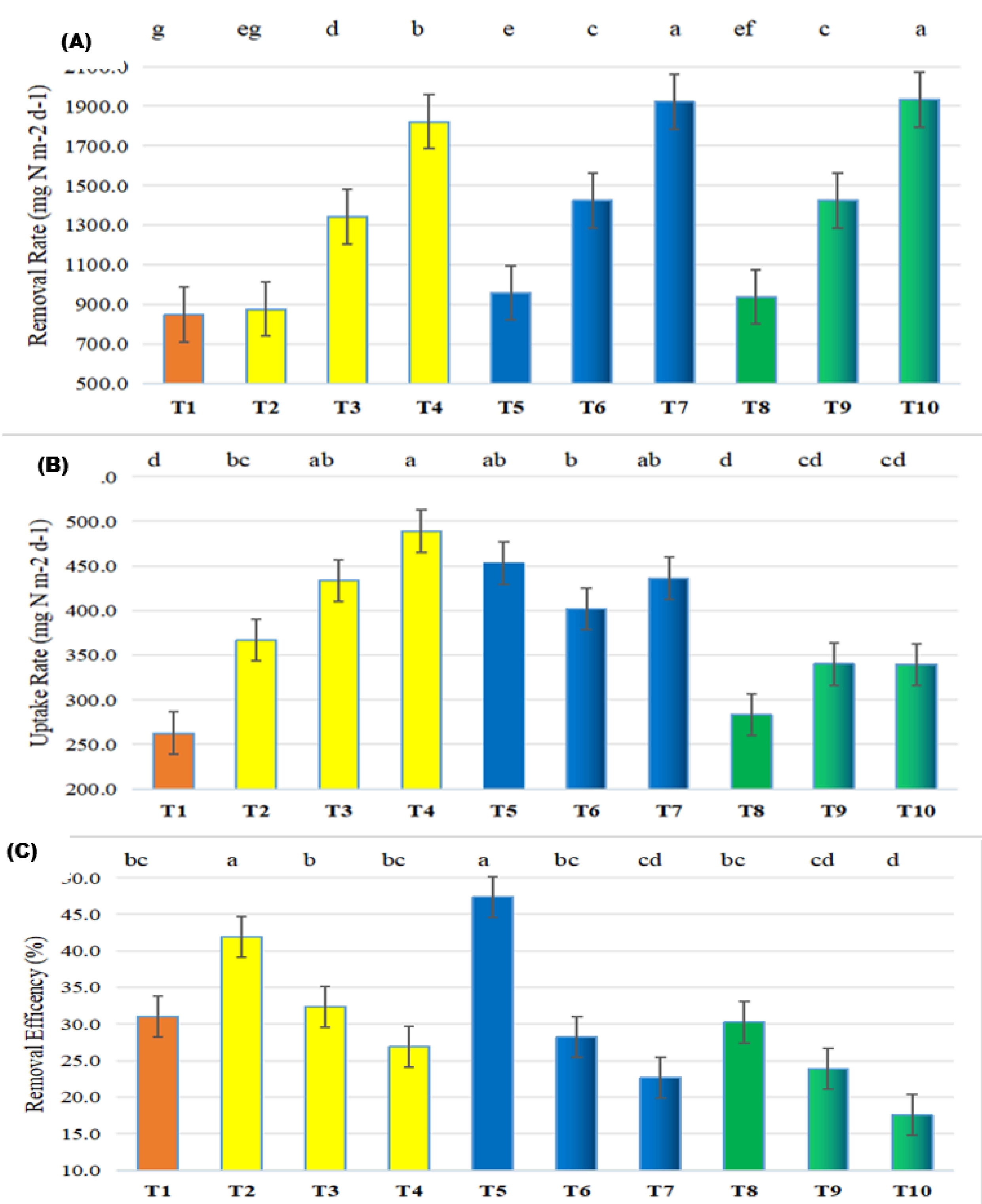



Figure 2

Nitrogen Removal Rate (A) N Uptake Rate (B) and N Uptake Efficiency (C) of *Lemna minor* fertilized by different manure types and application rate; **Legends:** Means (n=3) with different letters significantly different $p<0.05$; bar, standard error; T1, Urea-DAP inorganic fertilizer; T2, T3 and T4 represent PM at 16, 24 and 32 mg N $L^{-1}$, respectively; T5, T6, T7 represent PCM at 16, 24 and 32 mg N $L^{-1}$, respectively; T8, T9 and T10 represent CM at 16, 24 and 32 mg N $L^{-1}$, respectively.