**EFFECT OF *Nymphaea lotus* LEAVES ASH ON SOME PHYSICOCHEMICAL PROPERTIES OF WATER AND GROWTH OF POND ALGAE**

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**ABSTRACT**

| **Aims:** This research focuses on the effect of macrophyte ash on some physicochemical properties of water and the growth of pond algae  **Study design:** Using Randomized Complete Block Design (RCBD).  **Place and Duration of Study:** This study was conducted in the demonstration plot of the Department of Botany and Ecological Studies, Botanical Garden in the University of Uyo, Uyo, between April and June 2024.  **Methodology: M**acrophyte ash treatments (*Nymphaea lotus*) applied at various concentrations (0.6 g/L, 1.2 g/L, 1.8 g/L and 2.4 g/L), arranged in three replicates each and a control. Growth was assessed using different approaches, such as spectrophotometric readings (630, 645 and 660 nm) and dry biomass of cultured algae. Physicochemical properties (pH, turbidity and conductivity) of water quality were also assessed.  **Results:** The study reveals a significant difference at p˂0.05 in the growth of pond algae and water quality factors across all concentrations. *N. lotus* stimulated and enhanced algal production in all concentrations (0.6- 2.4 g/L) throughout the experiment, notably the 2.4 g/L concentration, where maximum growth stimulation was recorded. The study also reveals a similar outcome on physicochemical properties, such as pH (6.70 – 6.75), with no significant difference. Conductivity (0.18 ± 0.04 µS/cm - 1.24 ± 0.09 µS/cm) and turbidity (5.00 ± 0.00 NTU to 128.25 ± 19.30 NTU) showed a significant difference in their p-values (p ≤ 0.05). The values were comparable to World Health Organization (WHO) standard (pH: 6.5–8.5; Turbidity: <25 NTU; Conductivity: 100–2,000 µS/cm).  **Conclusion:** While the ashes of *N. lotus* plant have the potential to be as a nutrient supplement in aquaculture, their application must be carefully managed to avoid negative environmental impacts. |
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*Keywords: [Macrophyte, ash, Nymphaea lotus, physicochemical, spectrophotometric, aquaculture, microalgae.]*

**1. INTRODUCTION**

Aquatic ecosystems, particularly freshwater ponds, are complex and dynamic environments that play crucial roles in biodiversity and biogeochemical cycles. Algae and emergent aquatic macrophytes are key primary producers within these systems, forming the foundation of aquatic food webs (Hilt *et al.,* 2017).

Emergent aquatic macrophytes, such as *Nymphaea lotus,* are rooted plants that grow in shallow waters with their leaves and stems extending above the water surface. These plants play vital roles in aquatic ecosystems, including: Providing habitat and food for aquatic organisms, stabilizing sediments and reducing erosion, filtering pollutants and improving water quality, and competing with algae for nutrients (Villa *et al*., 2020; Rejmankova, 2011; Bamidele and Nyamali, 2008). However, they can also interfere with pond management practices if overgrown (Liu *et al.,* 2020). The interaction between emergent macrophytes and algae is complex and can significantly influence the dynamics of pond ecosystems. Adaptive mechanisms evolved by macrophytes allow them to optimally respond to environmental heterogeneity and inhabit various types of aquatic habitats, including freshwater bodies, watercourses, wetlands, swamps, seasonally flooded areas, as well as brackish and marine environments (Nakayama *et al,* 2017; Rejmánková, 2011; Wetzel, 2001).

In aquaculture ponds, the relationship between algae, emergent macrophytes, and fish production is complex and multifaceted. Algae are a natural food source for many fish species and contribute to oxygen production. Algae, especially phytoplankton, are essential components of pond ecosystems. They contribute significantly to primary production, oxygen generation, and nutrient cycling. However, excessive algal growth can lead to eutrophication, causing water quality issues and ecological imbalances (Wurtsbaugh *et al*., 2019). Also, excessive algal growth can lead to night-time oxygen depletion and off-flavour in fish (Boyd *et al*., 2020). The nutrient dynamics in aquaculture ponds are heavily influenced by fish feed inputs, making these systems prone to eutrophication and algal blooms (Verdegem, 2021; Iribarren *et al,* 2012)

Recent studies have shown that macrophytes can affect algal growth through various mechanisms, including nutrient competition, allelopathy, and alteration of light conditions (Grutters *et al*., 2016). The production of allelochemicals by certain macrophyte species also has a strong impact on phytoplankton (Körner and Nicklisch, 2002; Švanys *et al*., 2014) especially cyanobacteria, making macrophytes a useful tool in cyanobacteria management (Wang *et al*., 2012; Bakker and Hilt, 2016).

This study focuses on the potential effect of macrophyte residues, particularly their ash, on algal growth and water quality of the aquaculture pond system. Ash contains a range of minerals and nutrients that could potentially influence algal growth and water chemistry. The chemical composition of aquatic plant ash can vary depending on the species and environmental conditions. Generally, it contains essential nutrients such as phosphorus, potassium, calcium, and magnesium, as well as trace elements (Zhang *et al*., 2015). These nutrients, when released into the water, could stimulate algal growth. Conversely, the ash might also contain compounds that inhibit algal proliferation.

**2. materials and methods**

**Collection and Identification of Plant Materials**

Fresh and healthy leaves of *Nymphaea lotus* were harvested from a riverbank at Iko Oko Idio, Uyo, Akwa Ibom State, Nigeria, located between 5º2'37''N and 7º57'5''E in January 2024. The sample was taken to the Herbarium of the Department of Botany and Ecological Studies, University of Uyo, Uyo, where it was identified by a Plant Taxonomist, and voucher samples UUH4553 (Uyo) were deposited in the same herbarium. The samples were identified according to the identification protocols of Dutta (2011). The plant samples were harvested using the Hand-harvesting method (Quilliam *et al*., 2015; Madsen, 2000).

**Preparation of Ash**

Using the method of the U.S. Geological Survey (USGS, 2005). The mature aquatic macrophyte plants harvested were washed and dried in the sun and also under shade for one week. (Kumar *et al*., 2016). After drying, 30 g of the dried plant parts were weighed and crushed into small fragments (Napagoda *et al*., 2016; Patil *et al*., 2015).

After drying, the samples were placed in ceramic crucibles and weighed to determine the dry mass. They were then incinerated in a muffle furnace at 550°C for 4 hours, following the USGS standard ignition protocol for total organic matter removal and ash yield determination. This temperature is recommended to prevent the loss of inorganic nutrients by volatilization while ensuring complete combustion of organic matter (Heiri et al., 2001; USGS, 2005).

After combustion, the ash was cooled in a desiccator to avoid rehydration, then ground with a ceramic mortar and pestle and sieved through a 0.5 mm mesh to obtain fine ash particles. The final ash was stored in labelled airtight containers until used in water treatment experiments.

**Experimental Design**

The open pond system was used. Fifty-one (51) plastic buckets with a capacity of 20 litres each were filled with 12 litres of distilled water, which was obtained from the University of Uyo’s main campus.

**Treatment/Setup**: Four different concentrations of ash of the plant samples, 0.6 g, 1.2 g, 1.8 g, and 2.4 g, were each applied to plastic buckets containing 12 litres of water. After dilution, the 12-litre solution was then evenly distributed into three (3) replicates (4-litre solution). The 3 replicates were made up with 8 litres of water, to obtain 12 litres each. A control made of distilled water was also used. The buckets were placed in an open space in the demonstration plot for inoculation. The buckets were arranged in a Randomized Complete Block Design (RCBD), the experiment was monitored every six (6) days for one (1) month.

**Determination of physicochemical parameters**

Three key physicochemical parameters, viz, pH, conductivity and turbidity of the system, were estimated every 6 days for 18 days. Standard methods were followed as described by APHA (2017) and other established protocols

**pH:** The pH of the water samples was measured using a calibrated digital pH meter. The pH meter was first calibrated with standard buffer solutions (pH 4.0, 7.0, and 10.0) according to the manufacturer’s instructions. Samples were gently stirred before insertion of the probe, and readings were allowed to stabilize before being recorded. All measurements were taken at room temperature (~25°C) and performed in triplicate for accuracy (APHA, 2017).

**Conductivity**: Electrical conductivity, which reflects the ionic strength of the water, was measured using a digital conductivity meter. The instrument was calibrated using standard KCl solutions before use. Water samples were placed in clean beakers, and the electrode was immersed in 30 ml of water sample contained in a clean beaker. The results were expressed in microsiemens per centimetre (µS/cm) (APHA, 2017; Wetzel, 2001).

**Turbidity:** Turbidity was measured using a nephelometric meter, expressing turbidity in Nephelometric Turbidity Units (NTU). The device was calibrated with standard turbidity solutions before use. Water samples were gently shaken to resuspend any settled particles before measurement. Each reading was taken in triplicate, and the average was reported (Boyd, 1990; APHA, 2017).

**Algal Growth Assessment Method**

**Spectrophotometric**: Algal growth was measured spectrophotometrically at six-day intervals. Aliquots of 30 ml were collected every six days from each concentration. These were then subjected to spectrophotometric reading at three different wavelengths (630, 645 and 660 nm) according to Lichtenthaler and Wellburn (1983) (Hulkko *et al.,* 2022)

Total Algal growth was then estimated using the formula

Chlorophyll a (mg/L) = 11.64 D660-2.16 according to Lichtenthaler and Wellburn (1983) (Hulkko *et al.,* 2022). Where D630, D645, and D660 are absorbance at 630, 645 and 660 nm.

**3. results and discussion**

**Growth Response of Microalgae by Days**

The results presented in Table 1 show the effect of varying concentrations of *Nymphaea lotus* ash on algal growth over 18 days, with growth estimated every six days. A significant variation in algal growth was observed across the different treatments, as indicated by the p-values (p ≤ 0.05) on Days 6, 12, 18, and for the overall means.

There was no observable algal growth in any treatment, including the control, in the first few days (0-4), suggesting that *Nymphaea lotus* ash had no immediate stimulatory or inhibitory effect within the time under review. This may be attributed to the time lag typically required for algal acclimatization and response to environmental changes (Wetzel, 2001).

Significant differences in algal growth were evident (*P <* 0.001) at Day 6 of the study. The control showed minimal growth (0.06 ± 0.02), while treatments with ash displayed a dose-dependent increase in algal growth. The 2.4 g/L ash concentration resulted in the highest growth (1.03 ± 0.13), followed by 1.8 g/L (0.51 ± 0.07). This suggests that the ash may have released growth influence into the water, which is known to promote algal proliferation (Smith *et al*., 1999; Maúre *et al.,* 2021).

At Day 12, a sharp increase in algal growth was observed across all ash treatments, with values of 5.00 ± 0.00 in the 1.2 g/L, 1.8 g/L, and 2.4 g/L treatments. The control remained significantly lower (0.57 ± 0.08), confirming the ash’s stimulatory effect. This dramatic increase could be attributed to the cumulative growth influence, which led to enrichment over time (Reynolds, 2006).

At Day 18, 2.4 g/L concentration maintains high algal growth (5.00 ± 0.04), while 1.2 g/L and 1.8 g/L also recorded similarly high values (4.58 ± 0.04 and 3.15 ± 0.04, respectively). Interestingly, the 0.6 g/L treatment resulted in relatively high algal biomass (4.37 ± 0.04), showing that even at lower concentrations, the ash had a lasting effect on promoting growth. The control treatment, in contrast, showed minimal growth (0.66 ± 0.04), further emphasizing the influence of N. lotus ash in the algal growth.

The overall mean values reinforce these observations, with significant differences (*P* = 0.049) between the control (0.32 ± 0.09 mg/L) and all treatments containing *N. lotus* ash (2.21- 2.76 mg/L). The highest mean algal growth was observed in the 2.4 g/L treatment (2.76 ± 0.69), indicating that higher concentrations of the ash had a more pronounced and sustained impact on algal proliferation. These findings are consistent with previous studies indicating that plant ash can act as a fertilizer by releasing macro- and micronutrients into aquatic environments (Asaolu *et al*., 2010; Kalff, 2002).

However, the reduction of growth in 1.8 g/L after Day 12 (from 5.00 to 3.15 mg/L) suggests possible nutrient saturation or onset of self-shading due to excessive algal biomass, which is a common feedback mechanism in eutrophic systems (Anderson *et al*., 2002).

**Table 1. Growth Response of Microalgae According to Age of Culture**

| **Ash Concentration** | **Day 1** | **Day 6** | **Day 12** | **Day 18** | | **Overall** |
| --- | --- | --- | --- | --- | --- | --- |
| Control (no ash) | 0.00±0.00 | 0.06±0.02d | 0.57±0.08c | 0.66±0.04c | 0.32±0.09b | |
| 0.6 | 0.00±0.00 | 0.33±0.02bc | 4.13±0.52b | 4.37±0.04b | 2.21±0.64a | |
| 1.2 | 0.00±0.00 | 0.18±0.03cd | 5.00±0.00a | 4.58±0.04a | 2.44±0.72a | |
| 1.8 | 0.00±0.00 | 0.51±0.07b | 5.00±0.00a | 3.15±0.04a | 2.16±0.62a | |
| 2.4 | 0.00±0.00 | 1.03±0.13a | 5.00±0.00a | 5.00±0.04a | 2.76±0.69a | |
| **Total** | **0.00±0.00** | **0.42±0.09** | **3.94±0.47** | **3.55±0.04** | **1.98±0.28** | |
| p Value | NA | <.001\* | <.001\* | <.001\* | .049\* | |

*NA – Not applicable, ns – Not significant at P>0.05, \* - Significant at P≤0.05.*

**Spectrophotometric Estimation of Growth of Microalgae**

Table 2 presents the growth response of microalgae measured at three different light wavelengths (630 nm, 645 nm, and 660 nm) under varying concentrations of *Nymphaea lotus* ash. The wavelengths correspond to absorption peaks typically associated with chlorophyll a and b, which are essential pigments in photosynthesis (Lichtenthaler, 1987).

Across all concentrations, microalgal growth increased in ash-treated groups compared to the control, suggesting that *Nymphaea lotus* ash positively influences algal proliferation. However, the statistical analysis reveals no significant differences among high concentrations (1.8 and 2.4 g/L) as the culture aged, as seen in days 6 to 18. This indicates that while ash enhances overall growth, the light absorption response of chlorophyll pigments does not significantly differ with an increase in ash concentration. The absence of significance could be due to the large standard deviations, implying variability within replicates (Kalff, 2002).

The overall mean growth across all wavelengths showed a statistically significant difference among treatments (*P* = .049), with the 2.4 g/L treatment enhancing the highest algal growth (2.76 ± 0.69 mg/L). This suggests that *N. lotus* ash application significantly enhances algal production.

The control group maintained relatively low absorbance values across all wavelengths (mean = 0.32 ± 0.09), consistent with limited algal biomass. In contrast, treatments from 0.6 to 2.4 g/L consistently recorded higher values, indicating increased algal density due to nutrient enrichment. Plant ash is known to contain minerals such as phosphorus, calcium, magnesium, and potassium, which can stimulate algal growth when released into aquatic systems that nutrient enrichment can lead to increased pigment content in microalgae, enhancing light absorption and growth (Reynolds, 2006; Asaolu *et al*., 2010; Smith *et al*., 1999).

**Table 2. Spectrophotometric Estimation of Growth of Microalgae**

| **Ash Concentration** | **630** | **645** | **660** | **Overall** |
| --- | --- | --- | --- | --- |
| Control (no ash) | 0.35±0.20a | 0.28±0.15a | 0.34±0.17a | 0.32±0.09b |
| 0.6 | 2.37±1.30a | 1.67±0.86a | 2.58±1.40a | 2.21±0.64a |
| 1.2 | 2.54±1.42a | 2.25±1.26a | 2.54±1.42a | 2.44±0.72a |
| 1.8 | 2.26±1.19a | 1.95±1.15a | 2.29±1.19a | 2.16±0.62a |
| 2.4 | 2.70±1.34a | 2.81±1.29a | 2.77±1.31a | 2.76±0.69a |
| **Total** | **2.04±0.51** | **1.79±0.45** | **2.10±0.52** | **1.98±0.28** |
| p Value | .628ns | .522ns | .604ns | .049\* |

*NA – Not applicable, ns – Not significant at P>0.05, \* - Significant at P≤0.05.*

**Effect of *N. lotus* Ash on Physicochemical Parameters**

Table 3 shows the influence of varying concentrations of *Nymphaea lotus* ash on three physicochemical parameters of water—pH, turbidity, and electrical conductivity.

**pH**

The pH values ranged from 6.70 to 6.75 across all treatments, with no statistically significant differences (*P* = 0.544). These values indicate a slightly acidic to near-neutral water environment. The minimal variation in pH suggests that the ash from *N. lotus* did not significantly alter the hydrogen ion concentration in the water. This may be due to a buffering capacity in the water or the weakly alkaline/neutral nature of the ash itself (Wetzel, 2001). Similar findings have been reported in studies where plant ash showed little or no effect on pH in moderately buffered aquatic systems (Asaolu *et al*., 2010). Also, for most freshwater organisms, a pH of 6.5 to 9.0 is considered optimum (Boyd, 1990; Hulkko *et al.,* 2022). Turbidity increased with an increase in ash concentration (*P <* 0.001), indicating a clear dose-dependent effect. The control exhibited the lowest turbidity (5.00 ± 0.00 NTU), while the highest turbidity was recorded at the 2.4 g/L *N. lotus* ash concentration (128.25 ± 19.30 NTU). Turbidity reflects the presence of suspended particles in the water, which in this case likely consists of undissolved ash particles (Reynolds, 2006). The significant increase at higher N. lotus ash concentrations suggests that *Nymphaea lotus* ash may act as both a direct contributor to particulate matter and an indirect stimulator of algal growth through nutrient release (Smith *et al*., 1999).

**Electrical Conductivity**

Conductivity also increased significantly with rising N. lotus ash concentration (*P <* 0.001), ranging from 0.18 ± 0.04 µS/cm in the control to 1.24 ± 0.09 µS/cm at 2.4 g/L N. lotus ash concentration. Electrical conductivity is a measure of the water's ability to conduct electricity, which increases with higher concentrations of dissolved ions. This result supports the hypothesis that *Nymphaea lotus* ash release nutrients such as potassium, calcium, magnesium, and phosphate into the water, enhancing ionic concentration (Kalff, 2002; Asaolu *et al*., 2010). The elevated conductivity values at higher N. lotus ash levels align with previous studies indicating that plant-derived ashes can significantly increase water salinity due to mineral dissolution (Anderson *et al*., 2002).

Environmental Implications

These physicochemical changes, particularly in turbidity and conductivity, have ecological consequences. Elevated turbidity can reduce light penetration, potentially affecting photosynthesis in submerged aquatic plants ( Zhou *et al*., 2020). Meanwhile, increased conductivity and nutrient release can stimulate algal growth, leading to eutrophication if uncontrolled (Smith *et al*., 1999; Maúre *et al.,* 2021). Therefore, while *Nymphaea lotus* ash has potential as a nutrient source in aquatic systems, its use must be carefully managed to avoid negative environmental impacts.

**Table 3. Effect of *N. lotus* Ash on Physicochemical Parameters** 

| **Concentration/Microalgae** | **Ph** | **Turbidity** | **Conductivity (µS/cm)** |
| --- | --- | --- | --- |
| Control | 6.73±0.02a | 5.00±0.00c | 0.18±0.04c |
| 0.6 | 6.75±0.03a | 64.25±10.14b | 0.29±0.01bc |
| 1.2 | 6.75±0.03a | 70.00±5.24b | 0.44±0.05b |
| 1.8 | 6.70±0.00a | 93.75±9.73b | 1.07±0.08a |
| 2.4 | 6.75±0.03a | 128.25±19.30a | 1.24±0.09a |
| **Total** | **6.74±0.01** | **72.25±10.25** | **0.65±0.10** |
| p Value | .544ns | <.001\* | <.001\* |
|  |  |  |  |

*NA – Not applicable, ns – Not significant at p>.05, \* - Significant at p≤.05.*

**4. Conclusion**

The study demonstrates that *Nymphaea lotus* ash significantly enhances the growth of pond algae. Hence, its ash has potential as a nutrient supplement in aquaculture or algal cultivation, their application must be carefully managed to avoid negative environmental impacts, while stimulation of algal growth can be beneficial for aquaculture, excessive stimulation may lead to eutrophication. The significant increases in turbidity and conductivity with higher concentrations of *N. lotus* ash suggest that the ash does not strongly affect pH, but influences the turbidity and conductivity of water. These changes could potentially affect aquatic life, particularly in ecosystems where clear water and low conductivity are important for the health of aquatic organisms.

**Competing interests**

Declaration of competing interest should be placed here. All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If no such declaration has been made by the authors, SDI reserves to assume and write this sentence: “Authors have declared that no competing interests exist.”.

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Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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