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Characterization and Environmental Condition of Local *Wolffia* sp. Found in Uttaradit Province

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Abstract: Khai-Phum is a diminutive floating flowering plant that contains highly nutritious, high-protein, and essential amino acids but has a significant capacity to absorb environmental pollutants. This study examined natural Khai-Phum sources in Uttaradit Province and collected morphological data on Khai-Phum from natural and aquaculture situations. The data were used to choose a Khai-Phum source for water quality assessment, and the physical ecology and quality of the water were studied. It was found that all Khai-Phum samples were classified as *Wolffia globosa*. The water from NK, TR, and BD sources indicates the most suitable quality for the growth of Khai-Phum, with quality factors (pH, DO, and TKN) influencing the growth of Khai-Phum. However, pH, alkalinity, EC, TP, and TKN are structurally related to Khai-Phum dry weight and stomatal density.

Keywords: *Wolffia*; Khai-Phum; human food; water quality

1. Introduction

The global human population currently stands at 8 billion, reflecting an increase of approximately 1.5 billion over the past thirty years [1]. This increase in people has resulted in a significant demand for nutrient intake, particularly protein, which is predominantly sourced from animals and is costly for specific demographic groups. Moreover, individuals with constraints on meat consumption, including patients with animal protein allergies, those with limited meat intake, or vegetarians, face a considerable challenge in acquiring adequate protein to fulfill the body's requirements. Consequently, the utilization of alternative proteins derived from plants is particularly intriguing, given that the plant production process is straightforward and uncomplicated, and plants serve as a source of numerous vitamins and bioactive compounds advantageous to human health. Several duckweed species possess elevated concentrations of protein and essential amino acids and are currently utilized as ingredients in the production of dietary supplements for humans, including *Lemna* [2] and *Wolffia* [3-5].

Wolffia globosa, commonly referred to as "water meal", is a member of the Lemnaceae family of duckweed. It is a diminutive floating flowering plant, measuring 0.5 to 1.0 mm. It possesses leaf-like structures known as fronds and lacks roots. Water meal is locally called "Khai-Phum", "Phum" or "Khai-nam" in Thailand. It serves as an ingredient in traditional dishes rooted in local knowledge, especially in northern and northeastern Thailand, where it is highly nutritious, comprising up to 45.54% protein and essential amino acids such as threonine, arginine, and leucine [6]. Furthermore, it encompasses phytochemicals

exhibiting biological activity and advantages for human health, including antioxidant compounds [7], beta-carotene, ferulic acid, luteolin-7-O-β-D-glucoside [8], lutein, and sitosterol [9]. Khai-Phum possesses the potential to serve as raw materials for products across diverse industries, including dietary supplements, plant-based snacks, alternative proteins, and animal feed [3-4].

Factors influencing the growth of Khai-Phum encompass the purity of the water source, optimal light intensity and temperature, and sufficient nutrient availability. Khai-Phum is a highly nutritious aquatic plant suitable for consumption; however, it is crucial to understand that it is a floating organism with a significant capacity to absorb environmental pollutants, including toxins and heavy metals [10]. Fortunately, the Khai-Phum source is located near agricultural regions utilizing pesticides or near wastewater discharges from households or industries; it may absorb chemicals, heavy metals, or various toxins, accumulating them in its tissues in substantial quantities, which poses a risk to consumer health.

Uttaradit Province is in the lower northern part of Thailand. The topography features a central river basin resembling plains, linked to the intricate mountains of the northern region, yielding a comparatively diverse ecosystem. It possesses diverse natural water sources, including substantial reservoirs formed by dams on the Nan River, resulting in the creation of backwater regions. Medium and small reservoirs are present throughout the irrigated regions of Uttaradit Province and its adjacent areas. These encompass immobile water sources, including natural ponds and swamps, as well as artificial water bodies such as ponds and both small and large reservoirs. Uttaradit Province is located in a tropical monsoon climate zone marked by persistently elevated temperatures, conducive to the cultivation of Khai-Phum.

Nevertheless, the use of Khai-Phum from natural water sources encounters numerous challenges, notably the lack of control over its production and quality. This could result in Khai-Phum not adhering to food quality and nutritional standards during commercial production and exportation, such as the General Standard for Contaminants and Toxins in Food and Feed [11]. Surveying Khai-Phum in Uttaradit Province will therefore provide a wealth of information about its ecological characteristics, especially environmental factors related to geographical uniformity, which may indicate different strains of the bacterium. Finding plenty of Khai-Phum sources could improve farmers' financial prospects by making it simpler to establish and expand commercial Khai-Phum farming in the region. Data collection will also aid in assessing Khai-Phum's standing in the region and promote the conservation of its genetic resources, which are crucial for maintaining ecological balance and ensuring food security. Therefore, the water quality in these sources is most suitable for the growth of Khai-Phum. The researchers selected water from these sources for further water quality analysis.

2. Materials and Methods

This research selected Uttaradit Province as its focus, noted for its diverse natural water sources attributable to its position in the lower northern region. The terrain consists of a central plain linked to the intricate northern mountains. The procedural steps of the study are as follows:

Investigate and gather morphological characteristics data on Khai-Phum from natural habitats and aquaculture farms by conducting interviews with Khai-Phum vendors in Uttaradit Province, encompassing local markets, weekly farmers' markets, online platforms on social media, and agricultural networks. Obtain Khai-Phum and water samples from the identified sources from December, 2024 to March, 2025, and transport them to the laboratory promptly on the day of collection. A morphological examination of Khai-Phum was performed by randomly sampling for characteristics such as frond size and stomatal count, with 30 replicates per site. The morphology of Khai-Phum was analyzed with a light microscope (Olympus, Japan) to assess size, stomatal count, characteristics (shape, form, and color), and additional features (flowers) at a total magnification of 40–400 times, comparing the findings with the reference literature [12-16]. The remaining Khai-Phum samples were cultured in 8-ounce tissue culture bottles with 1 g of Khai-Phum in ½ Schenk & Hildebrandt (SH) medium supplemented after being rinsed in purified water [14]. The samples were kept in a naturally lit laboratory with an average midday light intensity of 301.29±29.44 lux. Loosen the bottle cap slightly to facilitate air exchange. Maintain an average room temperature of 29±2 °C for a duration of 7 days to reassess the morphology. The data were utilized to identify a Khai-Phum source appropriate for subsequent water quality analysis.

An investigation into the physical ecology of aquatic environments inhabited by Khai-Phum was performed by collecting water samples from locations where Khai-Phum were present in surface water resources randomly throughout the region (grab sample) using 1-liter polyethylene (PE) bottles. The samples were preserved in accordance with the specified analytical parameters, and water quality analysis was conducted as detailed below: temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), alkalinity, dissolved oxygen (DO), total phosphorus (TP), and total Kjeldahl nitrogen (TKN). As per the established protocols outlined in the Standard Methods for the Examination of Water and Wastewater, 24th edition [17]. The temperature, pH, EC, and TDS were measured on-site using a thermometer, pH meter (Mettler Toledo model F20), and TDS and EC meter (Eutech model CON450), respectively. Other parameters (DO, alkalinity, TKN, and TP) were measured immediately after the samples were transported to the laboratory. Details are presented in Table 1.

Table 1. Quality Index for Inspected Water and Analysis Method

Indicators	Unit	Method
Temperature	°C	Thermometer
pH	-	Electrometric method (pH meter)
EC, TDS	mS	Conductivity meter
DO	mg/L	Azide modification
TKN	mg/L	Digestion, distillation and titration
TP	mg/L	Sulfuric acid – nitric acid digestion

The morphological variation among sampling sites was demonstrated with a One-way ANOVA followed by Tukey's HSD test. Examine variance and contrast the mean differences utilizing multivariate statistics through Spearman's rank correlation to assess the relationship between source characteristics and water quality variables.

3. Results and Discussion

3.1 Source of Khai-Phum

With a focus on community use, this study investigates natural Khai-Phum sources in Uttaradit Province. Khai-Phum is used for personal consumption by locals, who also sell it at local and nearby markets. At present, there are no documented surveys or findings regarding Khai-Phum in Uttaradit Province, nor is there definitive information concerning the origins of Khai-Phum distribution. Several possibilities exist, including individuals within the community or those near Khai-Phum sources introducing Khai-Phum from external origins for cultivation, resulting in inadvertent dissemination during the flood season, or the natural occurrence of Khai-Phum in the area. The survey identified five sources of Khai-Phum. All locations where Khai-Phum was discovered were earthen ponds, and the Khai-Phum from these locations has been utilized for a duration ranging from 2 to over 10 years. The majority of locations where Khai-Phum was discovered are public areas.

3.2 Characteristics of Khai-Phum

Following an analysis of the floating plant species identified within Uttaradit Province, the investigation revealed that all collected samples exhibited the following attributes: Fronds are leaf-like structures, predominantly round to oval in shape, with a greater portion of the plant submerged beneath the water's surface than exposed above it. The frond edges are translucent, the central region is opaque, and there are no roots (Figure 1). One aspect of the frond features a structure with a circular cavity known as the reproductive pocket (RP). Budding occurs at the center of the cavity, resulting in the formation of new plants (daughter fronds; DF) (Figure 2).

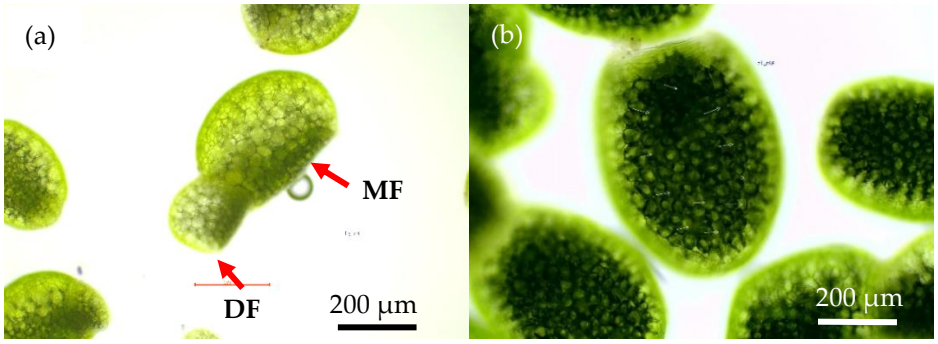


Figure 1. General appearance of the lateral part where a new frond (daughter frond; DF) is attached to the original frond (mother frond; MF) (a) and the dorsal part (b) of the Khai-Phum, magnified 40 times.

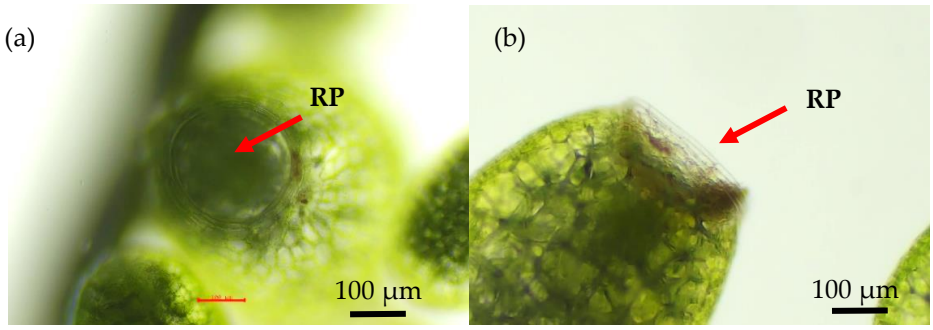


Figure 2. Reproductive pocket (RP of the frond, lateral view (a) and top view (b), magnified 100 times

Comparing the characteristics of the Khai-Phum fronds found in Uttaradit Province, it was found that all specimens have intensely green fronds that are nearly round to oval in shape, with a convex dorsal surface, an average width of 0.6 mm, an average length of 0.8 mm, and stomata found only on the dorsal surface and arranged in 2-3 rows along the length, with 5-20 stomata. Khai-Phum from the BD source exhibits the highest average stomatal density at 20.75±0.50 units/frond, which is significantly different ($p<0.05$) from all other Khai-Phum sources. The length-to-width ratio (L/W ratio) indicates the frond's shape, with fronds with a low L/W ratio (close to 1) being more rounded compared to those with a high L/W ratio. The length-to-width ratio of the fronds varies from 1.26 to 1.51 (Table 2). According to Khai-Phum, propagation occurs by the generation of new fronds (daughter fronds, DF) from the original fronds (mother fronds, MF) (Figure 3a) while the flower is present in the middle of the frond (Figure 3b). From this study, when considering the physical characteristics, Khai-Phum with large fronds (low L/W ratio) has a higher stomatal density than it does with small fronds (high L/W ratio). Stomata regulate transpiration and respiration and are crucial structures for water and mineral transport in plants. Stomatal density increases with plant development [18]. Additionally, Khai-Phum from NK, TR, TD, and BD sources has high dry weights (4.75, 4.46, 4.80, and 4.20% of fresh weight, respectively), indicating greater growth compared to Khai-Phum from other sources.

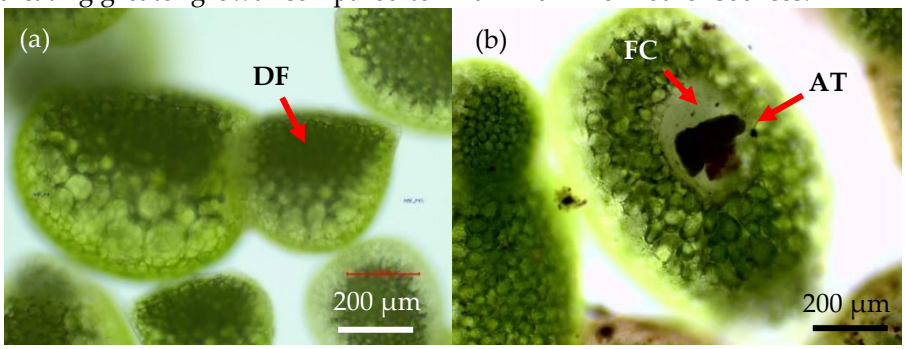


Figure 3. Characteristics of Khai-Phum with a daughter frond; DF (a) attached to the Khai-Phum at the flowering stage, showing anther; AT (b) inside the floral cavity; FC in the middle of the frond.

The morphology of the Khai-Phum species revealed significant differences in frond width among sampling sites ($p<0.05$). The TD population exhibited significantly greater frond width than WY, whereas the remaining populations did not differ significantly. Dry weight was also significantly different among sites ($p<0.001$), with TD, NK, and TR forming the highest statistical group, BD forming an intermediate group, and WY exhibiting the lowest biomass. Stomatal density differed markedly among locations ($p<0.001$), with BD showing significantly higher stomatal density than all other sampling sites, while NK, TR, WY, and TD did not differ significantly.

Table 2. Physical characteristics of Khai-Phum in Uttaradit Province (N = 30)

Sources	Length (mm)	Width (mm)	L/W ratio (fold)	Dry weight (%)	Stomatal density (Unit/frond)
NK	0.65 ± 0.09^{a}	0.51 ± 0.07^{ab}	1.29 ± 0.03^{a}	4.75 ± 0.13^{a}	11.20 ± 1.89^{b}
TR	0.63 ± 0.09^{a}	0.50 ± 0.04^{ab}	1.26 ± 0.05^{a}	4.46 ± 0.10^{a}	9.70 ± 1.86^{b}
WY	0.66 ± 0.08^{a}	0.43 ± 0.05^{b}	1.51 ± 0.10^{a}	3.78 ± 0.05^{c}	9.40 ± 2.30^{b}
TD	0.78 ± 0.15^{a}	0.58 ± 0.09^{a}	1.34 ± 0.08^{a}	4.80 ± 0.15^{a}	9.67 ± 1.53^{b}
BD	0.62 ± 0.12^{a}	0.52 ± 0.06^{ab}	1.36 ± 0.16^{a}	4.20 ± 0.05^{b}	20.75 ± 0.50^{a}

Note: Mean value±Standard error; letters in the same column. There is no significant difference ($p<0.05$).

The external morphological classification of Khai-Phum may be inadequate for definite species identification, as *Wolffia* is a small floating plant exhibiting overlapping morphological traits among species. Tissue complexity is diminished, and essential plant parts are absent, including roots, stems, shoots, fruits, and seeds. This study classifies the morphology of Khai-Phum from diverse sources in Uttaradit Province as *Wolffia globosa*. The morphology of all Khai-Phum specimens corresponds with prior investigations, displaying the following traits: Fronds are non-lustrous, pale green to strongly green with prominent translucent margins at the apex, predominantly possessing fewer than 20 stomata [13]. Stomata were primarily located in the top epidermis (dorsal side), while chloroplasts were present in both the mesophyll cells and the epidermis [14]. This contrasts with the morphology of *Wolffia arrhiza*, another species of Khai-Phum located in Thailand [19-20], which has a similar shape [15]. The principal characteristics are as follows: the frond is rather spherical and may exceed 1 mm in size [16] and possesses a flat dorsal surface with stomata arranged in 4-5 rows [12] from the dorsal to the lateral parts [15].

3.3 Water quality

The analysis of water quality derived from the Khai-Phum source encompasses this. The study intends to compare the chemical and physical parameters of water and investigate the correlation between environmental factors and Khai-Phum growth, utilizing key variables such as temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), alkalinity, dissolved oxygen (DO), total phosphorus (TP), and total Kjeldahl nitrogen (TKN), which are also included.

The water temperature was found to range between 32.5 °C and 33.5 °C based on water quality data collected from five sampling sites: Site 1, Na Nok Kok Subdistrict (NK); Site 2, Hat Song Khwae Subdistrict (TR); and Site 3, Ban Dan Subdistrict (BD). Khai-Phum grows best in the temperature range of 25 to 35 °C. An electrical conductivity (EC) between 101.06 and 236.60 μS/cm indicates low conductivity and few dissolved ions; this type of water is typically found in freshwater sources with minimal salt or chemical fertilizer buildup [21-22]. The BD source with the lowest EC value (101.06 μS/cm) has better water quality than the other sources and the pH value is between 6.0 and 7.0. Khai-Phum thrives in a pH range of 5.5 to 8.0, so it is neutral to slightly acidic and encourages nutrient absorption [23]. Total phosphorus (TP) concentrations were highest at the NK site, suggesting nutrient enrichment from either agricultural phosphate solutions or nearby organic sources. The range of TP concentrations was 0.29 to 1.37 mg/L. The WY site had the lowest TP value (0.13 mg/L), indicating a lack of essential nutrients that might limit the growth of Khai-Phum in this area. Total Kjeldahl nitrogen (TKN) concentrations ranged from 0.57 to 5.40 mg/L, with the NK site exhibiting the highest value, signifying a substantial presence of organic matter in the water (Table 3).

Table 3. Water quality from the Khai-Phum source

Water quality indicators	Unit	Sources				
		NK	TR	WY	TD	BD
Temperature	°C	33.50 ± 0.0	32.50 ± 0.5	30.30 ± 1.1	30.50 ± 1.0	33.00 ± 0.3
pH		6.50 ± 0.3	7.00 ± 0.0	6.52 ± 0.2	6.55 ± 0.5	6.00 ± 0.5
EC	μS/cm	146.00 ± 20.5	165.00 ± 0.7	192.15 ± 0.9	236.60 ± 0.5	101.06 ± 1.4
TDS	ppt	35.50 ± 2.9	71.50 ± 6.2	119.80 ± 0.6	147.30 ± 0.2	66.50 ± 9.1
Alkalinity	mg/L	170.00 ± 4.0	136.00 ± 8.1	114.60 ± 6.0	153.00 ± 4.0	68.00 ± 2.0
DO	mg/L	4.70 ± 1.9	4.00 ± 1.0	1.48 ± 0.3	1.05 ± 0.4	3.80 ± 1.7
TP	mg/L	1.37 ± 0.1	0.29 ± 0.1	0.13 ± 0.1	0.53 ± 0.1	1.23 ± 0.1
TKN	mg/L	5.40 ± 0.2	4.90 ± 0.3	0.57 ± 0.2	2.20 ± 0.9	5.00 ± 0.6

Note: Mean value±Standard deviation

The water quality measurements at the five sites revealed that site BD recorded the lowest EC value of 101.06 μS/cm, signifying a reduced concentration of dissolved substances in the water. The water pH ranged from 6.0 to 7.0, which is conducive to the proliferation of Khai-Phum and non-pathogenic microorganisms [24-25]. This range reduces the risk of heavy metal accumulation in Khai-Phum tissue by reducing the solubility and mobility of heavy metals from sediment to the water column [26]. The DO value was between 4.0 and 5.0 mg/L under typical freshwater conditions, which is sufficient and does not signify severe putrefaction. The Code of Hygienic Practice for Fresh Fruits and Vegetables [27] states that a moderate level of dissolved oxygen reduces the growth of anaerobic bacteria and microbiological hazards in the food supply. The NK source's higher alkalinity and TKN levels may encourage duckweed growth by offering a sufficient nitrogen source for protein synthesis and growth.

3.4 Relationship between the Khai-Phum morphology and environmental variables

The environmental factors indicated that frond morphology was associated with several water quality variables. Frond width showed a strong positive correlation with electrical conductivity (EC) ($r=0.80$, $p<0.01$) and alkalinity ($r=0.90$, $p<0.01$), suggesting that increased ionic concentration and buffering capacity promoted frond expansion. Dry weight was positively correlated with conductivity ($r=0.90$, $p<0.01$), total dissolved solids, and alkalinity, indicating that nutrient-rich environments favored biomass accumulation ($r=0.80$, $p< 0.01$). Conversely, dissolved oxygen was negatively correlated with frond width and dry weight, implying that larger fronds tended to occur in relatively nutrient-enriched waters with lower oxygen concentrations. Stomatal density exhibited weak to moderate relationships with most environmental variables and showed no clear association with nutrient availability.

The analysis found that the following water quality parameters—pH, alkalinity, electrical conductivity (EC), and nutrient levels (TP and TKN)—had a significant impact on the variation in Khai-Phum morphology. High average alkalinity and TP values of 170 and 1.37 mg/L, respectively, were found in the NK water source. Khai-Phum is positively correlated with higher dry weight and moderate stomatal density, suggesting a chemically stable environment with sufficient nutrients to allow Khai-Phum to effectively accumulate biomass. Khai-Phum has the highest L/W ratio (1.36) and an exceptionally high stomatal density (20.75 units/frond), whereas water source BD has low alkalinity (average 68 mg/L), relatively high TKN and TP values, and low EC (101.06 μS/cm). This suggests morphological adaptation to improve gas exchange and photosynthesis in a setting where nutrients and dissolved oxygen may fluctuate. Khai-Phum typically shows lower dry weight and stomatal density than BD for water source TR, which has the lowest TP level of 0.29 mg/L and a neutral pH of 7.00, indicating nutrient constraints influencing structural growth (Table 4).

Table 4. Relationship between the Khai-Phum morphology and environmental variables

Environmental variable	Length	Width	Dry weight	Stomatal density
Temperature	-0.30*	-0.10*	0.40*	0.50*
pH	-0.20*	0.10*	0.30*	-0.60*
EC	0.50*	0.80**	0.90**	-0.50*
TDS	0.40*	0.70*	0.80**	-0.30*
Alkalinity	0.20*	0.90**	0.80**	-0.60*
DO	-0.40*	-0.70*	-0.60*	0.20*
TP	0.10*	0.20*	0.30*	0.10*
TKN	0.30*	0.50*	0.60*	-0.20*

Note: * is a correlation (r) at the P-value level < 0.05, and ** is a correlation (r) at the P-value level < 0.01.

High phosphorus and nitrogen (NK) sources were linked to denser Khai-Phum growth, according to the relationship between water quality and Khai-Phum presence. Preliminary correlation analysis of the data showed that the amount of Khai-Phum was somewhat negatively correlated with pH and DO and positively correlated with TKN. Because oxygen solubility restricts the respiration of microorganisms in the ecosystem, Khai-Phum propagation might not be appropriate for the BD water source, which had lower DO values. This is in line with Ziegler et al.'s research [24], which discovered a direct relationship between the amount of nutrients and the development of tiny aquatic plants like Khai-Phum. Nitrogen and phosphorus in water, particularly TP and TKN, significantly increase the dry weight and leaf-to-width ratio (L/W ratio) of Khai-Phum. This indicates the possibility of increased yield and protein content based on the general features of duckweed. [25]. However, from the standpoint of food safety, an excess of nitrogen in the water can cause aquatic plant tissues to accumulate nitrate, which can cause health problems like methemoglobinemia if consumed in excess [28]. Ecologically, water quality not only affects the quantity of Khai-Phum but also directly influences morphological adaptation mechanisms, particularly changes in stomata and leaf proportions, which are key features in regulating photosynthetic rate and gas exchange. The findings are consistent with the concept of Chookhampaeng et al. [29], which states that Khai-Phum and floating aquatic plants are sensitive to changes in pH and nutrients, especially nitrogen and phosphorus, which can stimulate an increase in leaf surface area and stomatal density to support rapid growth. Additionally, high alkalinity helps stabilize pH and the availability of inorganic carbon in the water, which is conducive to Khai-Phum biomass production. The study results indicate that water bodies with high nutrient levels, such as NK sources, provide the most favorable environment for Khai-Phum biomass increase. Therefore, water bodies with suitable water quality, a neutral to moderately alkaline pH, and sufficient but not excessive nutrients are positively correlated with Khai-Phum characteristics conducive to its use as food, including high dry weight and leaf structure suitable for nutrient accumulation. The results of this study can therefore be used as scientific proof of Khai-Phum's potential for development as a substitute protein source for humans in water bodies with suitable water quality control. On the other hand, eutrophication brought on by high nutrient levels can upset the ecosystem. Thus, there is enormous potential for using Khai-Phum as a bioindicator to represent water quality in agricultural areas.

4. Conclusions

In summary, water quality factors such as pH, DO, and TKN influence Khai-Phum growth, especially in neutral conditions with sufficient nutrients. Water quality variables, including pH, alkalinity, EC, TP, and TKN, are structurally related to Khai-Phum morphological characteristics, particularly dry weight and stomatal density, which are significant factors affecting not only Khai-Phum growth but also its quality and safety as human food. This study suggested the potential of Khai-Phum for future research as a bioindicator of water quality and can be effectively applied to monitor water body status or manage freshwater ecosystems.

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