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IDENTIFICATION AND DISTRIBUTION OF COMMERCIALLY IMPORTANT FRESHWATER PLANT (AZOLLA, WOLFFIA AND LEMNA SPECIES) IN BINTULU, SARAWAK

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IDENTIFICATION AND DISTRIBUTION OF COMMERCIALLY IMPORTANT FRESHWATER PLANT (AZOLLA, WOLFFIA AND LEMNA SPECIES) IN BINTULU SARAWAK

By

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A Project Report Submitted in Partially Fulfilment of the Requirement for the Degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural and Forestry Sciences University Putra Malaysia Bintulu Sarawak Campus
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ABSTRACT

This study addresses a significant knowledge gap concerning the identification and distribution of commercially significant freshwater plants, specifically *Azolla, Wolffia*, and *Lemna* species, within the region of Bintulu, Sarawak. The absence of comprehensive information poses a critical challenge to understanding the ecological dynamics and potential economic impact of these plants. Our research aims to morphologically identify these commercially important freshwater species and document their distribution in Bintulu. Fieldwork involved the collection of macrophyte specimens from three sampling stations in Bintulu, Sarawak, revealing distinct patterns. *Azolla*, *Wolffia*, and *Lemna* species exhibited varied characteristics. Azolla sp. differed from existing studies, making species identification challenging. *Wolffia sp*. likely corresponds to *W. globosa*, but discrepancies suggest the need for further investigation. *Lemna sp*. closely aligns with *Lemna minor*, despite some differences observed in other *Lemna* species. The study emphasizes the complexity and variability within freshwater plant species, underscoring the importance of detailed morphological analyses for accurate identification.

ABSTRAK

Kajian ini menangani jurang pengetahuan yang ketara mengenai pengenalpastian dan pengedaran tumbuhan air tawar yang penting secara komersial, khususnya spesies *Azolla, Wolffia*, dan *Lemna*, dalam wilayah Bintulu, Sarawak. Ketiadaan maklumat yang komprehensif menimbulkan cabaran kritikal untuk memahami dinamik ekologi dan potensi kesan ekonomi tumbuhan ini. Penyelidikan kami bertujuan untuk mengenal pasti secara morfologi spesies air tawar yang penting secara komersial ini dan mendokumenkan pengedarannya di Bintulu. Kerja lapangan melibatkan pengumpulan spesimen makrofit dari tiga stesen pensampelan di Bintulu, Sarawak, mendedahkan corak yang berbeza. Spesies *Azolla, Wolffia*, dan *Lemna* mempamerkan ciri yang berbeza-beza. *Azolla sp*. berbeza daripada kajian sedia ada, menjadikan pengecaman spesies mencabar. *Wolffia* sp. berkemungkinan sepadan dengan *W. globosa*, tetapi percanggahan mencadangkan keperluan untuk siasatan lanjut. *Lemna sp.* sejajar rapat dengan *Lemna minor*, walaupun terdapat beberapa perbezaan dalam spesies *Lemna* yang lain. Kajian ini menekankan kerumitan dan kebolehubahan dalam spesies tumbuhan air tawar, menekankan kepentingan analisis morfologi terperinci untuk pengenalpastian yang tepat.

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APPROVAL SHEET

I clarify that this research project report entitled "Identification and Distribution of Commercially Important Freshwater Plant (*Azolla, Wolffia* and *Lemna* Species) in Bintulu, Sarawak" has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural and Forestry Sciences University Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1: INTRODUCTION

1.1 Background of the study

The identification and distribution of commercially important freshwater plants, specifically *Azolla, Wolffia*, and *Lemna* species, represent substantial research undertaking with wide-ranging consequences. These plants are essential contributors to aquatic ecosystems, playing a crucial role in maintaining biodiversity, regulating nutrient cycles, and promoting overall environmental well-being. According to (Poveda, 2022a) , freshwater macrophytes, including various plant categories like spermatophytes, pteridophytes, and bryophytes, possess the capability to thrive within or near aquatic habitats, underscoring the diverse nature of freshwater flora and their integral role in supporting the health and balance of aquatic environments.

Freshwater plants, particularly *Azolla, Wolffia*, and *Lemna* species, are intricate tasks due to their morphological similarities, as highlighted by (Ceschin et al., 2016; Oyedeji & Abowei, 2012) . Freshwater macrophytes can be classified based on various factors such as zonation, habitats, or taxonomic groups, with primary morphological characteristics including flowers, frond structure, stems, root structure, rhizomes, or stolons (Oyedeji & Abowei, 2012). However, accurate identification remains challenging due to the morphological similarities among different freshwater macrophyte species, creating difficulties for researchers alike. For instance, the confusion between *Lemna minuta* and *L. minor*, driven by their small size and similar morphology, exemplifies the difficulties encountered in distinguishing between different freshwater macrophyte species (Ceschin et al., 2016) . This emphasizes the need for comprehensive studies to enhance our understanding of the distribution and characteristics of commercially significant freshwater plants.

The study's significance is underscored by its contribution to filling existing gaps in knowledge concerning the taxonomy and geographical distribution of *Azolla, Wolffia*, and *Lemna* species. These aquatic plants exhibit unique characteristics valuable for diverse purposes, including biofertilization, bioenergy production, and water purification. A comprehensive investigation into their identification and distribution will not only enhance our understanding of their ecological roles but also provide insights into optimizing their utilization for economic and environmental benefits.

Azolla, Wolffia, and *Lemna* species have gained significant attention due to their notable commercial importance, with applications spanning diverse industries such as agriculture, wastewater treatment, and pharmaceuticals. The recognition of their economic significance underscores the need to comprehend the precise identification and distribution of these plants, ensuring not only the harnessing of their economic potential but also fostering sustainable utilization. This aligns with the findings of (Azim & Wahab, 2003) , who, in their pursuit of bolstering aquaculture profitability and alleviating escalating feed costs, conducted experiments utilizing specific indigenous aquatic plants as potential alternative feed sources for aquaculture purposes. Additionally, the multifaceted utility of macrophytes, highlighted by (Hasan & Chakrabarti, 2009; Rahman et al., 2007; Swapna et al., 2011) extends beyond aquaculture, encompassing their role as valuable sources of food, fodder, and fertilizers for various agricultural applications, thereby reinforcing their significance in sustaining both terrestrial and aquatic ecosystems.

In Malaysia, a wide variety of freshwater macrophytes are readily accessible from diverse water sources (Ong & Teng, 2022a) . Consequently, Bintulu, situated in the state of Sarawak, Malaysia, serves as an ideal study area for several reasons. Sarawak's rich biodiversity and unique ecological landscapes make it a hotspot for exploring freshwater plant diversity. Bintulu, specifically, is characterized by a diverse range of aquatic ecosystems, including rivers, lakes, and wetlands, providing an excellent setting for studying *Azolla, Wolffia*, and *Lemna* species. Moreover, Bintulu is a hub for various industries, including aquaculture and agriculture, where identified freshwater plants can potentially contribute to sustainable practices. In East Malaysia, specifically Sarawak, around 43 to 48 species of wild freshwater macrophytes from 28 families, classified as weeds, are gathered and employed for various purposes, including consumption as edible food and ingredients for food preparation, utilization in traditional medicine, crafting household items like pillows and mats, and even being transformed into souvenirs (Saupi et al., 2015; Zakaria et al., 2023) . This diversity of species underscores the importance of accurate identification, considering the challenges posed by morphological similarities among these plants.

The relevance of this research to Bintulu extends beyond its ecological significance. The economic and practical implications of identifying and understanding the distribution of these commercially important freshwater plants can contribute to developing innovative and sustainable practices in the region. This study aims to bridge the gap between scientific knowledge and practical applications, offering insights that can inform policies and practices related to freshwater resource management in Bintulu, Sarawak, and beyond.

1.2 Problem Statement

The current lack of comprehensive knowledge regarding the identification and distribution of commercially significant freshwater plants, specifically *Azolla, Wolffia*, and *Lemna* species, in Bintulu, Sarawak, poses a critical challenge. This knowledge gap hinders the development of effective strategies for harnessing the economic potential of these plants and implementing sustainable practices in the region.

1.3 Objectives

1. To morphological identify the commercially important freshwater plants (*Azolla, Wolffia*, and *Lemna* species), in Bintulu, Sarawak.
2. To document the distribution of freshwater plants (*Azolla, Wolffia*, and *Lemna* species), in Bintulu, Sarawak.

CHAPTER 2: LITERATURE REVIEW

2.1 Freshwater macrophytes

In the context of freshwater ecosystems, aquatic macrophytes constitute a diverse group of photosynthetic organisms residing in freshwater or brackish water, visible to the naked eye, and actively growing while permanently or periodically submerged, floating, or emerging through the water surface (Beger et al., 2010; Murphy et al., 2019) . Aquatic macrophytes encompass seven plant divisions, including Cyanobacteria, Chlorophyta, Rhodophyta, Xanthophyta, Bryophyta, Pteridophyta, and Spermatophyta. Aquatic macrophytes encompass various types of water plants, including emergent macrophytes (rooted in submerged or periodically inundated soils, with foliage extending above the water), floating-leaved macrophytes (rooted to the lake or stream bottom, with leaves floating on the water's surface), submersed macrophytes (growing entirely submerged under water, with roots or root-like structures attached to the substrate), and free-floating macrophytes (typically floating on or under the water surface) according to (Murphy et al., 2019) . A previous study represented by 33 orders and 88 families, comprises around 2,614 species across approximately 412 genera (Balian et al., 2008). A widely acknowledged hypothesis suggests that a majority of aquatic macrophytes exhibit broad geographical distributions, as supported by a recent investigation into macrophyte diversity and endemism patterns involving 3457 species (Murphy et al., 2019) . This emphasizes the global prevalence of these crucial components within freshwater environments. Their accessibility is underscored by their abundant presence and rapid proliferation in freshwater ecosystems, as noted by (Mandal et al., 2007) . Aquatic macrophytes vary in size, ranging from the large *Victoria amazonica*, boasting a leaf diameter of up to 2.5 meters, to the smallest angiosperms like Wolffia spp., which have frond diameters less than 0.5 mm (Chambers et al., 2008).

Freshwater macrophytes, also referred to as hydrobionts or aquatic plants, encompass all members of the Plantae kingdom thriving in water or its immediate vicinity, excluding microphytes like algae in rivers, basins, and lakes (Brogan & Relyea, 2015). This classification encompasses free-floating, floating-rooted, submerged, and amphibian plants, spanning spermatophytes, pteridophytes (ferns and fern allies), and bryophytes (mosses, liverworts, and hornworts). Although vascular plants constitute less than 2% of the total aquatic plant population, their ecological significance is substantial (Bornette & Puijalon, 2009; Brogan & Relyea, 2015). Macrophytes, as primary producers in freshwater ecosystems, serve as vital habitats for a diverse range of organisms, including periphytons, invertebrates (such as zooplankton), and vertebrates like fish and frogs. Beyond their role in biogeochemical cycles, contributing to organic carbon production through photosynthesis, macrophytes play a crucial role in nutrient cycling. They absorb excess nutrients like nitrogen and phosphorous, mitigating issues such as eutrophication, and influence water flows and sediment dynamics, thereby modifying hydrology and sedimentation processes (Bornette & Puijalon, 2009) . As emphasized by these findings, freshwater macrophytes are indispensable components of freshwater ecosystems, playing multifaceted roles in shaping the ecological balance and functioning of these environments.

2.1.1 Azolla sp.

Azolla is a water fern that floats freely and is found naturally in tropical and temperate paddies (Oyange et al., 2020.; Subedi et al., 2015) . There are seven officially recognized species of *Azolla* worldwide, which *are A. nilotica, A. pinnata, A. filiculoides, A. mexicana, A. rubra, A. microphylla*, and *A. caroliniana* (Pereira et al., 2011) . The species belong to the sub-genres Rhizosperma and Euazolla (Oyange et al., 2020.). *Azolla* is widely distributed in both temperate and tropical paddies. Human activities have led to the introduction of new *Azolla* species in different regions, resulting in the elimination of native species (Carrapico, 2002). Three global species, namely *A. pinnata R.Br., A. filiculoides Lam.,* and *A. mexicana Presl*, have been categorized as invasive (Oyange et al., 2020-b). *Azolla filiculoides* has been documented in Tanzania, South Africa, and Kenya according to (Oyange et al., 2020-b). *A. pinnata subsp. africana* is native to Africa, *whereas A. pinnata subsp. asiatica* is found in South Africa. Additionally, *A. cristata Kaulf.* (Including *A. mexicana* and *A. microphylla Kaulf.*) has established populations in South Africa, Mozambique, Zimbabwe, and Ghana, as reported by (Madeira et al., 2013). Morphological features have been used for a long time to identify and characterize Azolla (Pereira et al., 2011). The major distinguishing features which were relied upon included number of float capsules, type of glochidia, branching pattern and leaf trichomes (Pereira et al., 2011). However, presented 13 polymorphic descriptors used to characterize existing Azolla species thus: sporophytic shape and arrangement, rhizome indumentum and papillae, dorsal lobe apex, angle and shape, hyaline border cells, symmetry, and papillae, dorsal and ventral stomata. (Madeira et al., 2013) successfully used morphological characteristics to identify the sub-sections Rhizosperma Sadeb. and Euazolla Sadeb. and to distinguish the Asian Azolla pinnata and African Azolla pinnata.

Table 2.1.1: Taxonomy of *Azolla sp.*

Classification	**Taxonomy**
Kingdom	*Plantae*
Phylum	*Tracheophyta*
Class	*Polypodiosida*
Order	*Salviniales*
Family	*Salviniaceae*
Genus	*Azolla*
Sub Genus	*Eu-Azolla*

Figure 2.1.1: *Azolla sp.* plant. Adapted from (Riaz et al., 2022)

2.1.2 Wolffia spp.

Wolffia spp., commonly known as water meal, is recognized as the tiniest flowering plants worldwide. They possess rootless and freely floating thalli with green or yellow-green hues. The genus comprising 11 species, includes both the fastest-growing angiosperm and the tiniest flowering plants (Sree, Sudakaran, et al., 2015). The size of *Wolffia* plants varies, ranging from 1.5 cm in diameter for *Spirodela polyrhiza* (Giant Duckweed) to less than 1 mm for *Wolffia angusta*, the smallest angiosperm (Romano & Aronne, 2021) . According to (Hasan & Chakrabarti, 2009) , all *Wolffia* species are 2 mm or smaller in diameter. *Wolffia australiana* emerges as the largest species (1.0–1.5 × 0.5–0.8 mm) when viewed from above, whereas *W. angusta* claims the title of the smallest (0.5–0.8 × 0.2–0.4 mm) within the genus (Bog et al., 2013) . Similar to other species in the family, *Wolffia* plants consist of a single physical unit known as a frond or thallus, interpreted as a leaf and stem in an embryonic stage of development (Romano & Aronne, 2021). The fronds of *Wolffia* species have a globose, ovoid boat shape. *W. microscopica* exhibits unique features that distinguish it from other *Wolffia* species, notably its unusual frequent flowering (Sree, Maheshwari, et al., 2015). For *W. globosa* frond, it shaped like an oval, can be subdivided into dorsal, ventral, and lateral parts (Yang et al., 2021). Identifying *Wolffia* species can pose challenges, especially since relying on stomata counts seems unreliable without the use of Scanning Electron Microscopy (SEM) (Schmitz et al., 2014) . Despite this difficulty, numerous species, such as *W. columbiana* from the Americas, *W. globosa* from Asia, and *W. australiana* from Australasia, have been documented beyond their native habitats (Ivan & Katya, 2013; Schmitz et al., 2014).

Table 2.1.2: Taxonomy of *Wolffia*

Classification	Taxonomy
Kingdom	*Plantae*
Phylum	*Magnoliophyta*
Class	*Liliopsia*
Order	*Arales*
Family	*Lemnaceae*
Genus	*Wolffia*

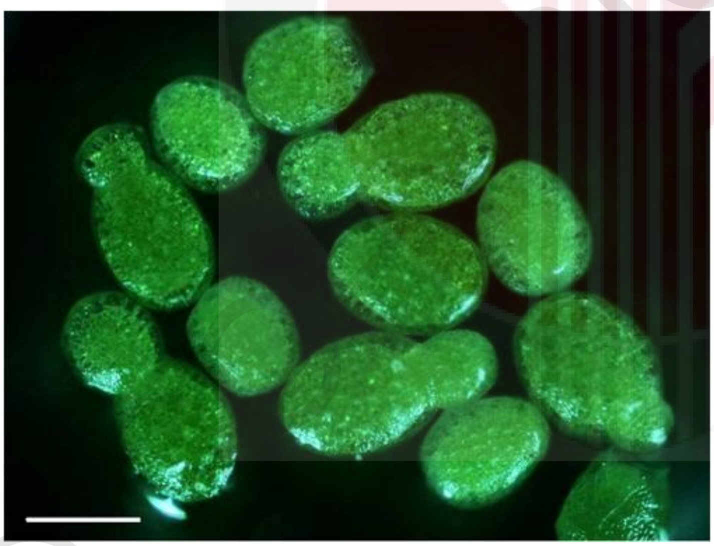


Figure 2.1.2: *W. globose*. Source from (Ruekaewma et al., 2015)

Table 2.1.2: Identification characteristic of *Wolffia* species known from Europe adapted from (Bog et al., 2013; Landolt, 2000; Lansdown et al., 2022)

Character	***W. arrhiza***	***W. australiana***	***W. columbiana***	***W. globosa***
Greatest width	just below surface	at surface	well below surface	well below surface
Length (mm)	(0.5)0.7-1.3(1.5)	(0.5)1.0-1.3(1.5)	(0.5)0.7-1.2(1.4)	(0.4)0.5-0.8(0.9)
Width (mm)	(0.4)0.6-1.0(1.2)	(0.3)0.5-0.7(0.8)	(0.5)0.6-1.1(1.2)	(0.3)0.4-0.6
Length: width	1-1.33	1.33-2	1-1.33	1.33-2.33
Depth: width	1-1.33	2-3	1-1.33	0.75-1.33
No. of stomata	(0)30-100	50-80	1-10(-30)	8-25(35)
Surface colour	bright green	bright green	pale green	pale green
Surface	opaque	opaque	translucent	translucent
Lower colour	translucent	?	green	green

2.1.3 Lemna spp,

Lemna spp., commonly known as duckweeds, form a group of petite flowering aquatic plants belonging to the Araceae family(Cabrera et al., 2008). Within the Lemnaceae family, five genera exist, including *Spirodela, Wolffiella, Landoltia, Lemna*, and *Wolffia*, totaling 38 species that thrive in aquatic ecosystems globally (Wang et al., 2011). These tiny plants, characterized by inconspicuous flowers, pose challenges in species identification due to their minute size. Azer, (2013) emphasized the difficulty in distinguishing duckweed species, leading to the reliance on examining vegetative morphological traits such as roots (root tip and sheath) and fronds (habit, number, shape, margin, length, branching, papilla, gibbousness, symmetry, and dimensions) (Azer, 2013; Ceschin et al., 2018). The research conducted by Andriani et al., (2019) specifically delves into the morphological characteristics of duckweeds, homing in on roots and fronds. The resulting findings are succinctly summarized in the table below, offering a comprehensive of the finding's outcomes.

Table 2.1.3: Morphological characters of duckweeds from Java, Indonesia. Source: (Andriani et al., 2019)

Characters	***S. polyrrhiza***	***L. perpusilla***	***L. gibba***
Root			
Tip	Pointed	Pointed	Rounded
Sheath	Not winged	Winged	Not winged
Length	10-15 mm	10-20 mm	10-15 mm
Number	Numerous (9-15) per frond	1 per frond	1 per frond
Frond			
Habit	Floating	Floating	Floating
Shape	Ovate to sub-orbicular	Obovate to oblong	Obovate
Apex	Obtuse to rounded	Obtuse	Obtuse to rounded
Number	Solitary (2-5 in group)	Solitary (2-5 in group)	Solitary (2-4 in group)
Margin	Entire	Entire	Entire
Length	8-16 mm	10-15 mm	7-12 mm
Branching	Unbranching	Unbranching	Unbranching
Scale	Present in ventral and dorsal	Absent	Absent
Gibbous	Present	Absent	Present
Nerves	Prominent, 7-12 nerve	(3) Indistinct nerve	Indistinct nerve
Symmetry	Symmetric	Symmetric	Asymmetric
Surface	Brown pigment in ventral, green in dorsal and slightly convex	Flat in dorsal and ventral	Gibbous in ventral and dorsal, brown pigment in ventral, shining in dorsal

Table 2.1.3: Taxonomy of Lemna spp.

Classification	Taxonomy
Kingdom	*Plantae*
Phylum	*Magnoliophyta*
Class	*Liliopsia*
Order	*Arales*
Family	*Lemnaceae*
Genus	*Lemna*

Figure 2.1.3: *Lemna perpusilla* Torr. A. Source: (Andriani et al., 2019)

2.2 Diversity of freshwater macrophytes in Malaysia

Malaysia boasts a rich diversity of freshwater macrophytes, reflecting the country's varied aquatic ecosystems and climatic conditions. The nation's water bodies, including rivers, lakes, and wetlands, host an extensive array of macrophytic species, contributing to the overall ecological health of these habitats. According to the existing literature, around 235 aquatic plant species inhabit the aquatic ecosystems of Malaysia, as documented in works such as those by (Chong et al., 2010) . Within this context, Malaysia harbors numerous representatives of freshwater macrophytes, such as the *Azolla, Wolffia*, and *Lemna* species, alongside a wide spectrum of other aquatic plants. The diversity of these macrophytes plays a crucial role in supporting biodiversity, providing habitats for aquatic fauna and influencing nutrient dynamics in aquatic ecosystems. Moreover, macrophytes have been locally utilized in Malaysia as food resources, as highlighted in studies by (Nordiah et al., 2012; Saupi et al., 2015; Zakaria et al., 2023). Notable macrophyte species, including E*ichhornia crassipes (Mart.) Solms, Limnocharis flava (L.) Buchenau,* and *Neptunia oleracea Lour*., are commonly ingested by local residents. Chew, (2010) documented the presence of Cabomba aquatica in the freshwater environment of Kota Tinggi, Johor, while (Chew, 2010) suggest that *C. furcata* likely became naturalized in Lake Chini within the past decade, akin to many introduced Cabomba populations. Additionally, (Nicolaus & Edelaar, 2018) underscore the status of water hyacinth, *Lemna*, and hydrilla as aquatic plant species considered noxious weeds in Malaysia. The study and conservation of freshwater macrophytes in Malaysia are not only scientifically significant but also imperative for the sustainable management of its aquatic resources.

2.2.1 Sarawak

In Sarawak, located in East Malaysia, a diverse range of 43–48 species of wild freshwater macrophytes, spanning 28 families and commonly perceived as weeds, have been identified. This rich array of aquatic plants is actively collected by local residents and serves various purposes such as being used as edible food, ingredients for food preparation, medicinal resources, and materials for crafting household items like pillows and mats, as well as souvenirs (Harah et al., 2005; Nordiah et al., 2012; Saupi et al., 2015; Zakaria et al., 2023) . Furthermore, these freshwater macrophytes are gradually gaining recognition as consumed vegetables, with local collectors offering them for sale in local markets (Saupi et al., 2015; Zakaria et al., 2023) . Despite being considered as weeds, aquatic plants like yellow velvet leaf (*Limnocharis flava(L.)*Buch) and water mimosa (Neptunia oleracea Loureiro) thrive in Sarawak's wetland habitats and are actively harvested by the local population. This includes the collection of various plant parts, such as young leaves with petioles and inflorescences of Eichhornia crassipes and Limnocharis flava, as well as young leaf shoot tips and immature pods of Neptunia oleracea, which are not only consumed as blanched or cooked vegetables but also sold in local markets for income generation. Ethnobotanical studies, including those by (Harah et al., 2005; Nordiah et al., 2012; Saupi et al., 2015; Zakaria et al., 2023) further validate the prevalence of these practices, particularly in the Bintulu community of Sarawak.

2.2.2 West Malaysia

West Malaysia, with its diverse ecosystems, is home to a range of macrophytes, sizable aquatic plants visible to the naked eye, playing vital roles in its aquatic environments. These macrophytes are crucial components of wetland ecosystems, offering habitat, oxygenation, and nutrient cycling. Notably, species like *Lemna minor*, commonly known as duckweed, dominate specific regions, such as the Pontian sampling site in Johor, covering a substantial 88.67% of the surveyed area (Ong & Teng, 2022b). Additionally, water hyacinth and water lettuce, studied by Jamion et al., (2021) in Kampung Parit Tinggi, Kuala Pilah, Negeri Sembilan, contribute to the rich biodiversity of macrophytes in constructed wetlands. The presence and distribution of these plants highlight the importance of comprehending and conserving West Malaysia's unique aquatic flora, as they play a crucial role in maintaining the ecological balance and overall health of the region's water bodies. However, despite the valuable insights from specific studies, there is a lack of comprehensive data distribution concerning macrophytes in various areas of West Malaysia.

2.3 Commercial application and uses

Freshwater macrophytes hold significant commercial value and find diverse applications across various industries. Freshwater macrophytes have various well-established applications today, serving as biomarkers, phytoremediators, producers of valuable metabolites (including antimicrobials, herbicides, insecticides, and drugs), or biomass for the generation of feed, biofuels, pellets, or ceramics, as extensively documented for exotic species like E. crassipes (Su et al., 2018). In aquaculture, freshwater macrophytes play a crucial role by providing habitat and food for aquatic organisms. To mitigate the rise in feed expenses and enhance the profitability of aquaculture, Azim & Wahab, (2003) conducted experiments utilizing specific locally abundant macrophytes as alternative sources of food that could be utilized in feed preparation. Consequently, some species, such as Lemna, are actively employed as feed for fish and other cultured species, thereby contributing significantly to the economic viability of aquaculture operations. Additionally, macrophytes are increasingly recognized for their potential in wastewater treatment. (Zhang et al., 2013) conducted a study on the effectiveness of macrophytes-based treatment systems for municipal wastewater treatment in China. They found that large-scale centralized wastewater treatment systems have been prevalent in industrialized countries and have been considered successful over the past century. Azolla species, for example, have garnered significant attention in research due to their rapid growth rates and exceptional bioremediation efficiency (Forni et al., 2012; Sood et al., 2012). Notably, Azolla is recognized for its capacity to absorb heavy metals and pollutants from water, presenting a sustainable and eco-friendly solution for water purification. Furthermore, macrophytes have found applications in the cosmetic and pharmaceutical industries, where extracts from these plants are utilized for their medicinal properties and in the production of herbal products. The economic potential of freshwater macrophytes continues to be explored, making them valuable resources for various commercial ventures and sustainable practices.

2.3.1 Aquafeed

Macrophytes, aquatic plants that play pivotal roles in freshwater ecosystems, have garnered increasing attention as valuable components in aquafeeds. *Azolla* is recognized in aquaculture because it has more protein (13% to 30%), according to studies by (Ojha et al., 2017). Likewise, research on *Lemna* found that the protein content averages between 16% and 40% (Mirzaeva et al., 2020) . This potential alternative to conventional fishmeal holds promise in meeting the increasing global protein demand. Researchers have extensively studied the feasibility of incorporating macrophytes into aquafeeds, with findings suggesting positive outcomes in terms of growth and nutritional benefits for various cultured fish species such as grass carp and Nile tilapia. Among the macrophytes explored for their potential use in aquafeeds, *Azolla* has been extensively studied, along with *Salvinia, Ipomoea, Trapa, Lemna, Pistia*, and duckweed (Abou et al., 2007; Naseem et al., 2021). Moreover, Majhi et al., (2023) observed a favorable response in grass carp (*Ctenopharyngodon idella*) fingerlings fed with *Azolla*, demonstrating a significantly greater final weight gain compared to the control group. Similarly, (El-Leboudi et al., 2008) achieved positive outcomes with Azolla-based diets for juvenile Oreochromis niloticus. (El-Leboudi et al., 2008) also noted efficient utilization of *Azolla* by young Nile tilapia. For Duckweed, highlighted by Mwale, (2013) as a valuable protein source, has a protein content ranging from 20% to 30%, surpassing that of cereals. Additionally, *Wolffia*, specifically *Wolffia arrhiza*, has been considered a potential substitute for soybean meal in tilapia diets, with optimal results achieved at a 15% substitution level. The exploration of alternative aquatic plants, their nutritional profiles, and their optimal incorporation levels in aquafeeds represent significant strides toward sustainable and eco-friendly aquaculture practices.

2.3.2 Wastewater treatment

Macrophytes play a crucial role in the realm of wastewater treatment, offering a natural and sustainable approach to mitigate water pollution. Macrophytes have proven to be effective in the treatment of various wastewater types, primarily attributed to their capacity for nutrient absorption, simplicity, cost-effectiveness in terms of construction, operation, and maintenance, low energy requirements, process stability, and the potential benefits of the harvested materials (Shah et al., 2014) . Their utilization in wastewater treatment processes is characterized by simplicity, cost-effectiveness in terms of construction, operation, and maintenance, as well as low energy demands. The efficiency of macrophyte-based treatment systems has been demonstrated in various studies, with documented success in treating different types of wastewaters. In China, (Zhang et al., 2013) investigated the efficiency of a macrophyte-based treatment system for Municipal Wastewater Treatment. According to Zhang's findings, large-scale centralized wastewater treatment systems have traditionally dominated in industrialized countries and have been considered a successful approach throughout the last century (Zhang et al., 2013) . Various macrophytes have proven instrumental in bioremediation efforts, with prominent members of the Lemnaceae family, commonly known as duckweeds (*Lemna spp., Spirodela spp., Wolffia spp*., and *Wolffiella spp*.), along with *Eichhornia crassipes, Hydrocotyle umbellata*, and the water fern *Azolla* (*Azolla filiculoides*), standing out as extensively employed species (Beger et al., 2010) . Duckweed species, recognized for their notable bioremediation capabilities, have been actively utilized for over three decades to recover nutrients from wastewater sources, as evidenced by studies conducted by (El-Leboudi et al., 2008; Muradov et al., 2014) . *Azolla* species have also undergone thorough examination due to their rapid growth rates and efficient bioremediation capacities, as highlighted in research by (Sood et al., 2012) . These findings collectively emphasize the valuable role of these macrophytes in environmental remediation, contributing to the sustainable management of wastewater and restoration of aquatic ecosystems.

2.3.3 Agricultural application

Macrophytes play a multifaceted and pivotal role in agriculture, contributing significantly to various aspects of crop cultivation and soil health. In rice farming systems, exemplified by rice-fish culture, the incorporation of Azolla during its linear growth phase serves as an effective fertilizer (Hasan & Chakrabarti, 2009) . As mentioned before, freshwater macrophytes are integral to nutrient cycling in aquatic ecosystems, serving as a significant reservoir of nutrients that can potentially be utilized as organic fertilizer in agriculture, particularly in nitrogen (N) and phosphorus (P) (Adzman et al., 2022; Subedi et al., 2015) . In freshwater environments, the natural release of nutrients from macrophyte tissues occurs through the decomposition of organic matter by various fungi and bacteria (Yang et al., 2021) . These microorganisms must interact with agricultural soils to facilitate the availability of nutrients for crops. This strategic application aligns with the phase of maximum productivity and low lignin content, facilitating rapid decomposition and nutrient release into the soil (Hasan & Chakrabarti, 2009). Additionally, the use of fresh tissues from diverse freshwater macrophytes, such as Hydrilla verticillata (waterthyme) or Phragmites australis (common reed), has proven beneficial in increasing crop productivity, particularly in crops like maize. The contribution of essential nutrients including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) from these macrophytes enhances soil fertility, thereby supporting sustainable and enriched agricultural practices (Jain & Kalamdhad, 2018; Jha, 2021).

CHAPTER 3: METHODOLOGY

3.1 Study area

The research was conducted in the freshwater habitats around Bintulu, Sarawak, Malaysia, which offer a rich environment comprising diverse habitats. To ensure a comprehensive sampling of macrophytes diversity within the region's freshwater ecosystems, specific habitats were strategically identified. Three sampling sites, labeled as station 1, station 2, and station 3 (refer to Figure 3.1 and Table 3.1), were selected for the study, as outlined in Table 3.1. The sampling encompassed these designated areas, ensuring a representative exploration of the macroalgal diversity present in the freshwater ecosystems of the Bintulu region.

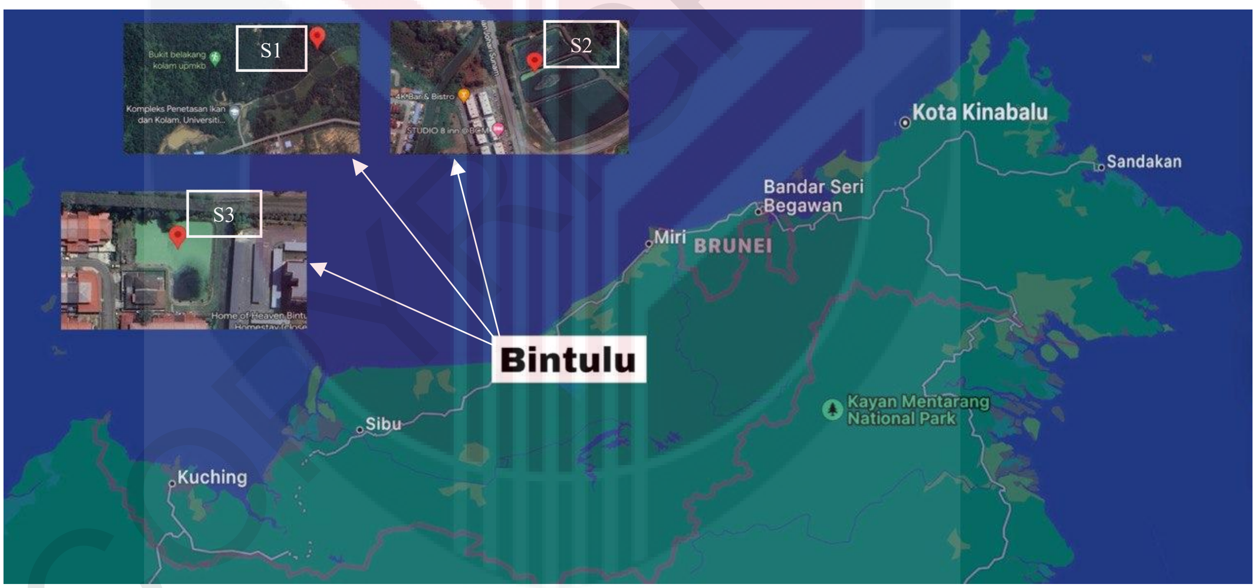


Figure 3.1: Sampling location in Bintulu, Sarawak.

Table 3.1: Coordinates of each sampling station.

Station	Latitude (N)	Longitude (E)
S1 (Hatchery UPMKB)	3˚21’22.8”	113˚08’29.9”
S2 (Wastewater Taman Putrajaya	3˚20’39.6”	113˚08’93.3”
S3 (Wastewater Taman Bandar Jaya)	3˚18’98.8”	113˚07’16.6”

3.2 Collection of macrophytes specimens

In the investigation of freshwater habitats within our study area, the collection of macrophyte specimens was conducted using a straightforward method specifically designed for immediate analysis. The Nasco Swing Sampler was employed as the primary tool to efficiently capture macrophytes in the freshwater environment. The meticulous collection process involved placing the macrophytes into sampling bottles and plastic zip-lock bags (6x9 inches) to ensure secure containment during transport. This expeditious approach not only guaranteed the preservation of the specimens' structural integrity but also facilitated their swift transfer to the laboratory for in-depth analysis. Careful handling of the sampling bottles was emphasized to prevent any damage to the macrophytes, ensuring an efficient and effective collection process within the freshwater research environment. This methodological precision contributed to the reliability and integrity of the collected macrophyte specimens for subsequent scientific investigation and analysis.

3.3 Laboratory processing

The laboratory processing phase for macrophytes was characterized by a meticulous and systematic approach aimed at the comprehensive analysis of the collected specimens, focusing particularly on morphological characteristics. Upon their arrival at the laboratory, the macrophyte specimens underwent careful sorting, cleaning, and preparation to ensure optimal conditions for subsequent examination. Preliminary assessments were conducted, the characteristic morphology of each species was carefully documented, encompassing features such as leaf arrangement, size, color, and root structures. For Wolffia, microscopic analysis played a pivotal role in discerning intricate details not visible to the naked eye. This thorough analysis provided a comprehensive overview of the morphological diversity present within the macrophyte communities in the study area. The emphasis on morphological characteristics during the laboratory processing phase facilitated the identification and classification of macrophyte specimens at various taxonomic levels.

3.4 Microscopic examination

In the comprehensive laboratory processing phase, the microscopic examination concentrated on the leaf structures of freshwater macrophyte specimens, with a specific focus on *Azolla, Wolffia,* and *Lemna* species in Bintulu, Sarawak. This specialized investigation utilized an inverted microscope, the (Olympus CKX53, Japan). Thin sections of the macrophyte leaves were meticulously prepared for detailed microscopic observation, providing an up-close exploration of cell types, arrangements, and other anatomical characteristics. The microscopic analysis was instrumental in discerning subtle variations in leaf structure among different macrophyte species, contributing to a more refined taxonomic classification and enhancing the overall comprehension of the ecological roles and adaptations of these plants within the freshwater ecosystem. The integration of the Olympus CKX53 microscope in the analysis of macrophyte leaves played a pivotal role in generating detailed and accurate information, thereby enriching the dataset for subsequent scientific interpretations and ecological assessments.

3.5 Data recording

The data recording process for the identification and distribution of commercially important freshwater plants, specifically Azolla, Wolffia, and Lemna species in Bintulu, Sarawak, was systematically conducted. The collected specimens were then transported to the laboratory, where they underwent rigorous processing, including microscopic examinations using the Olympus CKX53 model microscope from Japan. The acquired data, inclusive of detailed morphological features were systematically organized and catalogued for subsequent statistical analyses. This meticulous data recording phase not only facilitated the characterization of the targeted freshwater plant species but also provided a robust foundation for generating valuable insights into their ecological significance and commercial potential in Bintulu, Sarawak.

3.6 Statistical analysis

This study was conducted using SPSS version 25, and one-way analysis of variance (ANOVA) was performed to assess variations in macroalgal communities among different habitats. Calculations for species richness, evenness, and diversity indices were carried out. Additionally, data on the width, length, and root length of the three species were collected from three sample areas. The presence of these three species in the sample areas and associations between environmental variables and macroalgal diversity were explored as part of the research methodology.

CHAPTER 4: RESULT

The three distinct stations chosen for this study exhibited varying environmental conditions. Station 1, located at Hatchery UPMKB, is situated at a latitude of 3˚21’22.8” N and a longitude of 113˚08’29.9” E. Situated in a shaded spot surrounded by a forest. The nutrient levels in the unused pool, specifically in terms of macrophyte-friendly nutrients may include essential elements like nitrogen and phosphorus. The combination of a shaded environment and suitable nutrient levels creates an ideal habitat for macrophytes grow. Station 2, positioned at Wastewater Taman Putrajaya (latitude: 3˚20’39.6” N, longitude: 113˚08’93.3” E), contrasted with the first station, being an open area exposed to sunlight. In such open areas with sunlight exposure, macrophytes thrived due to the capability of light penetration suitable for photosynthesis, primarily facilitated by the availability of essential nutrients. Similarly, Station 3 at Wastewater Taman Bandar Jaya (latitude: 3˚18’98.8” N, longitude: 113˚07’16.6” E) was an open area exposed to sunlight and wastewater environments. The thriving of macrophytes in these locations could be attributed to factors such as nutrient availability and light penetration. These diverse sampling sites aimed to provide a comprehensive understanding of macrophyte dynamics in different environmental contexts.

4.1 Identification freshwater macrophytes species

Identification of freshwater macrophyte species involves a comprehensive analysis of several key morphological characteristics, including frond width and length, color, and root length.

Table 4.1 Presence of freshwater macrophytes species in three sample areas in Bintulu

Species Name	Hatchery UPMKB	Wastewater Taman Putrajaya	Wastewater Taman Bandar Jaya
Azolla	*		
Wolffia globosa		*	*
Lemna spp			
Lemna minor L.		*	*
Spirodela polyrhiza		*	*
Total	1	3	3

4.1.1 *Azolla sp.*

Azolla sp, when measured, displays individual frond length ranging from 0.1 to 0.2 cm (0.15 ± 0.05 cm), individual frond width spanning 0.05 to 0.1cm (0.075 ± 0.025), and the number of contiguous fronds ranging from 6 to 10 (8 ± 1.00). Azolla had a brown color near the root and green color in the fronds. Root length ranges from 1.0 cm to 2.5 cm, with an average of 1.75 cm ± 0.75 cm. In terms of shape, the fronds are intricate and feather-like, with a bilateral symmetry. *Azolla sp.* possessed a complex root structure.

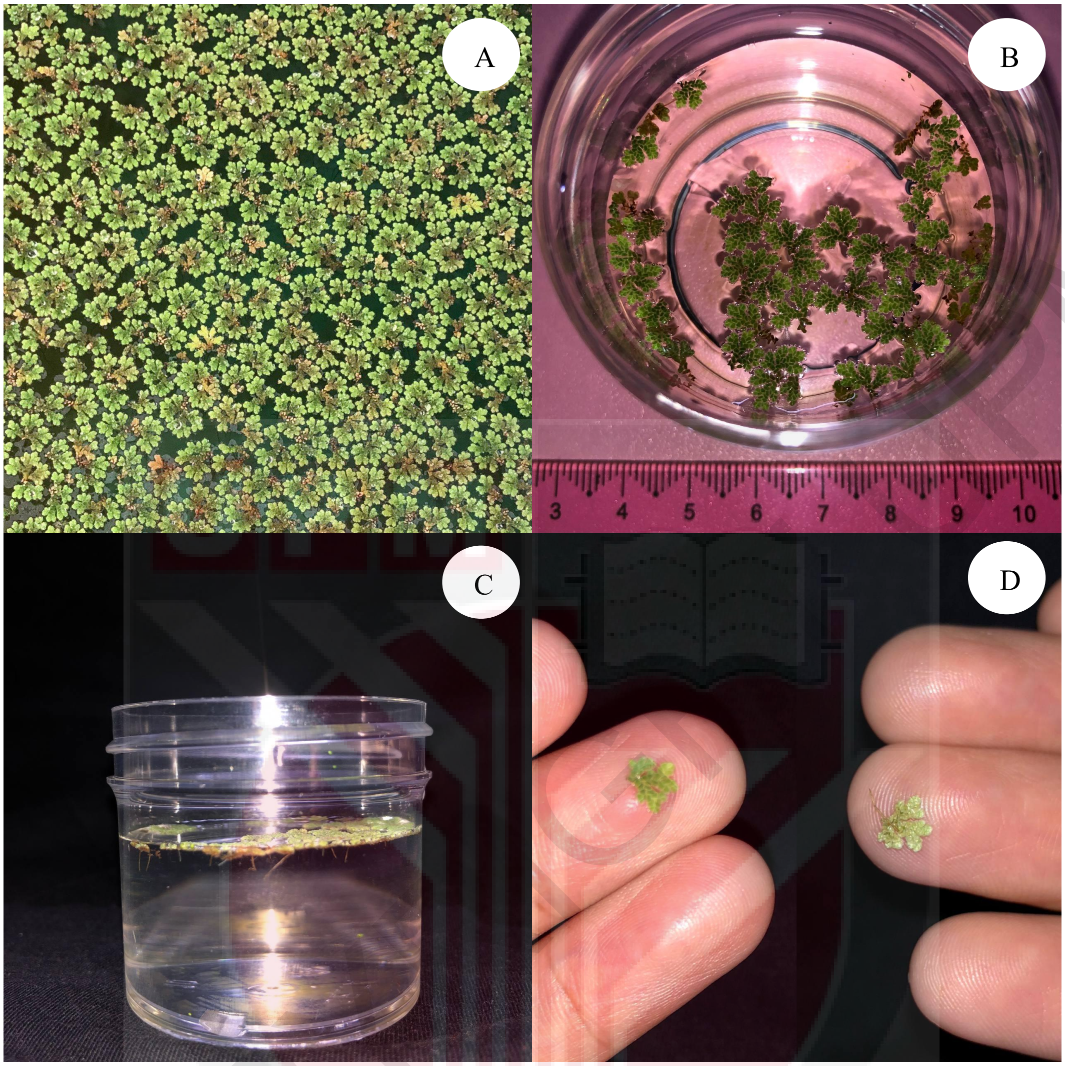


Figure 4.1.1: (A) Closer look of collected *Azolla sp*. (B) Top view of collected *Azolla sp*. (C) Side view and closer look into the root of collected *Azolla sp*. (D) Top and bottom view of collected *Azolla sp*.

4.1.2 *Wolffia globosa*

Wolffia globosa measured 0.05 – 0.08 cm (0.065 ± 0.015) frond length, 0.04 – 0.06 cm (0.05 ± 0.01) frond width. *Wolffia globosa* had light green colour.

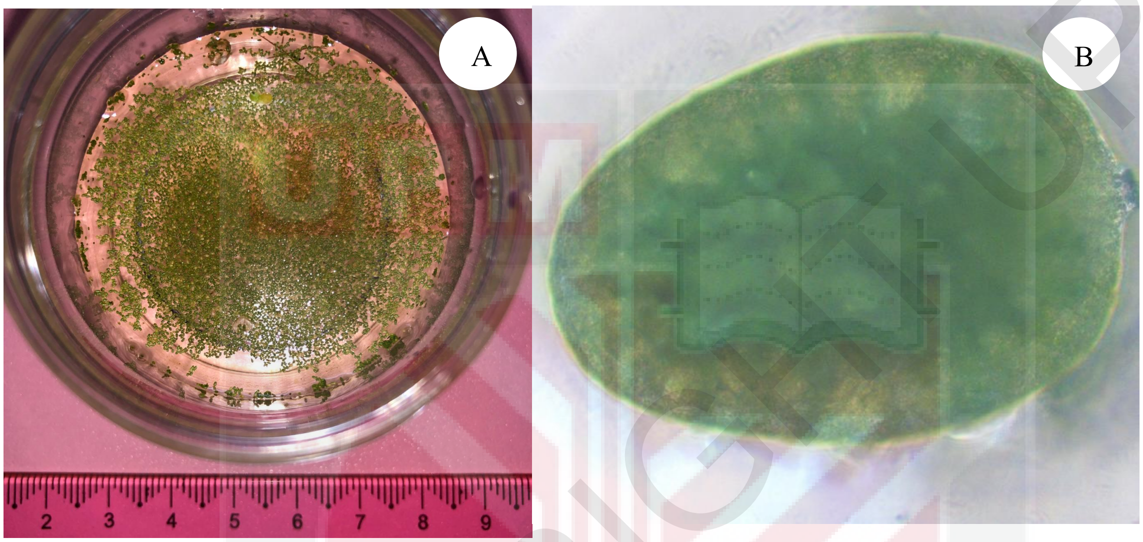


Figure 4.1.2: (A) Top view of collected *W. globosa.* (B) Closer look using microscope of collected *W. globosa.*

4.1.3 *Lemna minor L.*

Lemna minor L. measured 0.3 – 0.4 cm (0.35 ± 0.05) frond length, 0.2 – 0.25 cm (0.22 ± 0.02) frond width and 0.25 – 0.3 (0.27 ± 0.03cm) in the number of contiguous fronds. Have 1 root per frond. *Lemna minor L.* fronds were a pale green color, and they had obovate and symmetrical shapes. The roots were simple but also had stolons.

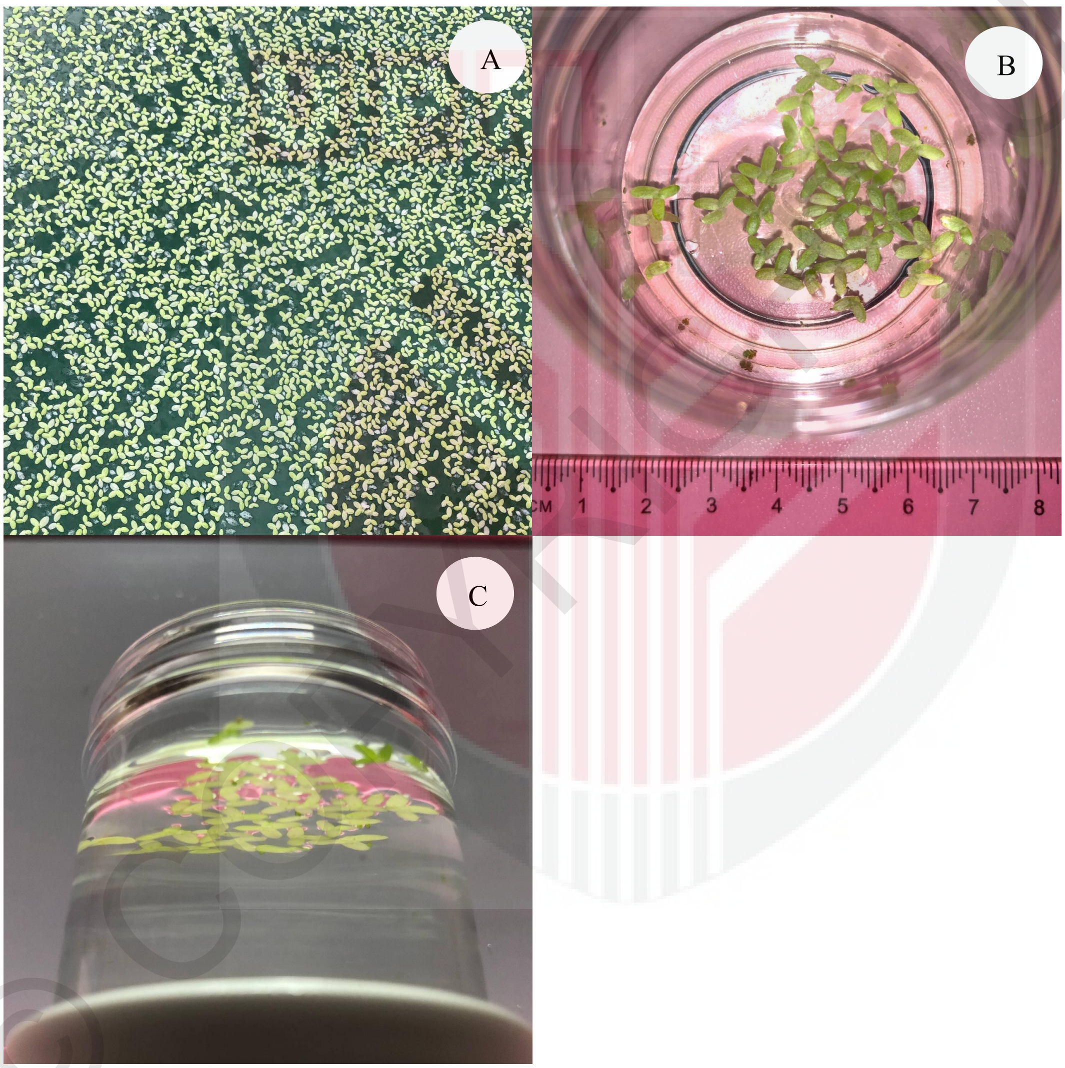


Figure 4.1.3: (A) Closer look of collected *Lemna minor L*. (B) Top view of collected *Lemna minor L*. (C) Bottom view of collected *Lemna minor L*.

4.1.4 ***Spirodela polyrrhiza***

Spirodela polyrrhiza measured 0.9 – 1.5 cm (1.2 ± 0.3) frond length, 1 – 1.5 cm (1.25 ± 0.25) root length. The fronds had bright green colour while the frond shape was obvate and symmetrical. The root structure was complex and have (7-12 roots) per frond.

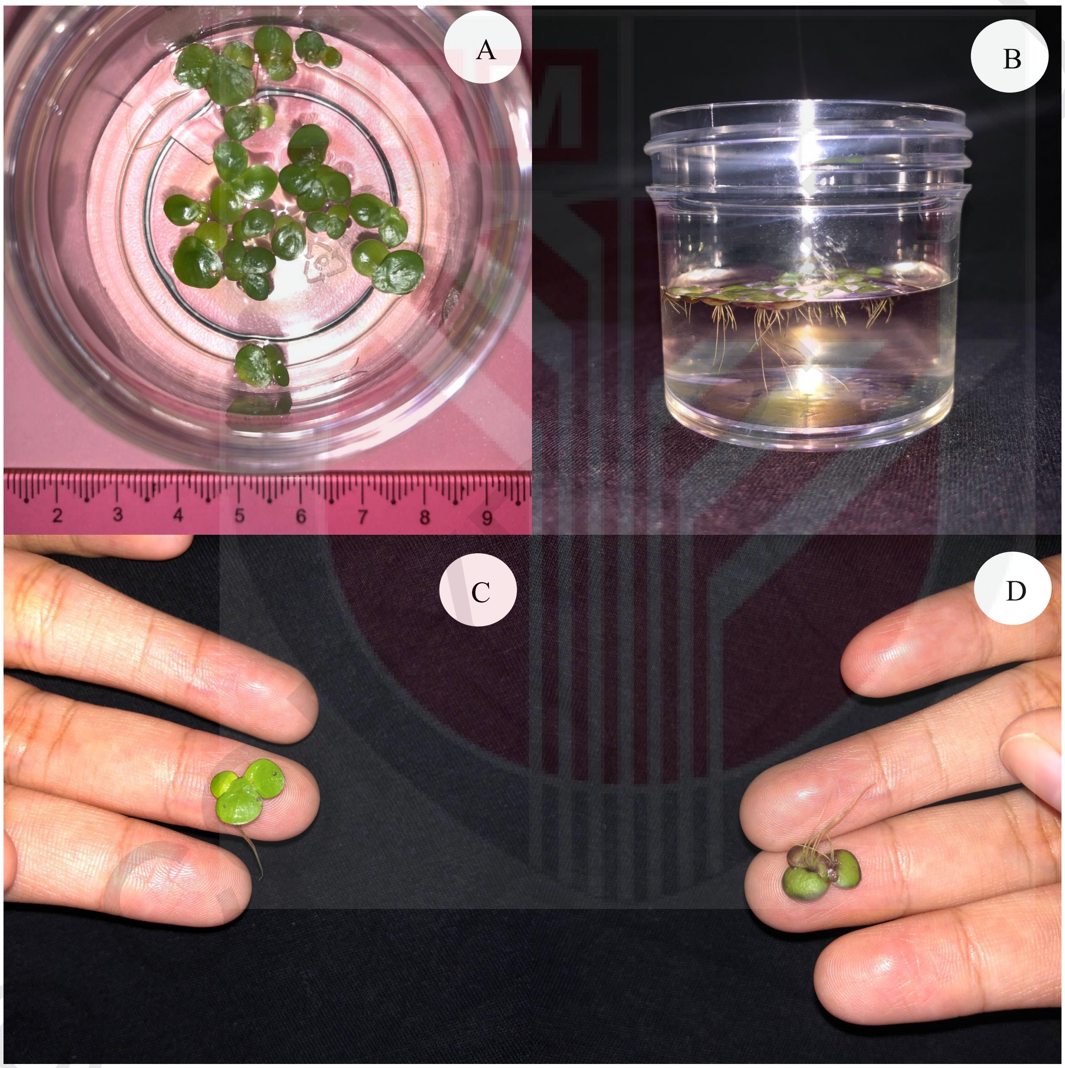


Figure 4.1.4: (A) Closer look of collected *Spirodela polyrrhiza*. (B) Side view and closer look into the root of collected *Spirodela polyrrhiza*. (C) Top view of collected *Spirodela polyrrhiza*. (D) Bottom view of collected *Spirodela polyrrhiza*.

CHAPTER 5: DISCUSSION

The results obtained from the three different sample areas, namely Hatchery UPMKB, Wastewater Taman Putrajaya, and Wastewater Taman Bandar Jaya, reveal distinctive patterns in the presence of freshwater plant species. In Hatchery UPMKB, Azolla is identified as present, while *Wolffia* and *Lemna* species are absent. Conversely, in Wastewater Taman Putrajaya, *Azolla* is absent, but Wolffia and *Lemna* species are observed. Notably, *Spirodela polyrhi*za is also present in this wastewater, introducing an additional element to the aquatic plant community. Similarly, Wastewater Taman Bandar Jaya exhibits the absence of Azolla but the presence of Wolffia and Lemna species, along with Spirodela polyrhiza.

5.1 *Azolla*

The results obtained in this study provide detailed morphological characteristics of *Azolla sp.,* specifically focusing on individual frond length, width, and the number of contiguous fronds, as well as root length and overall shape. The findings indicate a frond length ranging from 0.1 to 0.2 cm, a frond width spanning 0.05 to 0.1 cm, and 6 to 10 contiguous fronds. The roots vary in length from 1.0 to 2.5 cm. The coloration of *Azolla sp*. is described as brown near the root and green in the fronds, exhibiting intricate and feather-like shapes with bilateral symmetry. When comparing these results with existing studies, there are notable differences. Pereira et al., (2011) describe *Azolla pinnata* leaves as 1-2 mm long, overlapping in a two-ranked pattern, which contrasts with the findings of this study. Additionally, *Azolla filiculoides* is reported to have leaves that are 1-2 mm long, overlapping in 2 ranks, and displaying a feathery appearance, differing from the intricate shapes observed in the current study. The characteristics of *Azolla nilotica*, such as a frond length of 2-5 cm and horizontal growth, also differ from the results obtained here. Considering these disparities, it is challenging to definitively identify the species of Azolla in the current study.

5.2 *Wolffia*

In comparing the measured characteristics of *Wolffia sp*. in this study with the identification criteria provided by (Bog et al., 2013; Landolt, 2000; Lansdown et al., 2022) for various *Wolffia* species in Europe, it becomes evident that the frond length (0.05 – 0.08 cm) and frond width (0.04 – 0.06 cm) align closely with the specifications for *W. globosa*. The observed light green color of *Wolffia* sp. is a distinctive feature in accordance with *W. globosa*, described as having a pale green color with a translucent surface and green lower color. Additionally, the length-to-width ratio (1.33 to 2.33) and depth-to-width ratio (0.75 to 1.33) observed in *Wolffia sp*. fall within the specified ranges for *W. globosa*. Despite distinct characteristics outlined for other *Wolffia* species, the combined evidence from frond dimensions and coloration strongly supports the conclusion that the *Wolffia* species in this study is likely *W. globosa*.

However, when contrasting the measured dimensions and coloration of *Wolffia sp*. with the specified characteristics of *W. arrhiza, W. australiana, and W. columbiana*, discrepancies emerge. Notably, *W. arrhiza* displays a larger size range with a length of 0.7 to 1.3 mm and width of 0.6 to 1.0 mm, featuring a bright green coloration with an opaque surface – characteristics not observed in *Wolffia sp*. Similarly, *W. australiana*, characterized by a surface color of bright green and an opaque surface, differs in size (1.0 to 1.3 mm in length, 0.5 to 0.7 mm in width) from the measured dimensions of *Wolffia sp*. Finally, *W. columbiana*, known for its pale green color and translucent surface, exhibits dimensions (0.7 to 1.2 mm in length, 0.6 to 1.1 mm in width) inconsistent with the findings of this study. These discrepancies underscore the need for further investigation to determine the precise species of Wolffia in this study, possibly indicating the presence of an unidentified or yet-to-be-described species within the Wolffia genus.

5.3 *Lemna spp.*

The comprehensive comparison between the obtained results for *Lemna sp*. and other studies on *Lemna* species reveals both intriguing differences and notable similarities. The measurements obtained for *Lemna sp*. showcase fronds with lengths ranging from 0.3 to 0.4 cm, widths from 0.2 to 0.25 cm, and a mean number of contiguous fronds at 0.27 ± 0.03 cm, each with a single root. These findings align closely with characteristics attributed to *Lemna minor* in (Landolt, 2000) , particularly in terms of frond shape, size, and the presence of one root per frond. Additionally, the pale green color and symmetrical, obovate shape of Lemna sp.'s fronds correspond with the descriptions provided for *Lemna minor*. Yet, differences are clear in the information provided by (Andriani et al., 2019) about other *Lemna* species such *as L. gibba* and *L. perpusilla*. In these species, frond lengths, root numbers, and asymmetric shapes are different from what was found in *Lemna sp*. *Lemna gibba*, differs in frond shape (asymmetric) (Landolt, 2000) and the presence of gibbous fronds, which contrasts with the observed symmetrical fronds in the study. *S. polyrrhiza*, on the other hand, demonstrates differences in root characteristics, having numerous roots (9-15) per frond (Andriani et al., 2019), which contrasts with *Lemna sp*'s single root per frond. The comparison also highlights the variability within species, as mentioned by Landolt, especially in the length/width ratio, which could overlap between *L. minor* and *L. gibba*, making precise species determination challenging without genetic analysis. Considering the consistency in features with *Lemna minor* and the absence of significant disparities, the *Lemna* species in the result findings appears to align most closely with *Lemna minor*.

CHAPTER 6: CONCLUSION

In conclusion, the investigation into the presence and morphological characteristics of freshwater plant species, namely *Azolla, Wolffia*, and *Lemna*, across three distinct sample areas in Bintulu, Sarawak, has revealed noteworthy patterns and variations. In Hatchery UPMKB, *Azolla* is identified, while *Wolffia* and *Lemna* species are absent. Conversely, Wastewater Taman Putrajaya exhibits the absence of *Azolla* but the presence of *Wolffia, Lemna* species, and *Spirodela polyrhiza*. Similarly, Wastewater Taman Bandar Jaya lacks *Azolla* but hosts Wolffia, Lemna species, and *Spirodela polyrhiza.*

The detailed morphological analysis of *Azolla* species highlighted variations in frond length, width, the number of contiguous fronds, root length, and overall shape. These findings, when compared with existing studies, revealed discrepancies in characteristics, making definitive species identification challenging. *Wolffia* species, particularly in Wastewater Taman Putrajaya, displayed characteristics aligning closely with *W. globosa,* but inconsistencies with *W. arrhiza, W. australiana, and W. columbiana* suggest the possibility of an unidentified or yet-to-be-described species within the *Wolffia* genus. For *Lemna* species, the results closely resembled *Lemna minor*, with variations from other *Lemna* species such as *L. gibba* and *L. perpusilla*. However, the inherent variability within species, especially in the length/width ratio, underscores the complexity of precise species determination without genetic analysis.

In summary, this study contributes valuable insights into the presence and morphological characteristics of commercially significant freshwater plant species in Bintulu, Sarawak. The identified patterns and variations provide a foundation for further research and underscore the need for a comprehensive understanding of these aquatic plants' ecology, diversity, and potential implications for environmental management in the region. Future investigations incorporating genetic analysis may aid in refining species identification and contribute to a more nuanced understanding of the biodiversity within the studied freshwater habitats.

REFERENCES

Abou, Y., Fiogbé, E. D., & Micha, J. C. (2007). Effects of stocking density on growth, yield and profitability of farming Nile tilapia, Oreochromis niloticus L., fed Azolla diet, in earthen ponds. *Aquaculture Research*, *38*(6), 595–604. https://doi.org/10.1111/j.1365-2109.2007.01700.x

Adzman, N., Goh, S. J., Johari, A., Zainal Alam, M. N. H., & Kamaruddin, M. J. (2022). Preliminary study on Azolla cultivation and characterization for sustainable biomass source. *Journal of Physics: Conference Series*, *2259*(1). https://doi.org/10.1088/1742-6596/2259/1/012018

Alahuhta, J., Lindholm, M., Baastrup-Spohr, L., García-Girón, J., Toivanen, M., Heino, J., & Murphy, K. (2021). Macroecology of macrophytes in the freshwater realm: Patterns, mechanisms and implications. In *Aquatic Botany* (Vol. 168). Elsevier B.V. https://doi.org/10.1016/j.aquabot.2020.103325

Andriani, Y., Irawan, B., Iskandar, Zidni, I., & Partasasmita, R. (2019). Short communication: Diversity of duckweed (Araceae-Lemnoideae), morphological characteristics and its potentials as food sources for herbivorous fishes in West Java, Indonesia. *Biodiversitas*, *20*(6), 1617–1623. https://doi.org/10.13057/biodiv/d200618

Azer, S. A. (2013). Taxonomic revision of genus Lemna L. (Lemnaceae Gray) in Egypt. *Annals of Agricultural Sciences*, *58*(2), 257–263. https://doi.org/10.1016/j.aoas.2013.07.015

Azhari, N. A. A., Shamsudin, R., Kasim, S., & Anuar, M. S. (2023). Effect of Different Drying Methods on Physical Properties of Azolla pinnata Powder. *Advances in Agricultural and Food Research Journal*, *4*(2). https://doi.org/10.36877/aafrj.a0000356

Azim, M. E., & Wahab, M. A. (2003). Development of a duckweed-fed carp polyculture system in Bangladesh. *Aquaculture*, *218*(1–4), 425–438. https://doi.org/10.1016/S0044-8486(03)00012-7

Azolla growth in farm dams. (2011). www.dpi.vic.gov.au

Balian, E. V., Segers, H., Lévèque, C., & Martens, K. (2008). The Freshwater Animal Diversity Assessment: An overview of the results. In *Hydrobiologia* (Vol. 595, Issue 1, pp. 627–637). Springer Netherlands. https://doi.org/10.1007/s10750-007-9246-3

Beger, M., Grantham, H. S., Pressey, R. L., Wilson, K. A., Peterson, E. L., Dorfman, D., Mumby, P. J., Lourival, R., Brumbaugh, D. R., & Possingham, H. P. (2010). Conservation planning for connectivity across marine, freshwater, and terrestrial realms. In *Biological Conservation* (Vol. 143, Issue 3, pp. 565–575). https://doi.org/10.1016/j.biocon.2009.11.006

Bog, M., Schneider, P., Hellwig, F., Sachse, S., Kochieva, E. Z., Martyrosian, E., Landolt, E., & Appenroth, K. J. (2013). Genetic characterization and barcoding of taxa in the genus Wolffia Horkel ex Schleid. (Lemnaceae) as revealed by two plastidic markers and amplified fragment length polymorphism (AFLP). *Planta*, *237*(1), 1–13. https://doi.org/10.1007/s00425-012-1777-9

Bornette, G., & Puijalon, S. (2009). Macrophytes: Ecology of Aquatic Plants. In *Encyclopedia of Life Sciences*. Wiley. https://doi.org/10.1002/9780470015902.a0020475

Brogan, W. R., & Relyea, R. A. (2015). Submerged Macrophytes Mitigate Direct and Indirect Insecticide Effects in Freshwater Communities. *PLOS ONE*, *10*(5), e0126677. https://doi.org/10.1371/journal.pone.0126677

Brownsey, P. J. ; P. L. R. (2013). *Azolla rubra*.

Cabrera, L. I., Salazar, G. A., Chase, M. W., Mayo, S. J., Bogner, J., & Dávila, P. (2008). Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. *American Journal of Botany*, *95*(9), 1153–1165. https://doi.org/10.3732/ajb.0800073

Carrapico, F. J. (2002). Azolla-anabaena-bacteria system as a natural microcosm. In R. B. Hoover, G. V Levin, R. R. Paepe, & A. Yu. Rozanov (Eds.), *Instruments, Methods, and Missions for Astrobiology IV* (Vol. 4495, pp. 261 – 265). SPIE. https://doi.org/10.1117/12.454763

Ceschin, S., Abati, S., Leacche, I., & Zuccarello, V. (2018). Ecological comparison between duckweeds in central Italy: The invasive *Lemna minuta* vs the native *L. minor*. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, *152*(4), 674–683. https://doi.org/10.1080/11263504.2017.1317671

Ceschin, S., Leacche, I., Pascucci, S., & Abati, S. (2016). Morphological study of Lemna minuta Kunth, an alien species often mistaken for the native L. minor L. (Araceae). *Aquatic Botany*, *131*, 51–56. https://doi.org/10.1016/j.aquabot.2016.01.005

Chew, M. Y. (2010). *Ecological Implications from the Naturalisation of Noxious Cabomba Waterweeds in Malaysia*. https://www.researchgate.net/publication/265123724

Chong, V. C., Lee, P. K. Y., & Lau, C. M. (2010). Diversity, extinction risk and conservation of Malaysian fishes. *Journal of Fish Biology*, *76*(9), 2009–2066. https://doi.org/10.1111/j.1095-8649.2010.02685.x

Delnavaz, B., & Ataei Azimi, A. (2009). Alien and exotic Azolla in northern Iran. *African Journal of Biotechnology*, *8*, 187–190.

El-Leboudi, A. E., Abd-Elmoniem, E. M., Soliman, E. M., & El-Sayed, O. F. M. (2008). *The 3 rd International Conference on Water Resources and Arid Environments Removal of Some Heavy Metals from Treated Waste Water by Aquatic Plants*.

Forni, C., Braglia, R., Harren, F. J. M., & Cristescu, S. M. (2012). Stress responses of duckweed (Lemna minor L.) and water velvet (Azolla filiculoides Lam.) to anionic surfactant sodium-dodecyl-sulphate (SDS). *Aquatic Toxicology*, *110–111*, 107–113. https://doi.org/10.1016/j.aquatox.2011.12.017

Freshwater Animal Diversity Assessment. (n.d.-a).

Hasan, M. R., & Chakrabarti, R. (2009). *Use of algae and aquatic macrophytes as feed in small-scale aquaculture: a review Food and Agriculture Organization of the United Nations*. https://www.researchgate.net/publication/288653588

Hasmah Mohtar, S., Othman, R., Suliman, A., Azni Harmin, S., Akmal Suliman, N., Alias, R., Hanim Isa, M., Haulisah, S., & Nathan Meganathan, S. (n.d.). *Assessing the potential of duckweed as protein source for the establishment of halal fish feed*. https://www.researchgate.net/publication/348430453

Ismail, S. N., Abd Hamid, M., & Mansor, M. (2018). Ecological correlation between aquatic vegetation and freshwater fish populations in Perak river, Malaysia. *Biodiversitas*, *19*(1), 279–284. https://doi.org/10.13057/biodiv/d190138

Ivan, K., & Katya, V. (2013). wolffia globosa (roxburgh) hartog et plas (lemnaceae): a new species in bulgarian flora. *Journal of Biological & Scientific Opinion*, *1*(4), 356–357. https://doi.org/10.7897/2321-6328.01416

Jain, M. S., & Kalamdhad, A. S. (2018). Efficacy of batch mode rotary drum composter for management of aquatic weed (Hydrilla verticillata (L.f.) Royle). *Journal of Environmental Management*, *221*, 20–27. https://doi.org/10.1016/j.jenvman.2018.05.055

Jamion, A., Idden Shah Ismail, F., Salwani Ab Wahid, N., & Sofiah Abu Kassim, N. (2021). A Preliminary Study of Potential Aquatic Macrophytes in Phytoremediation of Lead in The Muar River. In *Malaysian Journal of Chemistry* (Vol. 23, Issue 2).

Jha, Y. (2021). Macrophytes as a potential tool for crop production by providing nutrient as well as protection against common phyto pathogen. *Highlights in BioScience*, bs202103. https://doi.org/10.36462/h.biosci.202103

Landolt. (2000). *Lemnaceae Gray. In: Flora of North America Editorial Committee (eds.)*. Flora of North America North of Mexico, Vol. 22: Magnoliophyta: Alismatidae, Arecidae, Commelinidae (in Part), and Zingiberidae. New York and Oxford.

Lansdown, R. V, Kitchener, G., & Jones, E. (2022). Wolffia columbiana and W. globosa (Araceae) new to Britain. In *British & Irish Botany* (Vol. 4, Issue 1).

Madeira, P., Center, T., Coetzee, J., Pemberton, R., Purcell, M., & Hill, M. (2013). Identity and origins of introduced and native Azolla species in Florida. *Aquatic Botany*, *111*, 9. https://doi.org/10.1016/j.aquabot.2013.07.009

Majhi, P., Gogoi, P., Lianthuamluaia, L., Jana, C., Tayung, T., Kumari, S., Sarkar, U. K., & Das, B. K. (2023). Assessing spatio-temporal dynamics of phytoplankton assemblage and ecohydrological interaction in a community managed reservoir using GIS for sustainable fisheries management. *Ecohydrology and Hydrobiology*, *23*(1), 109–126. https://doi.org/10.1016/j.ecohyd.2022.11.002

Mandal, R. N., Datta, A. K., Sarangi, N., & Mukhopadhyay, P. K. (n.d.-a). *Diversity of aquatic macrophytes as food and feed components to herbivorous fish-a review*.

Mirzaeva, D. A., Maksumkhodjaeva, K. S., Khujamshukurov, N. A., Gazieva, Sh. Q., Abdullaev, X. O., Iskhakova, Sh. X., & Kuchkarova, D. Kh. (2020). Nutritional Value of Lemnaceae Macrophytes. *International Journal of Current Microbiology and Applied Sciences*, *9*(4), 3233–3242. https://doi.org/10.20546/ijcmas.2020.904.376

Muradov, N., Taha, M., Miranda, A. F., Kadali, K., Gujar, A., Rochfort, S., Stevenson, T., Ball, A. S., & Mouradov, A. (2014). Dual application of duckweed and azolla plants for wastewater treatment and renewable fuels and petrochemicals production. *Biotechnology for Biofuels*, *7*(1). https://doi.org/10.1186/1754-6834-7-30

Murphy, K., Efremov, A., Davidson, T. A., Molina-Navarro, E., Fidanza, K., Crivelari Betiol, T. C., Chambers, P., Tapia Grimaldo, J., Varandas Martins, S., Springuel, I., Kennedy, M., Mormul, R. P., Dibble, E., Hofstra, D., Lukács, B. A., Gebler, D., Baastrup-Spohr, L., & Urrutia-Estrada, J. (2019). World distribution, diversity and endemism of aquatic macrophytes. *Aquatic Botany*, *158*. https://doi.org/10.1016/j.aquabot.2019.06.006

Mwale, M. (2013). *Characteristics of duckweed and its potential as feed source for chickens reared for meat production: a review*. https://doi.org/10.5897/SREX12.003

Naseem, S., Bhat, S. U., Gani, A., & Bhat, F. A. (2021). Perspectives on utilization of macrophytes as feed ingredient for fish in future aquaculture. In *Reviews in Aquaculture* (Vol. 13, Issue 1, pp. 282–300). Wiley-Blackwell. https://doi.org/10.1111/raq.12475

Nath Mandal, R. (n.d.). *Central institute of freshwater aquaculture*.

Nicolaus, M., & Edelaar, P. (2018). Comparing the consequences of natural selection, adaptive phenotypic plasticity, and matching habitat choice for phenotype–environment matching, population genetic structure, and reproductive isolation in meta-populations. *Ecology and Evolution*, *8*(8), 3815–3827. https://doi.org/10.1002/ece3.3816

Nordiah, B., Muta Harah, Z., Japar Sidik, B., & Wan Hazma, W. N. (2012). Azolla pinnata growth performance in different water sources. *Pakistan Journal of Biological Sciences*, *15*(13), 621–628. https://doi.org/10.3923/pjbs.2012.621.628

Ojha, M. L., Saini, V. P., Subodh, D., Sharma, K., Kumari, R., Ojha, M. L., & Sharma, S. K. (2017). Effect of azolla supplementation on growth of rohu (Labeo rohita) fingerlings. *Article in journal of entomology and zoology studies*, *5*(4), 1116–1119. https://www.researchgate.net/publication/319101842

Ong, Y. S., & Teng, S. T. (2022a). Morphological Characterisation and Documentation of Freshwater Macrophytes in Pontian, Johor's Water. *Borneo Journal of Resource Science and Technology*, *12*(1), 1–22. https://doi.org/10.33736/bjrst.4140.2022

Oyange, W. A., Kanya, J. I., Chemining'wa, G. N., & Njiruh, P. N. (n.d.-a). *Tropical and Subtropical Agroecosystems 23 (2020): #70 morphological and molecular characterization of azolla accessions in kenya † [caracterización morfológica y molecular de accesiones de azolla en kenia]*. www.cabi.org/isc

Oyedeji, A. A., & Abowei, J. F. N. (2012). The Classification, Distribution, Control and Economic Importance of Aquatic Plants. *International Journal of Fisheries and Aquatic Sciences*, *1*(2), 118–128.

Pereira, A. L., Martins, M., Oliveira, M. M., & Carrapiço, F. (2011). Morphological and genetic diversity of the family Azollaceae inferred from vegetative characters and RAPD markers. *Plant Systematics and Evolution*, *297*(3–4), 213–226. https://doi.org/10.1007/s00606-011-0509-0

Poveda, J. (2022a). The use of freshwater macrophytes as a resource in sustainable agriculture. In *Journal of Cleaner Production* (Vol. 369). Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2022.133247

Rahman, A. H. M. M., Rahman, A. H. M. M., Islam, A. K. M. R., Naderuzzaman, A. T. M., Hossain, M. D., & Afza, R. (2007). Studies on the Aquatic Angiosperms of the Rajshahi University Campus. In *Research Journal of Agriculture and Biological Sciences* (Vol. 3, Issue 5). https://www.researchgate.net/publication/268415423

Riaz, A., Khan, M. S., Saeed, M., Kamboh, A. A., Khan, R. U., Farooq, Z., Imran, S., & Farid, M. U. (2022). Importance of Azolla plant in poultry production. In *World's Poultry Science Journal*. Taylor and Francis Ltd. https://doi.org/10.1080/00439339.2022.2054752

Romano, L. E., & Aronne, G. (2021). The world smallest plants (Wolffia sp.) as potential species for bioregenerative life support systems in space. In *Plants* (Vol. 10, Issue 9). MDPI. https://doi.org/10.3390/plants10091896

Ruekaewma, N., Piyatiratitivorakul, S., & Powtongsook, S. (n.d.). Culture system for Wolffia globosa L. (Lemnaceae) for hygiene human food. In *Songklanakarin J. Sci. Technol* (Vol. 37, Issue 5). http://www.sjst.psu.ac.th

Saupi, N., Zakaria, M. H., Bujang, J. S., & Arshad, A. (2015). The proximate compositions and mineral contents of Neptunia oleracea Loureiro, an aquatic plant from Malaysia. *Emirates Journal of Food and Agriculture*, *27*(3), 266–274. https://doi.org/10.9755/ejfa.v27i3.16876

Schmitz, U., Köhler, S., & Hussner, A. (2014). First records of American Wolffia columbiana in Europe – Clandestine replacement of native Wolffia arrhiza? *BioInvasions Records*, *3*(4), 213–216. https://doi.org/10.3391/bir.2014.3.4.01

Shah, M., Hashmi, H. N., Ali, A., & Ghumman, A. R. (2014). Performance assessment of aquatic macrophytes for treatment of municipal wastewater. *Journal of Environmental Health Science and Engineering*, *12*(1). https://doi.org/10.1186/2052-336X-12-106

Sirirustananun, N. (2018). Appropriate Proportion of Water Meal (Wolffia arrhiza (L.)) and Commercial Diet in Combined Feeding for Tilapia Fingerlings Rearing. *International Journal of Agricultural Technology*, *14*(2), 249–258.

Sood, A., Uniyal, P. L., Prasanna, R., & Ahluwalia, A. S. (2012). Phytoremediation potential of aquatic macrophyte, Azolla. In *Ambio* (Vol. 41, Issue 2, pp. 122–137). https://doi.org/10.1007/s13280-011-0159-z

Sree, K. S., Maheshwari, S. C., Boka, K., Khurana, J. P., Keresztes, Á., & Appenroth, K.-J. (2015). The duckweed Wolffia microscopica: A unique aquatic monocot. *Flora - Morphology,* *Distribution, Functional Ecology of Plants*, *210*, 31–39. https://doi.org/10.1016/j.flora.2014.10.006

Sree, K. S., Sudakaran, S., & Appenroth, K. J. (2015). How fast can angiosperms grow? Species and clonal diversity of growth rates in the genus Wolffia (Lemnaceae). *Acta Physiologiae Plantarum*, *37*(10). https://doi.org/10.1007/s11738-015-1951-3

Srivastava, J., Gupta, A., & Chandra, H. (2008). Managing water quality with aquatic macrophytes. In *Reviews in Environmental Science and Biotechnology* (Vol. 7, Issue 3, pp. 255–266). https://doi.org/10.1007/s11157-008-9135-x

Su, W., Sun, Q., Xia, M., Wen, Z., & Yao, Z. (2018). The resource utilization of water hyacinth (Eichhornia crassipes [Mart.] Solms) and its challenges. In *Resources* (Vol. 7, Issue 3). MDPI AG. https://doi.org/10.3390/resources7030046

Subedi, P., Journals, A., & Shrestha, J. (2015). Improving soil fertility through Azolla application in low land rice: A review Azarian Journal of Agriculture Improving soil fertility through Azolla application in low land rice: A review. In *AJA* (Vol. 2). www.azarianjournals.ir

Swapna, M. M., Prakashkumar, R., Anoop, K. P., Manju, C. N., & Rajith, N. P. (2011). A review on the medicinal and edible aspects of aquatic and wetland plants of India. In *Journal of Medicinal Plant Research* (Vol. 5, Issue 33, pp. 7163–7176). https://doi.org/10.5897/JMPRx11.005

Thomas A, & Lumkin. (1981). *An Introduction to Azolla Nilotica*.

Wang, W., Kerstetter, R. A., & Michael, T. P. (2011). Evolution of Genome Size in Duckweeds (Lemnaceae) . *Journal of Botany*, *2011*, 1–9. https://doi.org/10.1155/2011/570319

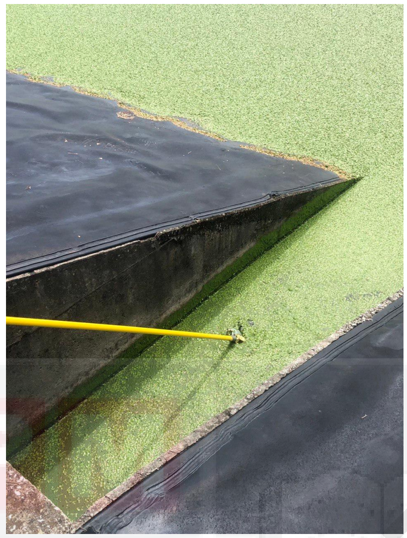
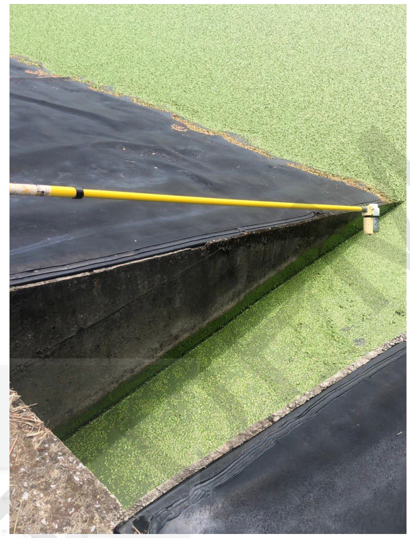
Yahaya, N., Hamdan, N. H., Zabidi, A. R., Mohamad, A. M., Suhaimi, M. L. H., Johari, M. A. A. M., Yahya, H. N., & Yahya, H. (2022). Duckweed as a future food: Evidence from metabolite profile, nutritional and microbial analyses. *Future Foods*, *5*. https://doi.org/10.1016/j.fufo.2022.100128

Yang, J., Zhao, X., Li, G., Hu, S., & Hou, H. (2021). Frond architecture of the rootless duckweed Wolffia globosa. *BMC Plant Biology*, *21*(1). https://doi.org/10.1186/s12870-021-03165-5

Zakaria, M. H., Ramaiya, S. D., Bidin, N., Syed, N. N. F., & Bujang, J. S. (2023). Nutritional values of wild edible freshwater macrophytes. *PeerJ*, *11*. https://doi.org/10.7717/peerj.15496

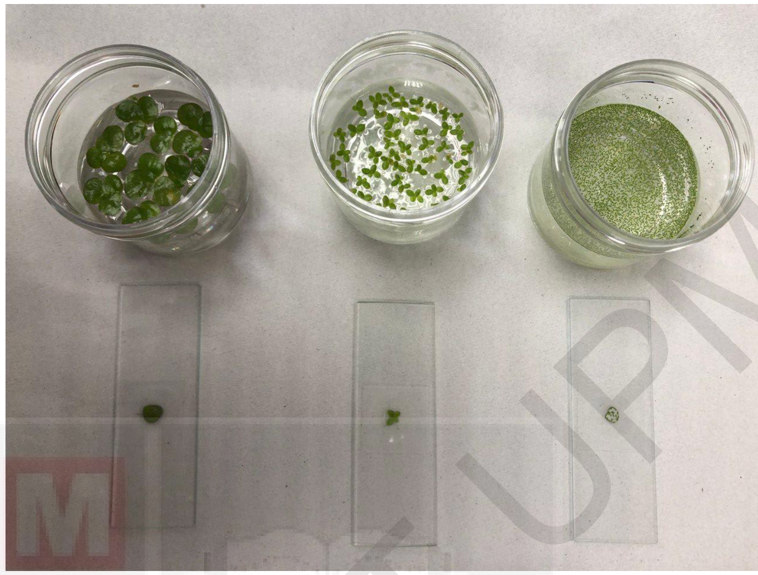
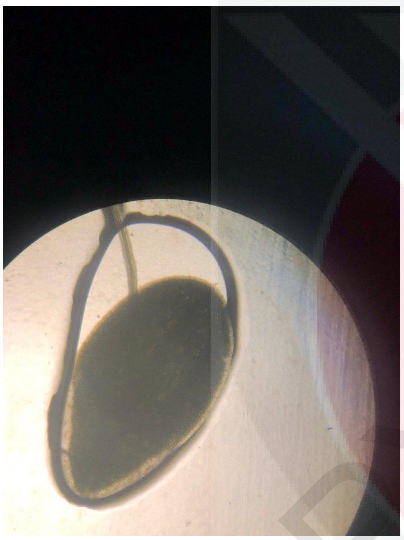
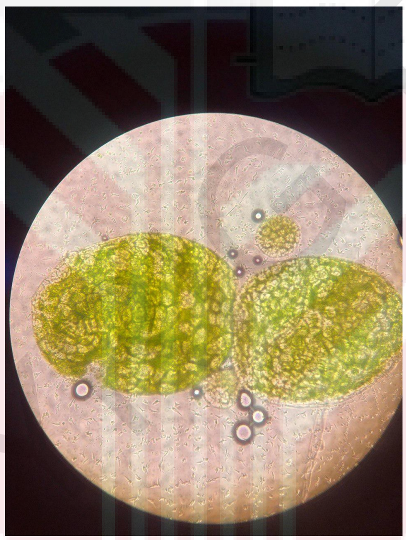
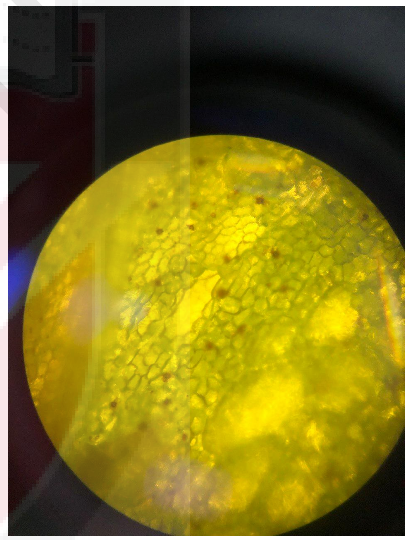
Zhang, Y., Liu, X., Wang, M., & Qin, B. (2013). Compositional differences of chromophoric dissolved organic matter derived from phytoplankton and macrophytes. *Organic Geochemistry*, *55*, 26–37. https://doi.org/10.1016/j.orggeochem.2012.11.007

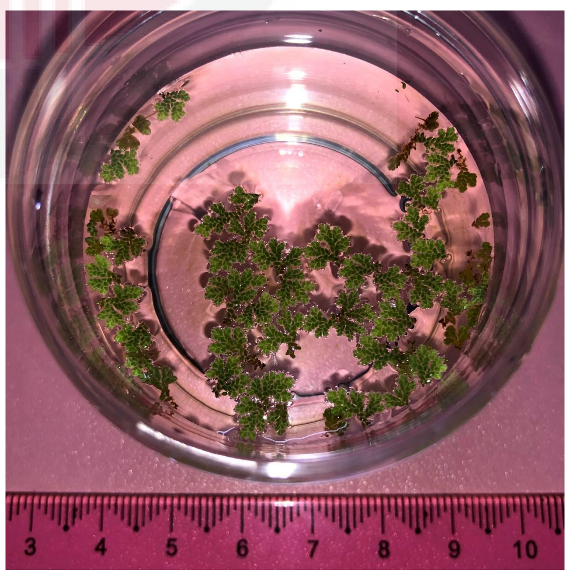
APPENDICES



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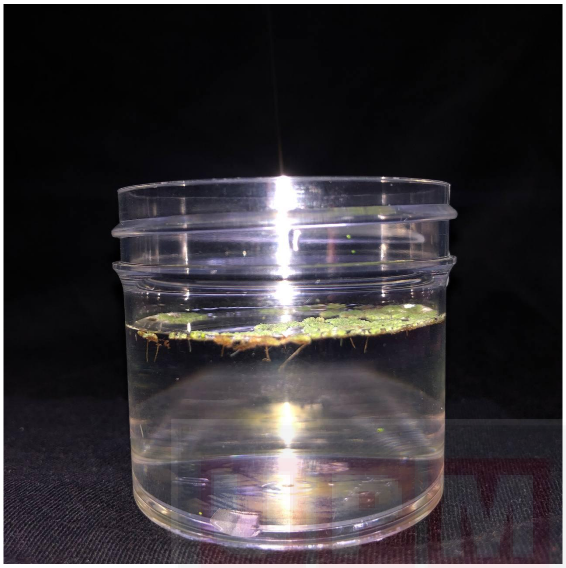

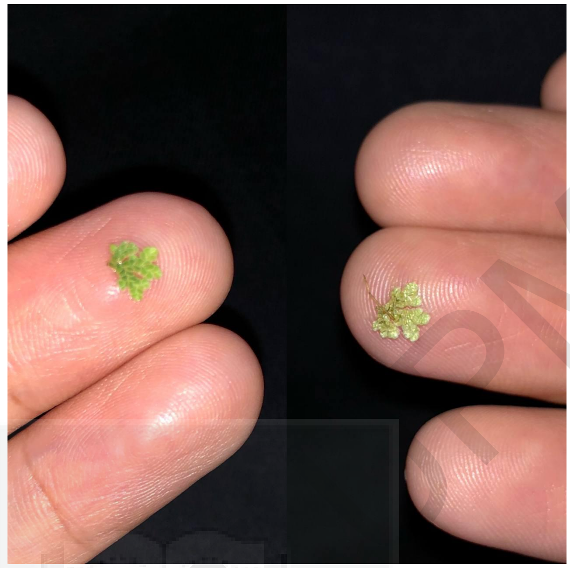

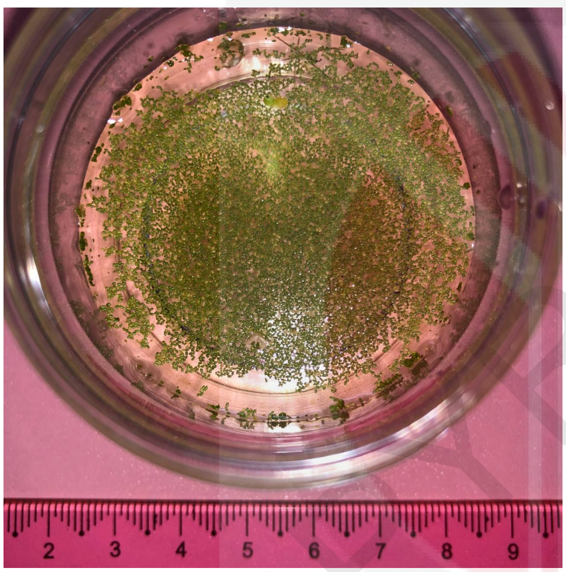
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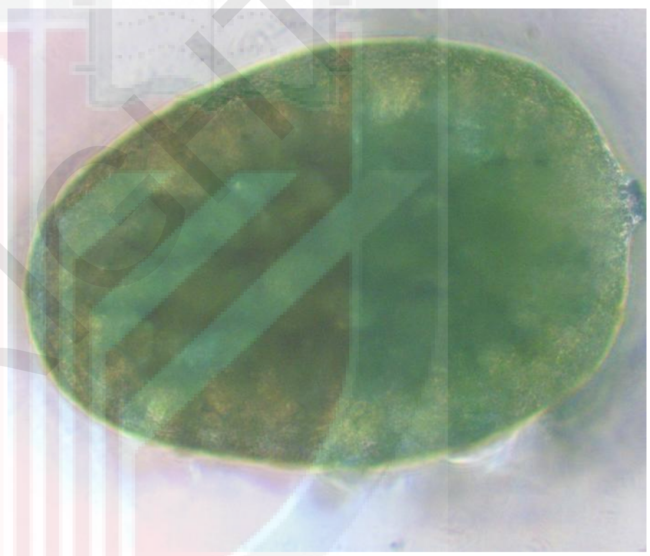

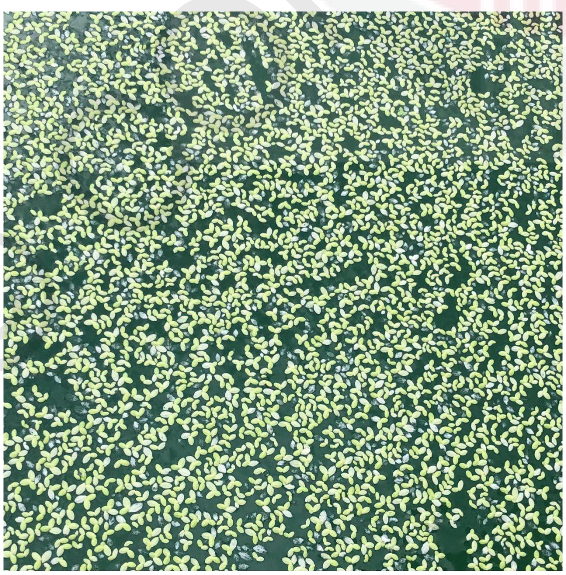

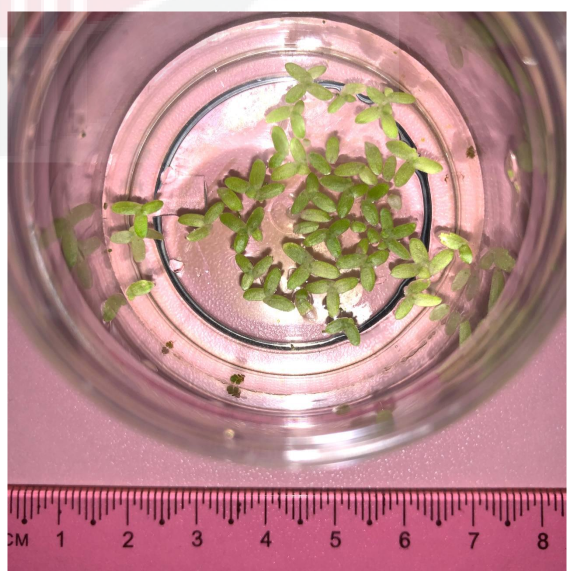
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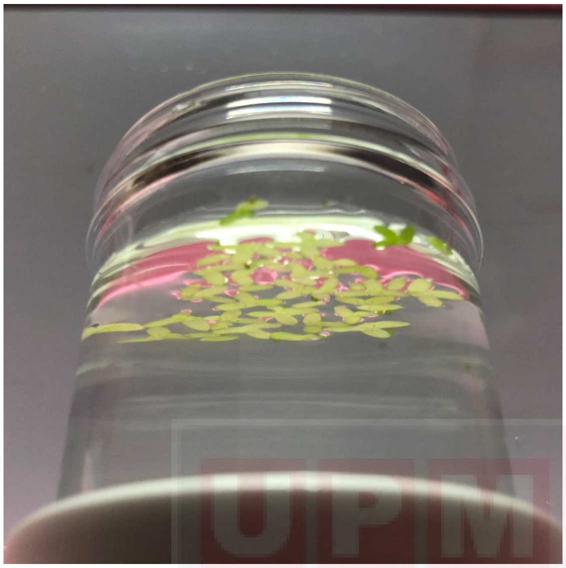

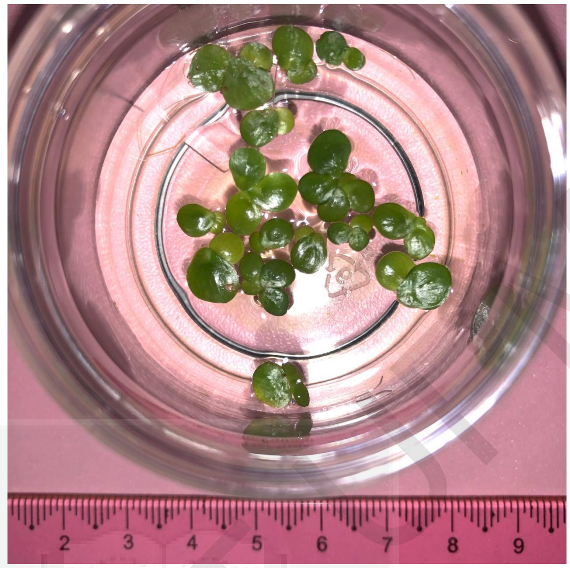
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