

Towards understanding the prospects of select macrophytes as potential fish feed

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Abstract

Aquaculture provides high-quality protein reasonably at cheap cost. But the intensive aquaculture is facing expensive feeding cost because the key aquadiet ingredient- fishmeal is becoming very costly. In this scenario application of aquatic plants as an alternative for fishmeal has the potential to revolutionize aquafarming in meeting protein demand globally. Macrophytes grow abundantly in all water bodies and are considered as a nuisance in most of eutrophic aquatic systems. In the current study the nutritional profile of five aquatic plants, namely *Azolla cristata*, *Ceratophyllum demersum*, *Nelumbo nucifera*, *Nymphaea mexicana* and *Trapa natans*, collected from the Dal Lake, Kashmir was evaluated. The proximate analysis was carried out as per standard methods of association of official analytical chemists (AOAC) and mineral analysis by atomic absorption spectroscopy. Results show that the crude protein lies in the range of 16–24% with *Nymphaea mexicana* showing the highest (24.13%) and *Ceratophyllum demersum* showing the lowest (16.07%) crude protein content. The crude lipid content ranges between 4–8% whereas ash content lies in the range of 11–37.74% with *C. demersum* showing the highest (37.74%). Among the investigated macrophytes, the highest levels of potassium, magnesium, iron, manganese, cadmium and copper were recorded in *C. demersum*. *Azolla cristata* was rich in sodium and zinc, and *Nelumbo nucifera* in cobalt. Omega-6 and omega-3 fatty acids were present in all the investigated macrophytes except *C. demersum*. Our findings revealed that macrophytes are rich sources of fatty acids, protein and minerals besides their readily availability and high productivity and it is argued here in this paper they can prove as viable candidates for aquafeed in the future.

Introduction

Feed input make up the major expenditure in commercial fisheries, constituting upto 70% of the total operational expenses (Naseem et al., 2020). Fishmeal has been accepted worldwide as key protein source in aquadiets owing to its high protein content and high essential amino acids (Perez-Velazquez et al., 2019). It is not a good news that utilization of fishmeal as the single protein source in aquafeeds is not possible as the yearly capture of silage fish used to make fishmeal declined from 23 million tons to 16 million tons between 2000 and 2017, and fishmeal prices have also consistently increased since 2012 (Naylor et al., 2021). Moreover, aquacultural industry competes with utilization of fishmeal in swine, poultry, and livestock diets and its productivity cannot go up without risking silage fish. Even with reduction in fishmeal inclusion in fish feeds, calculated scarcity ranging from 0.4 to 1.32 million metric tons of fishmeal might arise by 2050 (Jones et al., 2020). Consequently, increased price of fishmeal along with its dearth challenges the feed manufacturing industry and so the aquaculture as well. Further by the year 2100, the world population is projected to reach 11.2 billion which requires an increase of 200% in food production (Poveda, 2022). This growing demand leads to intensification of aquaculture. The sustainability of the fisheries and aquafarming depends on decrease in wild fish inputs into aquadiets (Stickney and Gatlin, 2022). Therefore, the current situation calls for identification of alternative protein sources for aquafeeds. However, alternate sources of protein should be able to be produced in large

amounts, economically competitive, contain required crude protein levels and balanced amino acid profiles and not jeopardise the growth and health of the fish.

Fish lipids provide all the essential fatty acids (EFAs) needed by humans for proper growth and development, as well as long chain polyunsaturated fatty acids (LC-PUFA), which have a variety of nutraceutical and pharmaceutical applications (Mukherjee et al., 2010). Although, aquafarming helps the world to produce enough PUFA to meet human nutritional needs; the only raw materials used to make aquafeeds that can give farmed fish long chain LC-PUFA are marine components- fishmeal and fish oil. It is not sustainable to use these expensive, scarce ingredients to maintain LC-PUFA levels in cultured fish (Tocher, 2015). Aquaculture presently consumes 75% of the world's fish oil, up from 55% (Bachis, 2017) creating more push for innovations in aquadiet formulation. It is possible to minimise the amount of fish oil used in aquafeeds while keeping the health advantages for consumers by using high omega-3 oils from algae or genetically engineered oilseeds, however, this is still economically ineffective and, in some areas, is limited by low consumer acceptance (Turchini et al., 2010). Therefore, aquaculture farmers must deal with the unanticipated environmental and social impacts of their feeding strategies for marketing sustainable goods (Naylor et al., 2021). In this regard, aquatic macrophytes are a viable non-traditional source of LC-PUFA for fish farming since they are readily available in abundance and provide the EFAs.

Scientists are therefore eying on macrophytes as the studies carried out in this line are showing early positive indications (Kumar et al., 2022; Haroon, 2020). Macrophytes are good choices as these have the ability to produce huge amount of biomass within a small span and have a promising biochemical profile.

Macrophytes being the main primary producers in aquatic systems contribute significantly to biodiversity at the ecosystem level (Hilt et al., 2022). These are normally found in nutrient enriched waters especially in shallow aquatic systems wherein they flourish and leading to consequences thereof. These plants have some merits also and converting these weeds to fecund use would be anticipated if it could somewhat compensate the mechanical removal expenses. Among other uses (Assad et al., 2020), there has been significant interest in utilizing aquatic plants as a feed source for livestock (Shah et al., 2010), poultry (Ara et al., 2018) and fish (Asimi et al., 2018).

Macrophytes serve as a base of aquatic food chain and are the potential food sources for humans like *Nelumbo nucifera*, *Nasturtium sp*, *Nymphaea mexicana*, *Ipomoea aquatica* and *Trapa natans* (Thomaz, 2021), and fodder for animals such as *Nymphoides peltata*, *Nymphaea alba*, *Phragmites australis*, *Hydrilla* (Shah et al., 2010). The protein derived from macrophytes could be used for monogastric animals or even human beings. Macrophytes are enriched with protein (11–32%), lipid (3-16.8%) in naturally grown macrophytes (Naseem et al., 2020), and up to 37% protein and 21% ash in cultivated macrophytes (Kumar et al., 2022). Aquatic plants possess a good range of amino acids, vitamins (Showqi et al., 2017), minerals and fatty acids (Haroon, 2020). Also, macrophytes are reported as a good carotenoids source, which include violaxanthin, cryptoxanthin, neoxanthin, xanthophylls, lutein epoxide, and β -carotene. These improve immunity and enhance the growth of fish (Ravelina et al., 2018). These

are the vital elements of food web dynamics (Dar et al., 2013). Macrophytes have been recognized as a natural resource for nutrient constituents (Haroon & Daboor, 2019) owing to their potential biochemical profile (Naseem et al., 2020). However, all the aquatic plants are not appropriate to be used as a source of food as they vary usually in their biochemical composition which depends upon part of plant used, species, location and season (Haroon, 2020). The assessment of proximate and mineral composition of macrophytes is vital for their preference as potent diet components for fish, poultry and livestock (Kumar et al., 2022). Therefore, study of biochemical composition of macrophytes is of importance so as to assay their potential as aquafeed ingredient. The objective of the study was to confirm whether the macrophytes can be used as an alternative for fishmeal or fish oil through evaluation of the proximate, mineral composition and fatty acid profile of these aquatic plants.

Aquatic macrophytes play an important role in preserving the hydrochemistry of aquatic systems by heavy metal removal (Eid et al., 2020). They take the benefit of root absorption, stem translocation, and entire body accretion and degradation. Most of the macrophytes have great capacity to scavenge heavy metals from wetland sediments and water. In comparison to the nearby waters, plants tend to accumulate relatively high heavy metal levels. Concerning their adverse effect on the dietary value, estimation of certain heavy metals was also carried out.

For the purpose of this paper the term fish represents all the commercially farmed aquatic animals which include prawns, shrimps and various types of fish.

Materials And Methods

Collection of sample plants

Macrophytes were collected manually in the month of October from the Dal Lake, Kashmir (Table 1, Plate 1). The plants were identified on the basis of morphological characters as *Azolla cristata*, *Ceratophyllum demersum*, *Nelumbo nucifera*, *Nymphaea mexicana* and *Trapa natans* and registered in the KASH Herbarium of Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, Hazratbal Srinagar under voucher specimen number 2856-(KASH), 2857-(KASH), 2858-(KASH), 2859-(KASH), 2860-(KASH) respectively. The macrophytes were cleaned to remove extraneous matter, air-dried for 72h and finally ground in the simple grinder. The ground powder was sieved with a fine mesh (1mm) and stored in zipper polythene bags at room temperature for analysis. The proximate analysis was carried out in the Aquatic Ecology Laboratory, Department of Environmental Science, University of Kashmir and mineral analysis was done at University Science Instrumentation Centre (USIC), University of Kashmir, and Research Centre for Residue and Quality Analysis (RCRQA), SKUAST-K.

Table 1

Macrophytes, with scientific, common names and flowering periods, which were collected from the Dal Lake for the evaluation of biochemical composition.

Plant	Scientific Name	Family	Common Name	Flowering Period
Free Floating	<i>Azolla cristata</i>	<i>Salviniaceae</i>	Mosquito Fern	May-September
Rooted Floating	<i>Nelumbo nucifera</i>	<i>Nelumbonaceae</i>	Lotus	May-September
	<i>Nymphaea mexicana</i>	<i>Nymphaeaceae</i>	Water Lily	July-September
	<i>Trapa natans</i>	<i>Trapaceae</i>	Water Chestnut	July-September
Submerged	<i>Ceratophyllum demersum</i>	<i>Ceratophyllaceae</i>	Hornwort	May-September

Proximate Analysis

The proximate analysis of plants was carried out using methods described by the Association of Official Analytical Chemists (AOAC, 2005). The crude protein (AOAC– 960.52) was calculated by Kjeldahl procedure ($N \times 6.25$), crude lipid (AOAC – 2003.05) was determined gravimetrically using Soxhlet Apparatus using petroleum ether (boiling point 60-80°C) as solvent. Ash content (AOAC – 923.03) was analysed by combusting the sample in muffle furnace at 540°C for 6h. Moisture content (AOAC – 925.10) was assayed by oven-drying the plant sample in petri plates at 105°C till constant weight was attained. The crude fibre content was evaluated by a method of acid and alkaline digestion.

Calculated Parameters

- Carbohydrate content (calculated as nitrogen free extract, NFE) was calculated by following the equation of Padua et al. (2004).

$$\% \text{ Carbohydrate} = 100 - (\% \text{ protein} + \% \text{ lipid} + \% \text{ ash})$$

- Digestible crude protein (DCP) was calculated by the equation of Demarquilly and Weiss, (1970).

$$\text{DCP (DW \%)} = 0.929 \times \text{CP} - 3.52$$

- Metabolizable energy (ME) was estimated by using the method followed by Haroon, (2020)

$$\text{ME (Kcal/gDW)} = \frac{3.4\text{NFE} + 8.1\text{CL} + 4.2\text{CP}}{100}$$

- Energy value of organic matter was calculated by using the method followed by Haroon, (2020)

$$\text{EV (Kcal/gDW)} = 9.45 \times \text{CL} + 4.10 \times \text{NFE} + 5.65 \times \text{CP}$$

Fatty Acid Analysis

The extracted crude lipids were esterified and the fatty acid methyl esters (FAMES) were quantified and identified by gas chromatography mass spectrometer (GC-MS, Shimadzu 17A GC, Japan). The esterification of fat was carried out by following work of Haroon, 2020. Briefly, 20mL of methanolic sulphuric acid (0.2M) was added to 0.2g extracted lipid in a sterilized screw capped tube. To this mixture, 2mL of benzene were added and mixed thoroughly. The tube was tightly closed and heated in a water bath at 90°C for 90min. Petroleum ether (10mL) was added to the cooled mixture, shaken vigorously and left undisturbed till the ether layer separated out. The ether layer was taken out in another sterilized dry tube, evaporated to dryness and analysis was performed in a GC-MS at Indian Institute of Integrative Medicine (IIIM), Jammu, under following conditions.

Detector: FID (Flame Ionization Detector)

Detector temperature: 250°C

Injection temperature: 250°C

Injection volume: 0.5µL

Column: DB-WAX (fused silica glass), 30 m × 250µm id, film thickness 0.25µm

Carrier gas: Helium, flow rate 1mLmin⁻¹

Oven program: 100°C

Fatty acids were recognised using standard retention times by comparing mass spectra to a standard library provided by the same institute. Results were calculated using the peak area and expressed as a proportion of each fatty acid (% total fatty acids).

Mineral Analysis

The mineral compositions of macrophytes were assayed using atomic absorption spectrometer (Agilent GTA 120, USA). The digestion of plants was carried out by the method followed by Ahmad et al. (2016). The powdered plant sample (1g) was digested with di acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 80°C till dense fumes appeared. The digested sample was allowed to cool at ambient temperature before being transferred to a 50ml volumetric flask, the volume was made by adding Milipore

ultrapure water. The digested samples were analysed for twelve metals (Mg, Ca, Na, K, Mn, Cu, Zn, Fe, Co, Cr, Ni, and Cd).

Results And Discussion

The proximate, fatty acid and mineral composition of the macrophytes revealed their nutritional importance (Table 2, Table 6a-c, Figs. 1 and 2). Proteins are vital for all biological processes and different organisms have varying requirement of protein as per age, species and culture environment (Lall and Dumas, 2022). In the current study, the protein content ranged between 16.06 to 24.12% which is lower than that required for aquadiets (32–52%). However, the protein requirement of fish depends on fish size, age, its physiological state, stocking density, temperature, protein quality, natural food availability, ration size, and non-protein energy levels. The optimum dietary protein requirement for grass carp and common carp lies in the range of 30–35% while for grass carp brood fish it is $\leq 25\%$ (www.fao.org/fishery/affris/species-profile). Though, the required protein content of shrimp is ranged between 27–35% but the commercial shrimp diets used in USA have 20–25% protein content. Some fish require much higher protein at early life stages such as *Penaeus japonicus* post larval stage (52–57%), *P. monodon* fingerling (55%), *Morone saxatilis* fingerling (55%) while others require low protein e.g., *Pangasius sutchi* juvenile (25%), *Ictalurus punctatus* grower (25%) *Mugil capito* juvenile (24%), *Macrobrachium rosenbergii* post larval stage (15%), *M. rosenbergii* fingerling (27%; Tacon, 1987). Therefore, macrophytes can be used as fishmeal substitute at one or other life stage either singly or in combination to suffice the requirement of fish.

While comparing with the previous studies, the protein content of *C. demersum* was higher than reported by Haroon, (2020) and similar to that reported by Olele, (2012). The protein content of *Azolla cristata* (20%) is lower than that reported by Ara et al. (2018), comparable to that reported by Kumari *et al.* (2018) and almost similar to the results shown by Bag et al. (2012) for *A. pinnata* (20.56%). On the other hand, the proximate composition of *N. mexicana* is close to the range reported by Stephen et al. (2017) for *N. lotus* (bulb), the little variation is due to difference at species level and also the part of plant assayed. The biochemical composition of *T. natans* is comparable to the results shown by Kalita et al. (2007), however, the protein content is higher in our study which is attributed to difference in geographical location since nutrient content in water affects crude protein content of the plant.

Lipids are a vital source of energy, as well as EFA and phospholipids, which act as a carrier for the absorption of fat-soluble vitamins. These are the precursors of various hormones and play a vital role in the cell and cell membrane structures. The optimum dietary requirement of crude lipid of fish lies in the range 7–15% (Craig et al., 2017). However, the dietary lipid requirement of common carp is 4.6%, grass carp 5%, mrigal 5–9%, and that of shrimps and prawns 2–8% (www.fao.org/fishery/affris/species-profile). In our study, the crude lipid content lies in the range of 4.6 to 8.22% showing that these macrophytes can meet dietary requirement of EFA of most of the farmed fish.

The quality of diets is ensured by studying the fatty acid profiles of feed items. Two critical fatty acids, n-3 (derived from alpha-linolenic acid, ALA), and n-6 (produced from linoleic acid, LA), cannot be synthesised by fish. Therefore, it is crucial to add these fatty acids to the diet to ensure proper growth and reproduction, as well as to maintain health and physiological functions. Diets rich in omega-3 fatty acids decrease lipid peroxidation and could enhance antioxidant ability in fish (Peng et al., 2016). In the current study omega-6 and omega-3 fatty acids were present in all the investigated macrophytes except *C. demersum*. Among SFAs, palmitic acid was the dominant one in all the macrophytes and highest content was found in *T. natans* (37.34%) followed by *A. cristata* (36.38%). Palmitic acid was also reported as dominant SFA by Kumar et al. (2022). Among the assayed macrophytes, MUFAs were highest in *Azolla cristata* while as in *Ceratophyllum demersum* and *Nelumbo nucifera* none of the MUFAs were determined. *Nymphaea mexicana* possessed the highest content of both n-3 and n-6 fatty acids among all the investigated macrophytes followed by *T. natans*. The fatty acid profile of the studied macrophytes supports their use as promising sources of nutraceuticals in aquafeed (Mukherjee et al., 2010).

Fibre is the key component of the plant cell wall and primarily constitutes carbohydrates. Lignin, cellulose and hemicellulose are types of primary constituents of plant fibre (Kung, 2014). Fibre is the ingredient that an animal cannot digest or absorb but it gives a diet physical bulk. Although, dietary requirement of fibre is not determined but high fibre diet has no reported negative impact on growth performance, gut histology and blood profile of fish (Bonvini et al., 2018). In contrary, leaf meal fibre revealed improved growth performance, indicating the prebiotic impact of using these leaf meals in hybrid lemon fin barbs (Jimoh et al., 2019). The freshwater prawns (*M. rosenbergii*) consume as high as 30% dietary fibre (Nesara and Paturi, 2018). The fibre content of five investigated plants lies in the range of 11–29%, with *N. nucifera* (29%) showing the highest fibre content and *N. mexicana* (11%) is having the least fibre content among all the five macrophytes.

Estimation of ash content is vital as it gives the mineral content in ingredients for quality assessment, nutrition labelling, microbiology stability and food processing as well (McClements et al., 2009). In our study, ash content was found in the range of 10.93 to 37.74%. Among all the investigated aquatic plants, ash content was higher in *C. demersum* which is attributed to its greater potential to accumulate metals. Its ash content was higher than reported by Haroon, (2020). Ash content of *A. cristata* (16.83%) is almost comparable to that reported by Das et al. (2018) for *A. pinnata* (17.3%) and that of *T. natans* is slightly lower than that reported by Kalita et al. (2007).

Carbohydrates are crucial for glycogen storage, and the production of fatty acids and steroids. Dietary carbohydrate requirement of *M. rosenbergii* for all stages is 25–35% (Nesara and Paturi, 2018). 35% dietary carbohydrate level is optimum for the hybrid lemon fin barb (Jimoh et al., 2019). Nile tilapias are capable of consuming high carbohydrate levels between 30 to 70 percent. The optimal dietary carbohydrate range for shrimp is 20 to 40% and for common carp it is 30–40 percent (Satoh, 2017). In the current study the carbohydrate content was found to be in the range of 38.36 to 64.78% which lies in the dietary requirement range of various aquatic animals.

Table 2
Mean values of proximate composition of the five macrophytes (%dry matter)

Macrophyte	Crude Protein	Crude Lipid	Ash	Moisture	Crude fibre	Nitrogen Free Extract	Organic Matter
<i>Azolla cristata</i>	20.00	7.25	16.83	12.14	26.50	55.92	83.17
<i>Ceratophyllum demersum</i>	19.30	4.6	37.74	7.75	23.78	38.36	62.26
<i>Nelumbo nucifera</i>	16.07	8.22	10.93	9.40	29.00	64.78	89.07
<i>Nymphaea mexicana</i>	24.13	5.1	19.16	11.25	11.02	51.16	80.84
<i>Trapa natans</i>	22.09	8.0	12.04	9.20	24.42	57.87	87.96

Table 3
Mean values of digestible crude protein (DCP), metabolizable energy (ME), energy value of organic matter (EV), (P/L), (P/NFE), (P/E) of the assayed aquatic plants expressed on dry matter basis

Macrophyte	DCP (%)	ME (Kcal/g)	EV (Kcal/g)	P/L (%)	P/NFE (%)	P/E (%)
<i>Azolla cristata</i>	15.06	3.38	323.43	2.76	0.36	0.062
<i>Ceratophyllum demersum</i>	14.41	2.49	225.70	4.20	0.50	0.085
<i>Nelumbo nucifera</i>	11.41	3.54	365.00	2.00	0.25	0.044
<i>Nymphaea mexicana</i>	18.89	3.17	287.73	4.73	0.47	0.084
<i>Trapa natans</i>	17.00	3.54	340.61	2.76	0.38	0.065

Table 4

Comparison of observed mean values of minerals of the five investigated plants with dietary requirement of fish

Macromineral (g/kg)	Element	Observed range	Dietary requirement	Reference
	Na	1.3 to 2.2	1 to 23	Snow and Ghaly (2008)
	K	22.3 to 30.63	5 to 15	Snow and Ghaly (2008)
	Ca	0.65 to 1.02	0.04 to 1.8	Hossain and Yoshimatsu, (2014)
	Mg	0.19 to 0.35	0.2 to 0.7	Dezfouli et al. (2019)
Microminerals (mg/kg)	Fe	200.5 to 2188	30–170	Lall and Kaushik, (2021)
	Cu	11.13 to 23.8 except <i>C. demersum</i> (28.77)	Upto 25	EFSA, (2016)
	Zn	19.14 to 46.33	20 to 115	Lall and Kaushik, (2021)
	Mn	10.83 to 136	15 to 240	Snow and Ghaly (2008)

Table 5

Comparison of heavy metal composition of the assayed macrophytes with the maximum permissible limit for feeding stuffs of fish

Heavy metal	Observed range (µg/g)	Maximum permissible limit (µg/g)	Reference
Cr	0.54 to 0.7	Not determined	Lall and Kaushik, (2021)
Cd	1.00 to 1.15	1.00	EFSA, (2016)
Co	0.04 to 0.4	1.04 (salmon feed)	EFSA, (2016)
		0.30 (trout feed)	EFSA, (2016)
Ni	0.55 to 0.8	Not determined	Lall and Kaushik, (2021)
Pb	Below detection level	0.07	EFSA, (2004)

Table 6a

Mean values of the saturated fatty acids of the macrophytes (%total fatty acids)

Macrophytes	Saturated fatty acids (SFA)					
	C14:0	C15:0	C16:0	C18:0	C20:0	Σ SFA
<i>Azolla cristata</i>	1.3	1.68	36.38	ND*	1.77	46.65
<i>Ceratophyllum demersum</i>	ND*	0.15	16.61	4.25	ND*	27.37
<i>Nelumbo nucifera</i>	ND*	ND*	29.15	6.03	4.22	42.11
<i>Nymphaea mexicana</i>	ND*	ND*	26.37	ND*	3.95	30.84
<i>Trapa natans</i>	0.94	0.13	37.34	0.52	ND*	38.93
*not determined						

Table 6b

Mean values of the monounsaturated fatty acids of the macrophytes (%total fatty acids)

Macrophytes	Monounsaturated fatty acids (MUFA)				
	C18:1 cis (n9)	C18:1 trans (n9)	C20:1 (n9)	C24:1 (n9)	Σ MUFA
<i>Azolla cristata</i>	7.29	1.7	ND*	ND*	8.99
<i>Ceratophyllum demersum</i>	ND*	ND*	ND*	ND*	0
<i>Nelumbo nucifera</i>	ND*	ND*	ND*	ND*	0
<i>Nymphaea mexicana</i>	0.98	ND*	ND*	1.70	2.68
<i>Trapa natans</i>	ND*	ND*	0.57	ND*	2.32
*not determined					

Table 6c
Mean values of the polyunsaturated fatty acids of the macrophytes (%total fatty acids)

Macrophytes	Polyunsaturated fatty acids (PUFA)			
	C18:2 (n6)	C18:3 (n3)	Σ PUFA	n3: n6
<i>Azolla cristata</i>	6.02	3.41	9.43	0.56
<i>Ceratophyllum demersum</i>	ND*	ND*	0	0
<i>Nelumbo nucifera</i>	7.91	11.87	19.78	1.50
<i>Nymphaea mexicana</i>	13.78	31.68	45.46	2.30
<i>Trapa natans</i>	13.65	22.90	36.55	1.67
*not determined				

Mineral Composition

The mineral composition of investigated five macrophytes is given in Figs. 1 and 2 and heavy metal concentration is shown in Fig. 3. Among the investigated macrophytes the highest levels of potassium (30.63g/kg), calcium (1.02g/kg), iron (2188 mg/kg), manganese (135mg/kg), cadmium (2.07µg/g) and copper (28.77mg/kg) were recorded in *C. demersum*. *Azolla cristata* was rich in sodium (2.17g/kg) and zinc (46.33mg/kg), and *Nelumbo nucifera* in cobalt (0.34µg/g). The magnesium was highest in *T. natans* (0.35g/kg).

Mineral nutrients are vital for nutritional quality. These are critical catalysts in a variety of biochemical processes. They are essential for growth and development, metabolism, and help animals to handle a variety of environmental settings (Skalnaya and Skalny, 2018). Minerals are categorised into three broad categories based on their contents in the human body namely, macro, trace, and ultra-trace minerals (Skalnaya and Skalny, 2018). Macrophytes are known for accumulation of metals in polluted water. The accumulation of metal in aquatic plants is function of growth and vitality, phenology, besides metal speciation and chemistry of water body (Cuadrado et al., 2019). The highest value for Ca was found in *C. demersum*; usually submerged plants have large quantities of macro elements probably due to extraneous metal precipitation in these plants. The comparison of observed values of minerals of the five investigated macrophytes with dietary requirement of fish is given in the Table 4. It is observed that the mineral composition of the macrophytes lies within the range of dietary requirement of fish except for K and Fe which are higher than that required by fish. However, the Fe content of *T. natans* (1011.71mg/kg) is comparable to that reported by Haroon (2020) for *Myriophyllum spicatum* (1060mg/kg). It can be inferred that the studied macrophytes could supply the essential minerals required for the development

and growth of fish. In fish, iron deficiency causes anaemia, magnesium deficiency causes skeletal deformities and nephrocalcinosis, manganese and cobalt deficiencies lead to poor growth; zinc deficiency results in depressed growth, reduced bone mineralization, skin and caudal fin erosion, and cataract, and deficiency of cobalt reduces haemoglobin formation (Kumar et al., 2022). Therefore, incorporation of macrophytes may be helpful to beat the mineral deficiency in fish. The comparison of observed values of heavy metals of the five investigated aquatic plants with maximum permissible limit for feed ingredients of fish feed is given in the Table 5. It is observed that heavy metals (Co, Cd, Pb) lie within the permissible limit and do not pose any threat to fish health.

Energy is vital for the maintaining life processes, so the potential of a diet to provide energy is essential in determining its nutrient value for animals. The growth of a healthy fish depends on providing them with the optimum energy in their diets. Since fish eat to meet their energy requirements, excessive dietary energy could result to elevated deposition of fat in the fish, reduced feed consumption, and lower weight gain. A diet with low energy content may result in decreased weight gain as the animal will use nutrients for energy provision rather than for tissue development or growth (Snow and Ghaly, 2008). The energy values of the organic matter of the investigated plants are shown in Table 3. In our study, metabolizable energy values (Kcal/g) were found to be higher in *Nelumbo* and *Trapa* (3.54 Kcal/g), *N. mexicana* (3.17 Kcal/g) and *Ceratophyllum* (2.49 Kcal/g) than that reported by Haroon (2010) for *P. stratiotus* and *N. lotus* (2.04, 2.05 Kcal/g, respectively). Energy requirements reported for *Penaeus indicus* is 3.50 to 4.00 Kcal/g (Alagarswamy and Ali, 2000) and that for common carp is 3.2–4.5 Kcal/g (Sato, 2017). The energy requirement of *M. rosenbergii* brood stock is 3.7-4.0 Kcal/g feed while for other stages it is 2.9–3.2 Kcal/g feed (Nesara and Paturi, 2018). Thus, macrophytes can provide the energy required for fish metabolism.

Protein to energy ratio (P/E) is critical for estimation of efficacy of feed ingredients, greater the ratio, more efficient is the diet (Mendez-Martinez et al., 2021). Among the studied plants, *N. mexicana* and *C. demersum* have higher P/E ratio. Thus, it can be inferred from our study that *N. mexicana* and *C. demersum* can be preferable for inclusion in fish feeds singly and others can be combined to achieve a higher P/E ratio.

The nutritional profile of the investigated macrophytes revealed that these can be substituted for fish oil and fishmeal in aquadiets for shrimps, prawns and fish at various life stages either individually or in combination with other macrophytes to meet the dietary needs of aquatic animals. Macrophytes are ideal option as the substitute of fishmeal with terrestrial plant protein sources (e.g., soybean) leads to higher pollution and ecotoxicity linked to the pesticides and fertilisers utilization during the plant growth phase (D'Agaro et al., 2022). However, macrophytes grow naturally in aquatic systems and do not involve use of any fertilizer or pesticide for their production. While it is of utmost significance to reduce environmental impacts to produce animal feeds sustainably, it is also desperately required to decrease competition with human food resources. Utilizing macrophytes as feedstuffs will play a vital role in an attempt to shift feed industry towards a viable future based on 'lower trophic' diet components (Parisi et al., 2020). Application of aquatic plant-based proteins primarily aims to offer an economical substitute for fishmeal

and fish oil. The economic efficacy of macrophyte derived proteins is based on increased profits, decreased production costs, and reduced environmental impacts. Utilization of untreated *Azolla* resulted in gradual reduction in feed cost as the supplementation levels of *A. pinnata* meal were increased in the diets of *Barbonymus gonionotus*. The prices of feed were reduced from 1337.61 to 97.58 US dollars because of lower production and processing cost of *Azolla* meal (Das et al., 2018). The substitution of 15% fishmeal in fish feeds with *Lemna minor* and *Ipomaea aquatica* showed positive impacts on the growth, feed consumption and productivity cost of *Oreochromis nilotica* and *Puntius gonionotus* (Yen et al., 2015). Also, incorporation of *Azolla* at 40% substitution level reduced feed production cost by 18.75% and 24.48% without compromising the growth and survival of *Cirrhinus mrigala* (Gangadhar et al., 2014) and *Labeo fimbriatus* respectively (Gangadhar et al., 2015). The above stated studies confirm the economic viability of macrophytes as fish feed. Despite the benefits offered by plant protein sources, there could be certain disadvantages such as presence of anti-nutrients and uneven nutritional profile. However, ANFs are reportedly found within tolerable range (Kalita et al., 2007) or otherwise can be brought to the acceptable limit by giving treatments such as solvent extraction, dry and wet heating, and solid-state fermentation (Naseem et al., 2020). Further, micronutrients and essential amino acids (EAAs) of plant based components can be enhanced by fermentation (Daniel, 2018) and certain macrophytes are reported to have EAA profile almost resembling to that of animal protein (Aslam et al., 2016; Asimi et al., 2018).

Despite the fact that fish have an antioxidant defence system to resist reactive free radicals, supplementing ingredients with radical quashing activity to diet can help to build up the defence mechanism. Macrophytes are reportedly enriched with flavonoids, polyphenols along with other bioactive phytochemicals. Polyphenol-rich feed improves immunological response, disease resistance, antioxidant defences and, reproductive and growth performance, digestive enzyme activities, gut microbial community diversity, and proximate body composition (Elumalai et al., 2020). Polyphenol chemicals prevent oxidation reactions by enhancing the activity of antioxidant enzymes or the production of antioxidant proteins, and can have synergistic antioxidant effects when combined with other substances. (Lv et al., 2021). Plant extracts acts as immunostimulants, anti-stressors, growth promoters, antimicrobial agent and stimulates appetite due to their bioactive components such as flavonoids, steroids, alkaloids, phenolics, carotenoids, terpenoids, and essential oils present in them (Chandan et al., 2020). In aquadiets, macrophytes could be a prospective source of nutraceuticals owing to their bioactive and fatty acid composition.

Aquaculture has the potential to have a direct environmental impact owing to the discharge of nutrient-rich waste waters into the adjoining environment—in general, aquatic habitats (Lennard and Goddek, 2019). The macrophytes can be involved in treatment of aquaculture wastewater, which can provide opportunities to recover the nutrients as a part of circular economy initiatives (Kurniawan et al., 2021b). The macrophytes can be grown either in hydroponic systems (Snow and Ghaly, 2008) or in ponds (Chakrabarti et al., 2018) or in outdoor cemented tanks (Kumar et al., 2022) and the resultant biomass can be used as feedstock. Aquaponics can have a decreased or no direct environmental impact from nutrient-rich waste streams as the fish- the major waste-generating component is connected with the

plant- a nutrient-use component (Lennard and Goddek, 2019, Goddek et al., 2015). Macrophytes such as *Eichhornia crassipes*, *Azolla pinnata*, *Carex virgata*, *Pragmites australis*, *Rotala rotundifolia*, *Pistia stratiotes*, *Ceratophyllum demersum*, *Trapa natans*, and *Lemna minor* have great potential for nutrient uptake from wastewater (Kurniawan et al., 2021b). *E. crassipes*, *P. stratiotes*, and *Myriophyllum aquaticum* were found to considerably reduce the pollution load of the aquaculture wastewater (Snow and Ghaly, 2008). The aquaculture effluent-raised *Azolla cristata* can substitute fishmeal up to 10% in *Pangasianodon hypophthalmus* diet, having improved growth performance, feed efficacy and nutrient utilization without compromising its feed consumption, survival and body composition (Rahmah et al., 2022). Therefore, macrophytes can be used for abatement of pollution caused by aquaculture effluents and simultaneously can be utilized as a protein input for cultured aquatic animals.

Conclusion

The proximate composition, macro and micromineral analysis and fatty acid profiling revealed that the macrophytes are reasonably rich in proteins, minerals and fatty acids. These could supply the protein and minerals necessary for fish growth, development and health in aquaculture ahead. The lipid profiles of these macrophytes suggest that they could be viable substitute for fish oil and thus have potential health benefits for fish and its consumers. The aquatic plants incorporated in the aquadiets could be an efficient, viable and inexpensive substitute for fishmeal to reduce the diet expenses.

Declarations

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

Authors declare that no ethical clearance is required for this work as no animal was involved in the study.

Authors' contributions

Sami Ullah Bhat and Adil Gani jointly conceived and designed the idea. Material preparation, data collection and analysis were performed by Shahida Naseem and wrote the draft of the manuscript after a thorough literature survey as well. Farooz Ahmad Bhat did some minor editing. Major editing was carried out by Sami Ullah Bhat however, at the end all authors read and approved the final manuscript before submission.

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article

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Plate

Plate 1 is available in the Supplemental Files section.

Figures

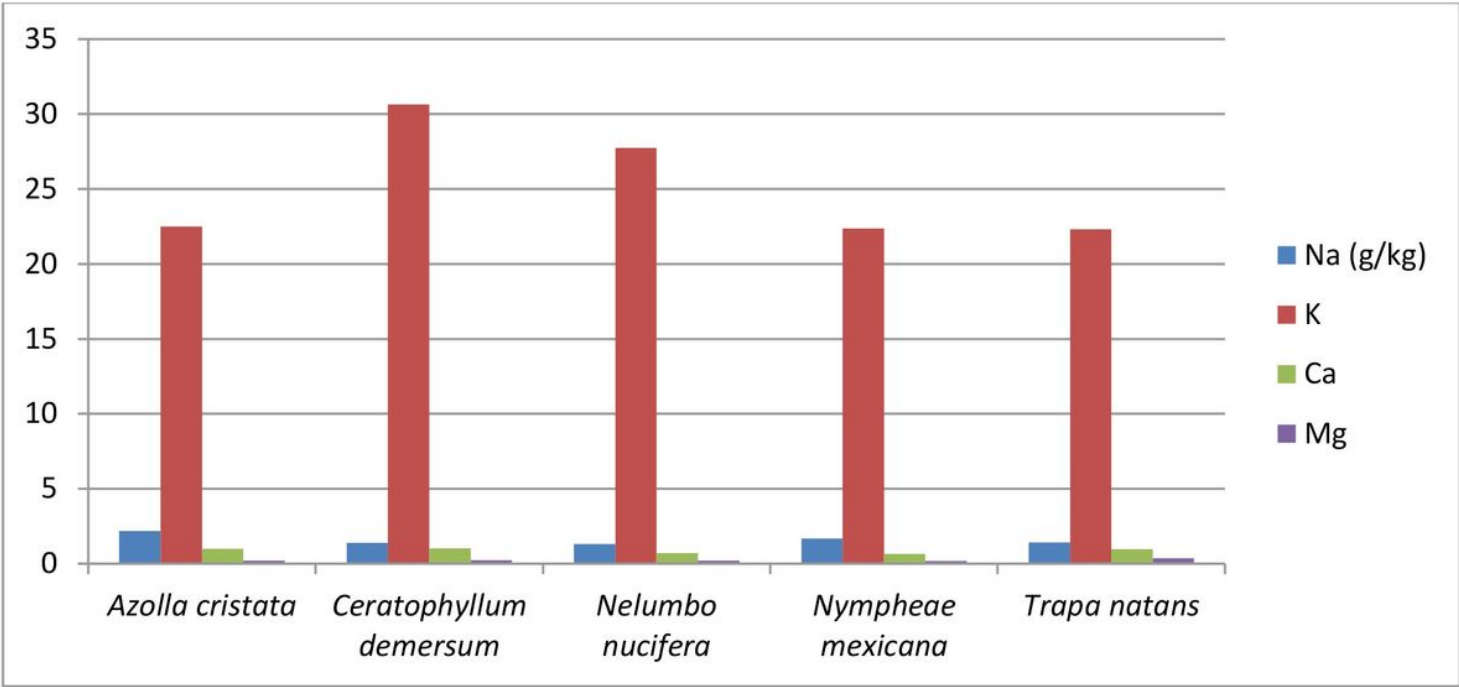


Figure 1

Macromineral contents of the five investigated macrophytes

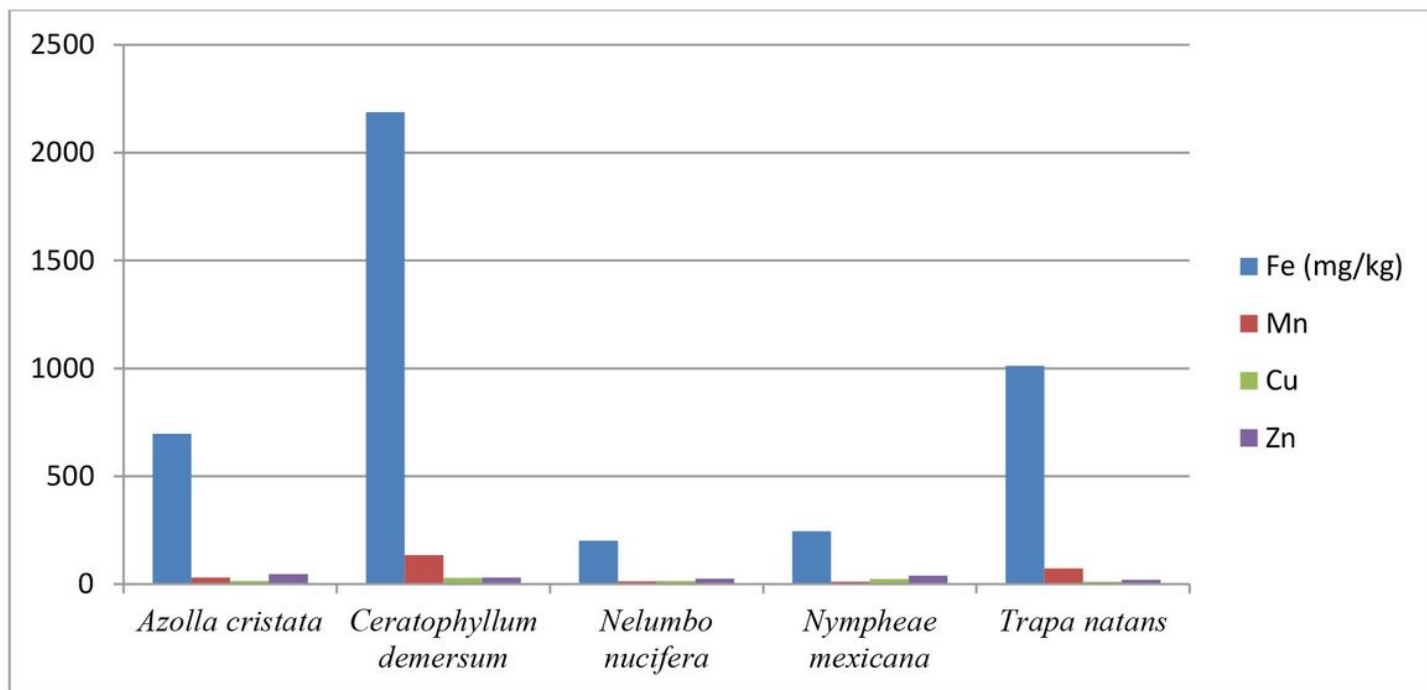


Figure 2

Micromineral contents of the five investigated macrophytes

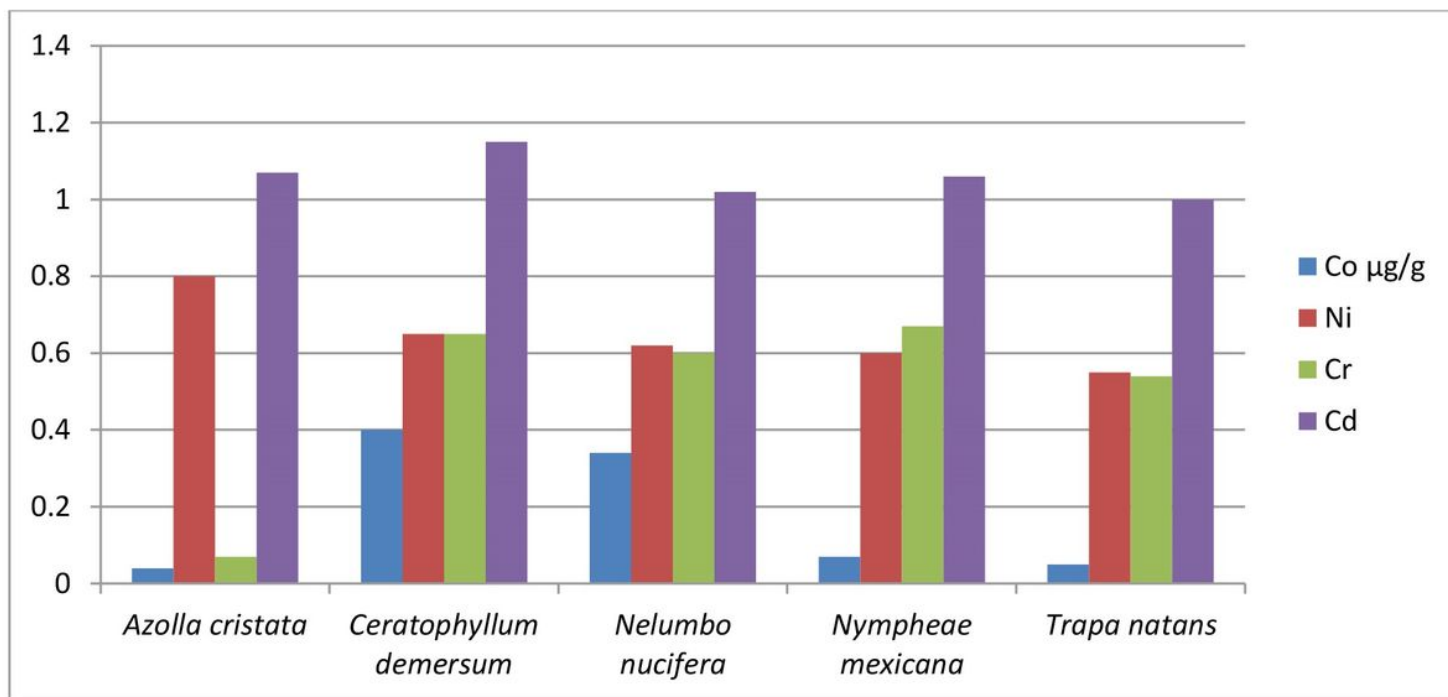


Figure 3

Heavy metal contents of the five investigated macrophytes

Supplementary Files

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- [Plate1.jpg](#)