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Determination of Mineral Content in Some Selected Aquatic Plants from Kalpani Beel of Assam, using Atomic Absorption Spectrometry

Jabin, C.R., Silistina, N. and Jatin, S.*

Department of Biotechnology, Bodoland University, Kokrajhar- 783370, Assam, India
**Email: jatinsarmahindia@gmail.com*

Abstract

Kalpani Beel is an oxbow lake formed by the river Manas and a tributary that originates from Bhutan and flows through Assam. Many aquatic plants of economic importance are confined to Kalpani Beel and often consumed by fishes. This study evaluated the mineral concentration of six selected aquatic plants, namely *Eichhornia crassipes*, *Hydrilla verticillata*, *Ipomoea aquatica*, *Nymphaea rubra*, *Pistia stratiotes*, and *Trapa natans*. A total of 11 elements, namely calcium (Ca), copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), and zinc (Zn), were analysed using an atomic absorption spectrometer. The concentrations of individual elements were: Ca: 0.045–0.093 mg/100 g, Cu: 0.001–0.012 mg/100 g, Co: 0.000–0.026 mg/100 g, Cr: 0.003–0.011 mg/100 g, Fe: 0.016–2.711mg/100 g, K: 0.335–37.362 mg/100 g, Mg: 0.004–6.759 mg/100 g, Mn: 0.004–0.428 mg/100 g, Mo: 0.001–0.016 mg/100 g, Na: 0.035–4.299 mg/100 g, and Zn: 0.011–0.041 mg/ 100 g. Results of the present study have provided vital data on the availability of essential elements in the fish food plants. The data possesses significant relevance in preparing low-cost value- added fish feed. The results suggest that *H. verticillata* may be used for commercial aquarium fish food production due to the amount and potential variety of nutrients it contains.

Keywords: Aquatic fish food plants, Oxbow lake, *Hydrilla verticillata*, Commercial aquarium fish food

1. Introduction

Kalpani Beel is an oxbow lake formed by the river Manas, a tributary of the river Brahmaputra originating in Bhutan (Ray and Sarmah, 2017). It is established as an ecologically suitable habitat for aquatic flora and fauna and fishes' production (Ray and Sarma, 2018). Minerals are essential for normal growth and other metabolic activities of the fishes. Inadequate supply of mineral salts leads to various deficiency-related diseases (Das and Chakraborty, 2007). Minerals are associated with several uses for animal health. Calcium (Ca), phosphorous (P), sodium (Na), molybdenum (Mo), chlorine (Cl), magnesium (Mg), iron (Fe), selenium (Se), iodine (I), manganese (Mn), copper (Cu), cobalt (Co), and zinc (Zn) are recognised as essential for body tissue in fishes (NRC, 1983). Fish may obtain these minerals from the diet and also from ambient water. The physiological importance of minerals is well documented for humans and some animals. All trace elements essential for higher animals are not necessary for fish. Information on nutritional requirements of trace elements for fish is scarce, particularly because many elements are needed only in trace amounts (Watanabe *et al*., 1997). In the last few decades, research relating to the mineral content of aquatic plants and mineral requirement of fish has been carried out in India and elsewhere (Watanabe *et al*., 1983; Edwards, 1980). This study is an attempt to analyse the mineral composition of six aquatic fish food plants of Kalpani Beel.

2. Materials and Methods

2.1. Sample collection and identification

The plant samples used in this study were collected from their natural habitat with the help of fisherman from Kalpani Beel of Assam, India. The collected plant samples were preserved individually in 10% formalin for taxonomic authentication by experts from the Botanical Survey of India, Shillong.

2.2. Sample preparation

The plant samples were washed thoroughly under tap water to remove contaminants and shade dried for two weeks. Subsequently, the samples were oven-dried for 24 hours at 40°C. The dried plant material was ground to form a fine powder and filtrated through a 345-micron pore sieve. The powder was stored in an air-tight container until further analysis.

2.3. Sample digestion

The samples for analysis of minerals were prepared by wet digestion method (Puwastein *et al*., 2011). A dry sample weighing 5.1 g was taken in a Teflon cup with screw cap. Concentrated nitric acid (HNO_3) (5 mL) and concentrated perchloric acid ($HClO_4$) (1 mL) (HNO_3:$HClO_4$ = 5:1) were added. The sample was pre-digested in a tightly closed cup overnight at room temperature. The cup was placed in an oven at 100°C for 5–8 hours, cooled to room temperature in a fume hood, transferred to a 50 mL volumetric flask, and diluted to mark with deionised (DI) water and mixed well. The obtained solution was filtered through Whatman filter paper No. 541 and transferred to Nalgene plastic bottles for mineral detection.

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2.4. Sample analysis

2.4.1 Determination of Ca and Mg

An aliquot of the test solution prepared in Section 3 was added to a volumetric flask 1% w/v $LaCl_3$. The solution was diluted to an appropriate volume with DI water. The solution was then measured for calcium and magnesium using an atomic absorption spectrophotometer (AAS) (GF-Atomic Absorption Spectrophotometer (AAS), Model: Analytik Jena Vario-6). The specific wavelength for each mineral was: (Ca = 422.7 nm and Mg = 285.2 nm) (Puwastein *et al*. 2011).

$$\text{Ca or Mg (mg/100 g)} = \frac{\text{(Co)} \times \text{total volume (mL)} \times \text{dilution} \times 100}{\text{Weight of sample (g)} \times 1000}$$

Where, Co = concentration of the sample in mg/L

2.4.2 Determination of Na and K

An aliquot portion of the sample solution prepared in Section 3 was added to the $CsCl_2$ solution to prepare a final dilution of 1% w/v $CsCl_2$ prior to the analysis to an appropriate volume. Analysis of the elements was performed using the AAS at their respective element wavelengths. (Na = 589.0 nm for 0.5–1.5 ppm and 330.4 nm for 10–100 ppm), (K = 766.5 nm) (Puwastein *et al*., 2011).

$$\text{Na or K (mg/100 g)} = \frac{\text{Co} \times \text{V} \times \text{D} \times 100}{\text{W} \times 1000}$$

where, Co = concentration of samples in mg/L; V = total volume in mL; D = dilution factor; W = weight sample, g

2.4.3 Determination of Iron (Fe), Copper (Cu), Zinc (Zn), Cobalt (Co), Chromium (Cr), Manganese (Mn), and Molybdenum (Mo)

The sample was prepared by wet digestion method, as mentioned in 3. An aliquot portion of the acidified sample was diluted to appropriate volume and analysed using AAS at relevant element wavelengths (Fe = 248.3 nm), (Cu = 324.7 nm), (Zn = 213.9 nm), (Co = 228.6 nm), (Cr = 267.7 nm), (Mn = 257.6 nm), and (Mo = 202.0 nm) (Puwastein et al., 2011).

$$\text{Trace element (mg/100 g sample)} = \frac{\text{Co} \times \text{V} \times \text{D} \times 100}{\text{W} \times \text{P} \times 1000}$$

where, Co = concentration of the sample in mg/L; V = total volume in mL; D = dilution factor; W = weight sample in g; P = sample solution taken, mL

2.5. Statistical Analysis

The results of all the analysis were calculated as the mean of triplicate analysis ±SD. The correlation coefficient of the two variables was estimated by Pearson's correlation coefficient (r) Correlation coefficient (r) test. The data obtained were then tested for statistical significance by comparing the calculated value with the value of r from the correlation test results. All the statistical analysis were performed in SPSS 26.0 version software.

3. Results and Discussion

Table 1 shows some selected aquatic herbs of Kalpani Beel of Assam, India, and their Botanical names, family, and part used along with their life form. The mineral contents of the different aquatic plants are presented in Table 2.

From the results, it was found that *P. stratiotes* showed the highest concentration of Ca (0.121 mg/100 g) whereas *H. verticillata* the lowest Ca (0.045 mg/100 g). Higher values for Ca (9.300 mg/100 g to 19.300 mg/ 100 g) were observed in *Ipomoea batatas* leaves grown on land (Monamodi *et al*., 2003) and aquatic Vietnamese *Ipomoea aquatica* leaves (101 mg/100 g) (Ogle et al.2001) compared to *P. stratiotes* (0.121±0.074 mg/100 g). In Beels the outflow and inflow of water depend on seasonal variations, so nutrient accumulation is not permanent in this water bodies. Only a small fraction of the available nutrients are integrated for biological interactions among the Beel communities (Winterbourn and Townsend, 1991). So, it is evident that aquatic plants can accumulate lesser amounts of minerals than those growing on the land. Nevertheless, fishes require only a small amount of Ca in their nutrition. Therefore the Ca content of selected aquatic plants in Kalpani Beel is adequate and can be used for fish nutrition. Calcium is a crucial trace element in fish nutrition because of its role in bones, teeth, muscle system development, and heart function (Brody, 1994). The interrelation of Ca and P is also crucial for the development and proper functioning of bones, teeth, and muscles (Dosunmu, 1997; Turan, 2003).

Cu is an ultra-trace element essential for animals. Cu is critical for animals as it is involved in the activity of enzymes, such as cytochrome oxidase, superoxide dismutase, lysyl oxidase dopamine hydrosylase, and tyrosinase. Investigations on Cu metabolism in fish by (Syed and Coombs, 1982) revealed similarities to mammals in the distribution of Cu and Cu-dependent enzymes. The highest Cu (0.012 mg/100g) concentration was estimated in *H. verticillata* and lowest in *Pistia stratiotes* (0.001 mg/100g). The Cu concentration of all the studied aquatic plants was also found to be lower than *Ipomoea aquatica* leaves (0.36±0.01 mg/100 g) reported by Umar et al. (2007). The optimal level of Cu in the diet as determined for several fish ranges from 3–5 mg/Kg of their diet (Gatlin and Wilson, 1986). Therefore, it is clarified that the studied aquatic plants are limiting in Cu and the fishes of the Beel need to depend on other sources for Cu. The permissible limit of Cu set by FAO/WHO in edible plants is 3.00 ppm. (FAO/WHO, 1984).

Cobalt is an important trace element required for the growth of living organisms. It is needed in very small amounts to the body (Khan *et al*., 2011). Among the investigated aquatic plants, cobalt was detected only in *H. verticillata*. Sridhar *et al*. (2020) observed that *H. verticillata* could efficiently accumulate low concentrations of cobalt in its tissues to increase its growth and detoxify reactive oxygen species (ROS) generation. For this reason, *H. verticillata* is considered a very efficient phytostabilizer. This may be the reason for the presence of cobalt in *H. verticillata* while found absent from the other selected aquatic plants in the study. Another reason for the existence of Cobalt in *H. verticillata* may be attributed to its submerged life form, as mineral accumulation efficiencies of aquatic plants generally are found to decrease from submerged to floating and then to the emergent plants (Bai *et al*., 2018). Besides *H. verticillata* all other mineral analysed plant samples

Table 1. Botanical name, family, part used and life form of some aquatic plants

Botanical name	Family	Part used	Life form
Eichhornia crassipes (Mart) Solma	Pontederiaceae	Lamina and petiole	FF
Hydrilla verticillate (L.f.) Royle	Hydrocharitaceae	Leaves and shoots	SA
Ipomoea aquatic Forssk	Convolvulaceae	Leaves and shoots	RFL
Nymphaea rubra Roxb. Ex. Salisb	Nymphaeaceae	Leaves	RFL
Pistia stratiotes L	Araceae	Leaves and shoots	FF
Trapa natans(Roxb.) Makino	Trapaceae	Leaves and shoots	RFL

FF = free floating, SA = submerged anchored, RFL = rooted with floating leaves

exhibited free-floating life form. The trace amount of cobalt in *H. verticillata* reveals its significance to fish nutrition as fish food. *H. verticillata* can be an essential natural plant ingredient in varieties of fish food formulation. Dietary cobalt supplementation in fish feed can improve growth, muscle composition and induce expression of growth and stress response genes in *Tor putitora* was proved by Younus and Zuberi, 2019). Co is a key element for fish nutrition. The presence of Co is critical because Co is a component of cyanocobalamin (Vitamin B_{12}), which constitutes nearly 4.5% of its molecular weight. Most animals need the element for the synthesis of the vitamin by intestinal micro flora, and such bacteria have also been isolated from the intestinal tract of fishes (Kashiwad *et al*., 1970). Co, as part of vitamin B_{12}, is associated with nitrogen assimilation and synthesis of haemoglobin and muscle protein. In addition, Co influences certain enzymes. Co binds to insulin (Cunningham *et al*., 1955) and also reduces plasma glucose levels (Roginski and Mertz, 1977). Co deprivation reduced the intestinal synthesis of vitamin B_{12} in catfish (Limsuwan and Lovell, 1981).

In the present study, the Cr content of the studied plant taxa varied from 0.003 mg/100 g (*Eichhornia crassipes*) to 0.011 mg/100 g (*Nymphaea rubra*) which is higher than that of the aquatic weed *Monochorria vaginalis* (0.0001 mg/100 g) of Tripura (Bhowmik *et al*., 2012). Cr is essential for normal carbohydrate and lipid metabolism (Anderson, 1981). The influence of dietary Cr on glucose metabolism of carp was investigated by earlier researchers (Hertz *et al*., 1989). It occurs in food as part of a biologically active molecule and as inorganic trivalent Cr. The range of Fe in the studied plants varied from 0.016 mg/100 g in *H. verticillata* to 2.711 mg/100 g in *Nymphaea rubra*. The permissible limit set by FAO/WHO (FAO/WHO, 1984) in the edible plant is 20 ppm.

Fe plays an active part in oxidation/reduction reactions and electron transport associated with cellular respiration (Watanabe *et al*., 1997). Fe is necessary for normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins, and fats (Adeyeye and Otokiti, 1999). The Fe content of fish is very low compared with that of mammals (Van, 1975) which indicates that fishes also require trace amount Fe. The highest concentration of Fe was found in *Nymphaea rubra* (2.711±2.012 mg/100 g). Ladan *et al*. (1996) reported high iron content (110-325 mg/100 g) in some land grown green leafy vegetables consumed in Sokoto. Even though a small amount of Fe was detected in the present study in comparison to land-grown green leafy vegetables, the amount is sufficient for fish nutrition. However, information on absorption and metabolism of Fe in fish is limited; however, the process of absorption and metabolism is generally considered the same as in other vertebrates (NIH, 2013).

K helps in the transportation of CO_2 by blood, contributes to the contraction and expansion of muscles and the heart. K helps in the metabolism of carbohydrates and proteins; K is essential for normal growth and body defence. Deficiency of K in fish may adversely affect the permeability of the cell membrane and the osmotic pressure of the cells and irregularity in the muscle and heart contractions (Das and Chakraborty, 2007). Of the minerals analyzed in aquatic plants, K was the most abundant secondary macro element ranging from 37.362 mg/ 100 g in *Pistia stratiotes* to 0.335 mg/ 100 g in *H. verticillata* is higher than the standard level (0.11%) in the living cell. Even though the concentration of K in Pistia stratiotes was 37.362 mg/100 g it was still found to be lower than that of land grown *Ipomoea batatas* leaves

Table 2. The concentration of mineral elements (mg/100 g dry weight)

Mineral	Plant code names					
mg/100g	EC	HV	IA	NR	PS	TN
Ca	0.067 ± 0.026	0.045 ± 0.009	0.093 ± 0.034	0.074 ± 0.013	0.121 ± 0.074	0.064 ± 0.025
Cu	0.004 ± 0.003	0.012 ± 0.002	0.004 ± 0.001	0.007 ± 0.008	0.001 ± 0.004	0.004 ± 0.002
Co	0.000 ± 0.000	0.026 ± 0.014	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Cr	0.003 ± 0.004	0.007 ± 0.001	0.007 ± 0.001	0.011 ± 0.006	0.005 ± 0.002	0.008 ± 0.003
Fe	0.234 ± 0.124	0.016 ± 0.05	0.257 ± 0.312	2.711 ± 2.012	0.242 ± 0.433	1.036 ± 0.174
K	35.186 ± 2.752	0.335 ± 0.017	36.721 ± 1.625	18.723 ± 0.768	37.362 ± 3.014	30.501 ± 1.308
Mg	3.372 ± 1.143	0.004 ± 0.006	3.880 ± 1.025	6.759 ± 2.112	5.514 ± 1.378	2.561 ± 1.410
Mn	0.135 ± 0.041	0.004 ± 0.003	0.206 ± 0.042	0.428 ± 0.064	0.219 ± 0.011	0.376 ± 0.023
Mo	0.016 ± 0.006	0.014 ± 0.020	0.002 ± 0.001	0.003 ± 0.003	0.002 ± 0.004	0.001 ± 0.005
Na	0.053 ± 0.018	0.035 ± 0.004	0.626 ± 0.112	1.233 ± 0.241	3.444 ± 1.032	4.299 ± 2.065
Zn	0.026 ± 0.010	0.011 ± 0.05	0.041 ± 0.016	0.022 ± 0.021	0.017 ± 0.008	0.035 ± 0.004

Value: Mean ± SD (standard deviation) EC = Eichhornia crassipes, HV = Hydrilla verticillata, IA = Ipomoea aquatica, NR = Nymphaea rubra, PS = Pistia stratiotes, and TN = Trapa natans

(750-4,953.49 mg/100 g) (Taiye and Asibey-Berko, 2001; Ishida *et al*., 2002; Monamodi *et al*., 2003).

The Mg content of the studied plant taxa varied from 6.759 mg/ 100 g in *Nymphaea rubra* to 0.004 mg/100 g in *H. verticillata*. Mg content in *Nymphaea rubra* (6.759 mg/ 100 g) was also found to be lower than land grown *Ipomoea batatas* leaves (79-107 mg/100 g) (Ishida *et al*., 2000). Mg concentration in this sample was expected to be high because Mg is a component of Chlorophyll (Dosunmu, 1997) and aquatic plants are a rich source of it. Mg acts as a co-enzyme in different enzymatic reactions and contributes to the normal functioning of muscles and nerves (Das and Chakraborty, 2007; Fleek, 1981).

Mn is a crucial component for bone structure, reproductive ability, and normal nervous system function (Fleek, 1981). Mn is essential for fish and is widely distributed in fish and animal tissue. Mn is necessary for the normal functioning of the brain and for optimal lipid and carbohydrate metabolism (Clark *et al*., 1987). The range of Mn varied from 0.004 mg/ 100 g in *H. Verticillata* to 0.428 mg/ 100 g in *Nymphaea rubra* which is similar to Mn contents ranging between 0.132 to 54.96 mg/g in *F. benjamina* leaves and *F. bengatensis* aerial roots (Ekinci *et al*., 2004). Except for *Nymphaea rubra* and *Trapa natans*, all other investigated taxa accumulated Mn below the permissible limit set by FAO/WHO (1984). The concentration of Mo in the studied aquatic plant ranges from 0.016 mg/ 100 g in *Eichhornia crassipes* to 0.001 mg/ 100 g in *Trapa natans*. The role of Mo in fish nutrition is not well known. Signs of Mo deficiency have not been established (Watanabe *et al*., 1997).

Na is a key constituent of blood, tissue fluid, and some enzymes. Na is associated with K in the body in maintaining optimal acid-base balance and nerve transmission (Setiawan, 1996). *H. verticillata* exhibit the lowest concentration of Na (0.035 mg/ 100 g) and *Trapa natans* exhibit the highest concentration of Na (4.299 mg/ 100 g). Highest concentration of Na (4.299 mg/100 g) was observed in *Trapa natans*, and it was also found to be higher when compared to other leafy vegetables available in Malaysia (3.00-65.00 mg/100 g) (Rukayah, 2002).

Zn is a key trace element in fish nutrition as it is involved in various metabolic pathways. It serves as a specific cofactor for several enzymes. In addition, Zn is an integral part of approximately 20 metallo-enzymes, such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase. Research on Zn–gene interactions has revealed a basic role of this element in growth control (Chesters, 1991). Among the studied taxa, *Ipomoea aquatica* exhibited the highest concentration of Zn (0.041 mg/ 100 g), whereas *H. verticillata* exhibited the lowest concentration (0.011 mg/ 100 g.

A correlation analysis was performed to investigate the relationship between different element concentrations in the aquatic plant samples. Table 3 reports the correlation matrix (r) between major and minor elements for 11 variables.

The environmental factors, including the atmosphere, pollution, season of sample collection, age of the plant, and soil conditions, affect the elements' concentration (Khan *et al*., 2011). Correlation analysis performed to investigate the relationship between different element concentrations in aquatic plant samples showed a positive correlation, whereas some analyses showed a negative correlation. Co and Cu, Fe and Cr, and Mn and Fe showed a significantly positive correlation.

4. Conclusion

The results of the mineral analysis showed that *H. verticillata* is a potential aquatic plant may be used for natural plant-based fish food as it is observed as the best source of essential minerals, Ca, Cu, Co, Cr, Fe, K, Mg, Mn, Mo, Na, and Zn. It is evident that the plant has essential minerals in reasonable concentrations, which is required in the food formulation to fish. The permissible limit for trace elements set by FAO/WHO (1984) in edible plants was 0.300 mg. In this study, the *H. verticillata* plant was found to contain trace elements within the permissible limit. However, more detailed analysis of the chemical composition of the plants is required. Minerals are essential elements involved in the normal metabolism of fish; though the relevant information is scarce. This is the first attempt that such exhaustive research on mineral content has been carried out on the aquatic plants of Assam, India.

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Table 3. The correlation coefficient between major and minor element concentrations of the aquatic plants

	Ca	Cu	Co	Cr	Fe	K	Mg	Mn	Mo	Na	Zn
Ca	1										
Cu	-0.77192	1									
Co	-0.60288	0.88576^{**}	1								
Cr	-0.15226	0.32695	-0.02661	1							
Fe	-0.07829	0.07118	-0.33355	0.88351^{**}	1						
K	0.71011^{*}	-0.97144	-0.8889	0.38604	0.13333	1					
Mg	0.67843	-0.5635	-0.76143	0.39237	0.62617	0.50783	1				
Mn	0.23951	-0.41244	-0.69359	0.68188	0.83065^{**}	0.33435	0.69936	1			
Mo	-0.57413	0.4763	0.51832	0.50936	0.39758	0.38566	0.5268	0.7284	1		
Na	0.40172	-0.55973	-0.42441	0.13738	0.16558	0.38643	0.24536	0.58192	0.72177	1	
Zn	0.16215	-0.52623	-0.64342	0.00626	0.05011	0.64948	0.15552	0.42659	0.44443	0.19407	1

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ (Value of the correlation coefficient (r) for different levels of significance)

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