ATOMIC ABSORPTION SPECTROSCOPIC DETERMINATION AND COMPARISON OF SOME MINERAL ELEMENTS IN ULVA RIGIDA C. AG. FROM HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

Dr. John Peter Paul, J.* and Muthu Sheeba, M.

PG and Research Department of Botany, St. Xavier’s College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

ABSTRACT
Seaweeds are potential sources of mineral contents that might represent useful leads in the development of new pharmaceutical agents. The present study was aimed to screen the important minerals such as Calcium, Magnesium, Manganese, Zinc, Chromium and Lead using Atomic Absorption Spectrophotometer in Ulva rigida C.Ag. collected from Hare Island, Thoothukudi, Tamil Nadu, India. The collected plant samples were air dried and digested by wet digestion method and analysed by Atomic Absorption Spectrometry. The essential metals and heavy metals in the plant extracts were quantified. Results obtained in the present study showed that the aqueous extracts of Ulva rigida C.Ag. investigated contain four essential elements namely Calcium (50.3084ppm), Magnesium (31.7284ppm), Manganese (0.1008ppm) and Zinc (0.0081ppm) and two heavy metals that is Chromium (0.0142ppm) and Lead (0.2946ppm). From the present study, it is concluded that the levels of mineral elements present in Ulva rigida C.Ag. fall in the permissible range.

Keywords: Seaweed, Ulva rigida, mineral, Thoothukudi.

INTRODUCTION
Seaweeds are commercially important marine living and renewable resources in many parts of the world which can be utilized for many purposes. Seaweeds are enriched with many mineral elements, protein, vitamins, several bioactive substances and secondary metabolites which are having economic importance as well as serve as feed for many cattle and fishes.
Seaweeds have been used since ancient times as food, fuel, fertilizer and as source of medicine. Seaweeds are also used as raw materials for many industrial productions like agar, algin and carrageenan. The edible seaweeds are found with low caloric value but rich in proteins, lipids, minerals, vitamins and daily fibers. They are nutritionally valuable as fresh or dried vegetables or as ingredients in a wide variety of prepared foods. Depending on the type of species, seaweed is generally suitable for making cool, gelatinous concoctions. In recent years, with enhanced awareness of the importance of mineral elements in health and disease, an increasing number of reports on the role of mineral elements in seaweeds have been reported in several countries by various techniques.

Manivannan reported that different group of seaweeds such as Chlorophyceae (Ulva lactuca and Enteromorpha intestinalis) Phaeophyceae (Turbinaria ornate and Padina gymnospora) and Rhodophyceae (Hypnea valentia and Gracilaria folifera) were collected from Mandapam coastal regions in the southeast coast of India for analyzing mineral composition. In the earlier study, Padina gymnospora showed the maximum content of mineral composition such as copper, chromium, iron, lead, sulphur and calcium content and potassium than other seaweeds. Hypnea valentiae showed the minimum level of mineral content such as cadmium, iron, magnesium and calcium. The highest level of Cr was observed in brown seaweed Padina gymnospora and the minimum content was observed in green seaweed Enteromorpha intestinalis. The highest Cu concentration was observed in the brown seaweed Padina gymnospora and the lowest level was observed in red alga Gracilaria folifera.

Karthikai Devi reported the elemental concentration in various seaweeds such as Codium tomentosum, Enteromorpha clathrata, Enteromorpha compressa, Turbinaria conoides, Colpomenia sinuosa, Sargassum tenerimum, Sargassum wightii and Acanthophora spicifera collected from Gulf of Mannar marine biosphere reserve, the southeast coast of India. It was reported that Sargassum wightii showed the highest level of elemental composition such as Cr, Cu, Mn, Ni, Pb and Zn content than other seaweeds and Acanthophora spicifera recorded the lowest level of element content such as Cr, Cu, Pb and Zn.

Ulva species are widespread green seaweeds occurring at all levels of the intertidal zone, in calm and protected harbours as deep as 10 meters and in northern climates and grows along rocky or sandy coasts of oceans and estuaries. In some parts of Britain and Asia, it is consumed by humans and livestock as it is considered valuable to human nutrition. Many of its nutrients include iron, proteins, iodine, vitamins and trace elements. Because of
antibacterial properties, it also has been recommended by treating skin irritations typically, including burns ⁸. Atomic spectroscopic techniques are widely used for analysis of trace metals. AAS is the most extensively used technique for determination of metals in plant samples. In the present work, an attempt has been taken to analyse the important trace elements such as Calcium, Magnesium, Manganese, Zinc, Chromium and Lead using Atomic Absorption Spectrophotometer in *Ulva rigida* C.Ag. collected from Hare Island, Thoothukudi, Tamil Nadu, India.

**MATERIALS AND METHODS**

**Collection of sample**

*Ulva rigida* C.Ag. (Figure 1) is green seaweed shows much attention in the recent years due to native vegetation. *Ulva rigida* C.Ag. was collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India during the month of January 2014. Samples were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. In the laboratory, the seaweeds are once again washed in fresh water and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further analysis.

![Figure 1. Natural Habit of Ulva rigida C.Ag.](image)

**Wet Digestion Procedure**

One gram of plant sample was digested with 5ml of 10M HNO₃ in the covered beakers to near dryness. It was necessary, another 5ml portion of 10M HNO₃ was further added each time until the sample solutions became clear. Five millilitres of 10M HCl were also added to ensure complete digestion. After cooling to room temperature, the digested solutions were
diluted to 100 ml with deionized water for the estimation of various mineral contents. The sample was analyzed using Atomic Absorption Spectrometry (AAS) for Calcium, Magnesium, Manganese, Zinc, Chromium and Lead. The procedure used is described by Okalebo.

**Preparation of Standard Solutions**
The stock solution for Ca, Mg, Mn, Zn, Cr and Pb were made from a metal salt of Analar grade (purity 99.9%). The metal salt was dissolved in dilute hydrochloric acid and diluted to the 1 litre mark using distilled water. The required concentrations were prepared by serial dilution of the stock solution.

**Calibration of Equipment**
For the elements under investigation, the following sensitivity and detection limits were established respectively for the use AAS apparatus. Ca 2.0, 4.0, 6.0 and 8.0 ppm, Mg 0.2, 0.4 and 0.6 ppm, Mn 1.0, 2.0 and 3.0 ppm, Zn 0.5, 1.0 and 1.5 ppm, Cr 1.0, 2.0, 3.0 and 4.0 ppm, Pb 2.0, 4.0 and 6.0 ppm. The blank solutions were undergoing the same digestion procedure as that of the samples.

**Atomic absorption spectroscopy for the analysis of mineral elements**
Instrument used was Spectra Atomic Absorption Spectrophotometer (AA-6300). The working standards of appropriate range were made from stock standard. The instrument was standardized with the above series of working standards. Samples were aspirated into the flame (combination of air and acetylene) and the corresponding absorption of characteristic radiation by the atomic vapor of the element was recorded. The minerals were analyzed by dissolving the ash (obtained in ash determination) in diluted Hydrochloric acid and estimated using Atomic Absorption Spectrophotometer with acetylene and air supplied in constant ratio for flame and hollow cathode lamp.

**RESULTS AND DISCUSSION**
An examination of the mineral elements using Atomic Absorption Spectrophotometer showed the presence of different elements like Ca, Mg, Mn, Zn, Cr and Pb in various proportions in Ulva rigida C. Ag (Table 1). The presence of these elements in different proportions was confirmed by measuring the absorbance of the species at its resonance wavelength by AAS technique. A number of trace elements play an important role in the metabolism. These elements are called essential. An element is considered essential for a
plant if the plant fails to grow normally and complete its life cycle in a medium adequately removed from the element whereas in the presence of the suitable chosen concentration of that element it grows and reproduces normally.

**Table 1: Standard conditions for Atomic Absorption measurement of Ulva rigida C. Ag.**

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Lamp current (mA)</th>
<th>Fuel gas Flow Rate (L/min)</th>
<th>Burner Height (mm)</th>
<th>Sensitivity check (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>442.7</td>
<td>0.7</td>
<td>10</td>
<td>2.0</td>
<td>6</td>
<td>50.3084</td>
</tr>
<tr>
<td>Mg</td>
<td>285.2</td>
<td>0.7</td>
<td>8</td>
<td>1.8</td>
<td>7</td>
<td>31.7284</td>
</tr>
<tr>
<td>Mn</td>
<td>279.5</td>
<td>0.2</td>
<td>10</td>
<td>2.0</td>
<td>7</td>
<td>0.1008</td>
</tr>
<tr>
<td>Zn</td>
<td>213.9</td>
<td>0.7</td>
<td>8</td>
<td>2.0</td>
<td>11</td>
<td>0.0081</td>
</tr>
<tr>
<td>Cr</td>
<td>357.9</td>
<td>0.7</td>
<td>10</td>
<td>2.8</td>
<td>9</td>
<td>0.0142</td>
</tr>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>0.7</td>
<td>10</td>
<td>1.8</td>
<td>7</td>
<td>0.2946</td>
</tr>
</tbody>
</table>

Calcium content of *Ulva rigida* C. Ag. (Figure 2) showed the highest range in the element studied (50.3084ppm). From the results obtained, it was observed that the concentration of Magnesium was 31.7284ppm (Figure 3). The comparative account of the concentration of Calcium and Magnesium was presented in the Table 4. Calcium is essential for healthy bones, teeth and blood. The health of the muscles and nerves depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as the pancreatic lypase. It helps to regulate the activity of skeletal muscle, heart and many other tissues. Deficiency of calcium causes rickets, osteomalacia and scurvy\textsuperscript{11}. Magnesium is an important trace element which is the central part of the chlorophyll pigment. In humans, Mg is required in the plasma and extra cellular fluid where it helps in maintaining osmotic equilibrium. It is required in many enzyme for catalysed reactions, especially those in which nucleotide participate where the reactive species is the magnesium salt, e.g., MgATP. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system\textsuperscript{12}. 
Figure 2: The Calcium (Ca) content curve and peak value of *Ulva rigida* C. Ag. using Atomic Absorption Spectrophotometer

Figure 3: The Magnesium (Mg) content curve and peak value of *Ulva rigida* C. Ag. using Atomic Absorption Spectrophotometer

Figure 4: Comparative study of Calcium and Magnesium content of *Ulva rigida* C. Ag.
The present study shows considerable Manganese content (0.1008ppm) presented in Figure 5. It is important here to note that Mn is an essential element required for various biochemical processes. The kidney and liver are the main storage places for the manganese in the body. Mn is essential for normal bone structure, reproduction and the normal functioning of the central nervous system. Its deficiency causes reproductive failure in both male and female. Mn is also important for several enzymatic processes. It helps in eliminating fatigue and reduces nervous irritability. A remarkable amount of Zinc (0.0081ppm) also found in the present study (Figure 6). The amount of Manganese and Zinc were compared in the Figure 7. The elements like Zn is essential trace elements (micro nutrients) for living organisms. Zinc is relatively non-toxic. Zinc deficiency is characterized by recurrent infections, lack of immunity and poor growth. Growth retardation, male hypogonadism, skin changes, poor appetite and mental lethargy are some of the manifestations of chronically zinc-deficient human subjects. Zinc is necessary for the growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis), for skin integrity, bone metabolism and functioning of taste and eyesight. It is a constituent of many enzymes and insulin. Zinc deficiency causes weight loss.

**Figure 5: The Manganese (Mn) content curve and peak value of *Ulva rigida* C. Ag. using Atomic Absorption Spectrophotometer**
A heavy metal, Chromium content was present in 0.0142ppm (Figure 8). Chromium is essential in carbohydrate metabolism. It also functions in protein and cholesterol synthesis. Deficiency of chromium causes glucose intolerance, impaired growth and decreased respiratory quotient. It plays an important role diabetes treatment. It is an important element required for the maintenance of normal glucose metabolism 16. The function of chromium is directly related to the function of insulin, which plays a very important role in diabetes. Chromium is found in the pancreas, which produces insulin. One usable form of chromium is the Glucose Tolerance Factor (GTF), an inorganic compound containing glutamic acid, cysteine and niacin 17. In the present study, the amount of Lead observed in Ulva rigida C. Ag. is 0.2946ppm (Figure 9). The content of Chromium and Lead is compared and presented in Figure 10. Lead has gained considerable attention as a persistent toxic pollutant of concern,
partly because it has been prominent in the debate concerning the growing anthropogenic pressure on the environment. Like various heavy metals, Pb treatment influences the activity behaviors of a wide range of enzymes of different metabolic pathways. Increasing attention has been paid to understand the action of Pb on plant enzymes.

Figure 8: The Chromium (Cr) content curve and peak value of *Ulva rigida* C. Ag. using Atomic Absorption Spectrophotometer.

Figure 9: The Lead (Pb) content curve and peak value of *Ulva rigida* C. Ag. using Atomic Absorption Spectrophotometer.

Figure 10: Comparative study of Chromium and Lead content of *Ulva rigida* C. A.
CONCLUSIONS
The extract of *Ulva rigida* C.Ag., investigated contain the important minerals namely Calcium, Magnesium, Manganese and Zinc that are considered essential elements followed by Chromium and Lead are non essential. The concentration (ppm) of essential mineral elements are present in the appropriate amount in the plant extract that was found to be as follows, Calcium (50.3084), Magnesium (31.7284), Manganese (0.1008) and Zinc (0.0081) and non essential heavy metals such as Chromium (0.0142) and Lead (0.2946). From the comparison of the results with the defined permissible concentration limits, it was concluded that the levels of essential mineral elements and non essential heavy metals present in the green seaweed fall in the permissible range for consumed seaweeds described for different countries.

REFERENCES