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Tracking Iodine Decrease in Commercially Sold *Caulerpa racemosa* (Forsskål) J. Agardh (Chlorophyta, Ulvophyceae) during Storage

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Iodine is known as an essential mineral for thyroid hormone production in humans. It is water-soluble and diffuses into the atmosphere, where it is thought to break ozone molecules. *Caulerpa racemosa* (Phylum Chlorophyta, Class Ulvophyceae, Order Bropsidales) is a common dietary seaweed believed to be a good source of iodine in the Philippines. As of this writing, there is no record of iodine concentration measurements in harvested *C. racemosa* from Philippine waters. There is an expected decrease in iodine concentrations from harvested *C. racemosa* through time. But how fast it decreases and how much of it remains in the seaweed if stored for days is also unknown. This study measured iodine concentration in *C. racemosa* samples from an area in the Philippines and calculated the daily changes in iodine level concentrations of harvested *C. racemosa* that were stored. Titration analyses were done every 24 h, with factors such as the algal age, temperature, light, among others, kept constant. Storage conditions were adapted from how local vendors store leftover seaweed merchandise. Results revealed a decrease in iodine levels over time ($y = –20.438x + 228.99$; $R^2 = 0.8937$). The initial concentration (at dry weight) of iodine measured was 196 ppm and dropped to 94.8 ppm on the seventh day. Iodine levels of freshly picked and stored *C. racemosa* were also compared with the recommended daily iodine intake for humans.

Keywords: algae, *Caulerpa racemosa*, iodine, macroalgae, seaweed

INTRODUCTION

Iodine, a naturally-occurring trace element, is important in human physiology. It is an essential mineral used in the production of thyroid hormones, especially thyroxine, and is important for the overall health of the thyroid gland (Risher *et al*. 2004). As thyroxine is necessary for many functions of growth and development (Cheng *et al*. 2010), the absence or deficiency of iodine in effect causes medical manifestations such as the increased risk of stillbirths, abortions, perinatal mortality, congenital abnormalities, cretinism, impaired growth, and illnesses such as breast cancer (Teas *et al*. 2007; Lazarus 2014). Worldwide, there are about 2 billion people who suffer from iodine deficiency, with 50 million having clinical manifestations (Lazarus 2015).

On the other hand, excess iodine consumption can lead to hyperthyroidism (Teas *et al*. 2004) and, in worst cases, thyroiditis (Risher *et al*. 2004). Iodine is soluble in water and alcohol. It enters the human body through the consumption of both natural and processed resources such as dairy products and iodized table salt (Risher *et al*. 2004).

Moreover, iodine and iodine derivatives have a variety of uses. It is added to food and salt for optimized health, used in making medicines and in purifying water, mixed

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with animal feeds, used in making soaps and bandages; used as disinfectants or as biocides; used in X-ray contrast media, and in polarizing film for liquid crystal display screens (Risher *et al*. 2004; Hora 2016).

Iodine is extensively found in marine organisms [see review by Dembitsky (2006)]. Seaweeds or marine algae are good sources of iodine (Mageswaran and Sivasubramaniam 1984; Ghosh 2004; Zava and Zava 2011). *Caulerpa racemosa* (Forsskål) J. Ag. or "lato" (local name in Visayas, Central Philippines) is a common dietary seaweed believed to be a good source of iodine. Most members of the genus *Caulerpa* have a wide distribution in the tropical and Mediterranean seas (Ghosh 2004). Also known as the sea grape, *C. racemosa* can be distinguished by its grape-like clusters called globose ramulli. It can thrive both in the reef and lagoon habitats (Collado-Vides and Robledo 1999). Mairh *et al*. (1989) measured iodine content in different species of seaweeds, including *C. racemosa*, in India. But there are no published iodine values of *C. racemosa* from the Philippine waters.

Molecular iodine (I_2) has been found to be emitted by brown algal species after being exposed to air, specifically to oxygen, termed iodovolatilization (Küpper *et al*. 1998; Ball *et al*. 2010). The biogeochemistry of iodine and its role in ozone destruction is being studied in temperate coastal ecosystems with iodine-rich macroalgae as drivers of iodine fluxes (Giese *et* al. 1999; Nitschke *et al.* 2018). But research on the stability of iodine in *C. racemosa* has not been done, nor a baseline value of iodine concentration of *C. racemosa* from Philippine waters has been published.

This study analyzed the iodine levels in different *C. racemosa* samples stored at different lengths of time, as would be found in local wet markets where this species is being sold. This provides information on the iodine content and how much iodine in *C. racemosa* is lost daily at a specific length of storage time. As one of the most consumed algal species in the Philippines, the results of this study can point to recommendations on how long *C. racemosa* can be stored and still contain enough iodine levels needed for normal bodily functions. Information obtained from this research will be useful especially for individuals who are iodine-deficient and, therefore, need more iodine, as well as for those who need only a certain amount of iodine in their diet. This will also serve as baseline data regarding the iodine concentrations of *C. racemosa* in Philippine waters, which could be useful in iodine biogeochemistry research.

MATERIALS AND METHODS

Sampling Station

All the seaweed samples of the same age and condition suitable for consumer consumption were collected from the coastal waters of Brgy. Uban, Babatngon, Leyte, Philippines, located at 11° 22' North, 124° 58' East (Figure 1) in an area with a depth of 3.5 m. The collection was done at around 06:00 AM, and the average temperature of the surrounding water at that time was 21.6 °C.

Sample Collection

Whole seaweed samples were handpicked and washed immediately with seawater to get rid of epiphytes, sand, and other foreign particles. Afterward, the seaweed samples were stored inside black trash bags to inhibit light exposure (Teas *et al.* 2007) while still submerged in seawater to mimic the conditions in the natural setting as much as possible and kept inside a Styrofoam box at ambient, room temperature (average: 27 °C). Verbal interviews with the local market vendors affirm that this is how they store *C. racemosa* for sale. All the samples were transported immediately to the laboratory at the University of the Philippines Tacloban College to arrive one hour later. The samples were put separately inside seven black trash bags with seawater, to be used one by one daily for iodine measurement later. The bags were sealed except when samples were being pulled out for the experiment. This was to represent the 7-d maximum storage duration of *C. racemosa* in the local market. This sampling design may not be ideal to generate replicate samples for statistical analysis, but it reflects how seaweed samples are being handled in the actual market situation most closely. Meanwhile, the bags were placed inside one Styrofoam box and seawater inside the bags with seaweeds was replaced daily. The temperatures of the laboratory room and of the seawater inside the bags were also monitored. Air-condition units and ceiling fans were not used during the entire experimentation period to simulate actual market conditions.

On the day of iodine analysis, the seaweed samples inside the bag were washed repeatedly with tap water to remove salt on its surface due to air exposure (Manivannan *et al.* 2008). Afterward, three 50-g seaweed samples for each trial were segregated and blotted with filter paper for air drying while covered in a black plastic sheet. After 20 min, the samples were weighed for moisture loss calculations and were minced in a food processor as preparation for the fusion procedure. The first batch of samples was prepared for experimentation approximately 4 h after collection and, for the succeeding batches, every 24 h thereafter.

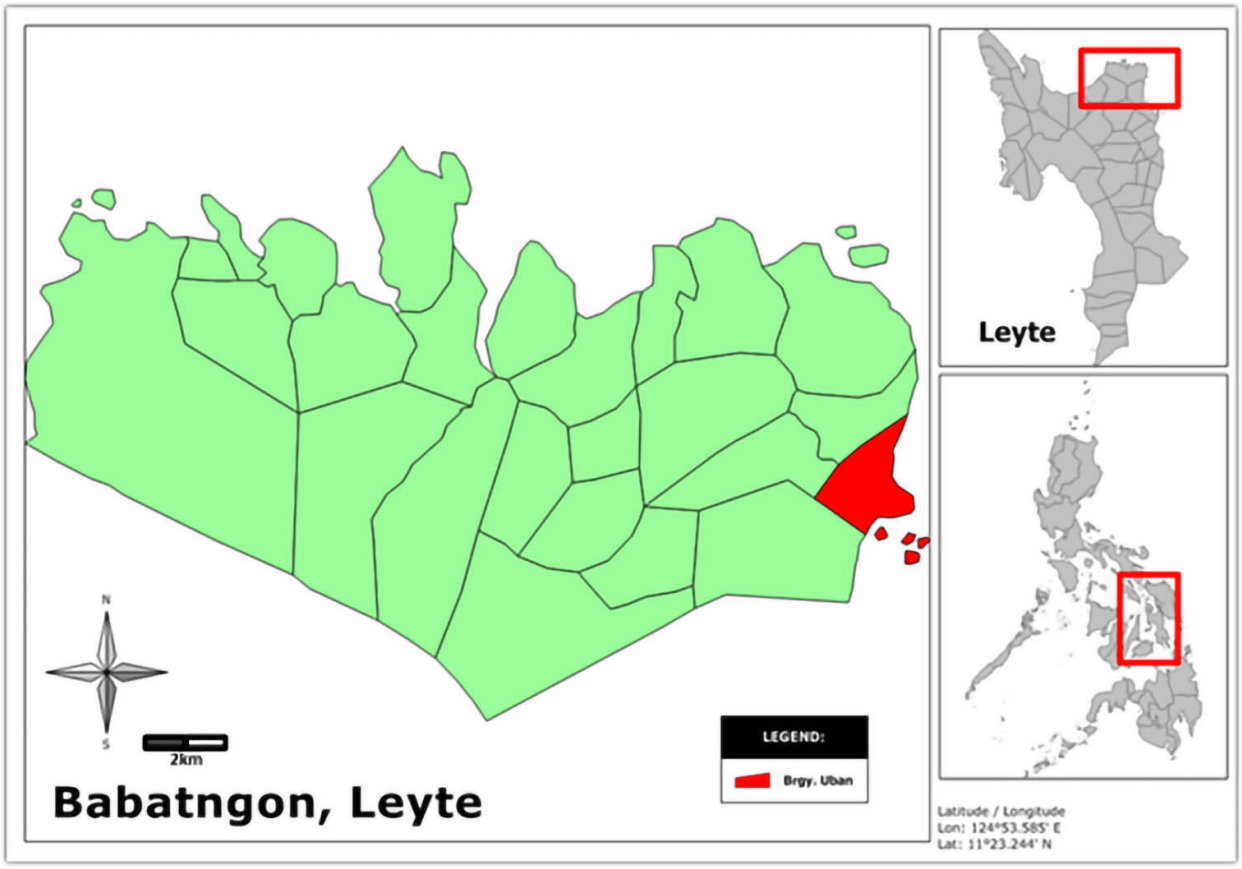

Figure 1. Collection site of *Caulerpa racemosa* at Brgy. Uban (in red), Babatngon, Leyte, Philippines.

Fusion Procedure

Fusion was conducted, following Kelly and Husband (1924). The minced seaweed samples were placed in a nickel crucible and were moistened with 0.25 mL of 30% sodium hydroxide (NaOH). It was heated at the medium level on an electric stove (at about 95–150 °C) to a thick, syrupy consistency until the melt had settled to the bottom. This was then added with 8 mg potassium nitrate (KNO_3) when no more foaming was observed. No direct heat was applied so as to avoid volatilization of iodine by placing the crucible containing the seaweed samples over another crucible with a 0.5-cm layer of sand at its bottom. Afterward, the organic material was allowed to cool. Then, 1 g of the cooled material was dissolved in 50-mL distilled water in a beaker by heating and subsequently transferred to a 250-mL Erlenmeyer flask for titration (Kendall and Richardson 1920). For trials with small amounts of insoluble residue remaining, the whole solution was filtered until a clear solution was obtained (Kelly and Husband 1924).

Titration Procedure for Iodine Analysis

This titration method for iodide content was based on the methods of Kendall and Richardson (1920), which was reviewed for accuracy by Leitch and Henderson (1926).

This was also described subsequently by de Maeyer *et al.* (1979) and the World Health Organization (WHO 2007). The reaction mechanism for this iodometric titration is as follows:

$$KI \rightarrow K^+ + I^-$$

$$Br_2 + 2I^- \rightarrow 2Br^- + I_2$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

The seaweed sample was added with 0.3 mL of methyl orange indicator to turn the solution to orange. The indicator was prepared by dissolving 0.01 g of methyl orange in 100-mL distilled water. To neutralize the solution, 2N H_2SO_4 – made by adding 6-mL concentrated H_2SO_4 to 90-mL water and diluted to 100-mL was added dropwise until the solution turned pink. Then, 0.5 mL of bromine water was added to change the solution to yellow. Sodium sulfite (Na_2SO_3) solution, prepared fresh

by dissolving 1 g of Na_2SO_3 in 100-mL water, was added dropwise until the solution in the flask turned pale yellow. Afterward, 0.15 mL of phenol solution was added, then 1 mL 2N H_2SO_4 and 5 mL of potassium iodide (KI) solution were prepared by dissolving 100-g KI in 1000-mL water. At this time, the solution in the flask became yellow-colored. A 10-mL burette was rinsed and filled with 0.005M sodium thiosulfate ($Na_2S_2O_3$) that was prepared earlier by dissolving 1.24 g of $Na_2S_2O_35H_2O$ in 1000-mL distilled water and was stored in a cool, dark place prior to use. The flask containing the seaweed sample solution was then titrated with the sodium thiosulfate solution until the solution in the flask turned pale yellow. Subsequently, 1 mL of freshly prepared starch solution was added, which led to a dark purple color. The starch solution was prepared by dissolving 1 g of soluble starch in 100-mL water while heating. All the reagents used were stored in dark-colored amber bottles with covers for the whole duration of the experiment. Starch powder and sodium thiosulfate were stored in amber bottles as well and were prepared fresh every day.

Titration with $Na_2S_2O_3$ was continued until the solution became colorless. The amount of thiosulfate used, in parts per million (ppm), was proportional to the amount of free iodine liberated from the sample, where 0.005 N sodium thiosulfate consumes 0.1058 mg iodine/mL. The conversion to mg/kg (ppm) was based on the following equation (WHO 2007):

$$\text{ppm} = \frac{\text{titration volume (mL) x 21.15 x normality of sodium thiosulfate x 1000}}{\text{sample weight (g)}}$$

The dry weight of the samples was obtained by heating three replicates of 100 g fresh *C. racemosa* in the oven at 110 °C for 10 min. The temperature was then lowered to 60 °C for at least 22 hours until a constant weight was achieved.

Statistical Analysis

Iodine analyses were done in triplicates per day, and the means per day were calculated. Regression analysis was carried out to investigate the relationship between storage duration and iodine levels in *C. racemosa*. Statistical tests were computed using the QI Macros 2013 Software in Microsoft Excel 2010, as well as the SPSS statistical software. The significance level was set at $p = 0.05$.

RESULTS

The sampling design of obtaining samples from each bag of seaweeds each day may not be ideal; nonetheless,

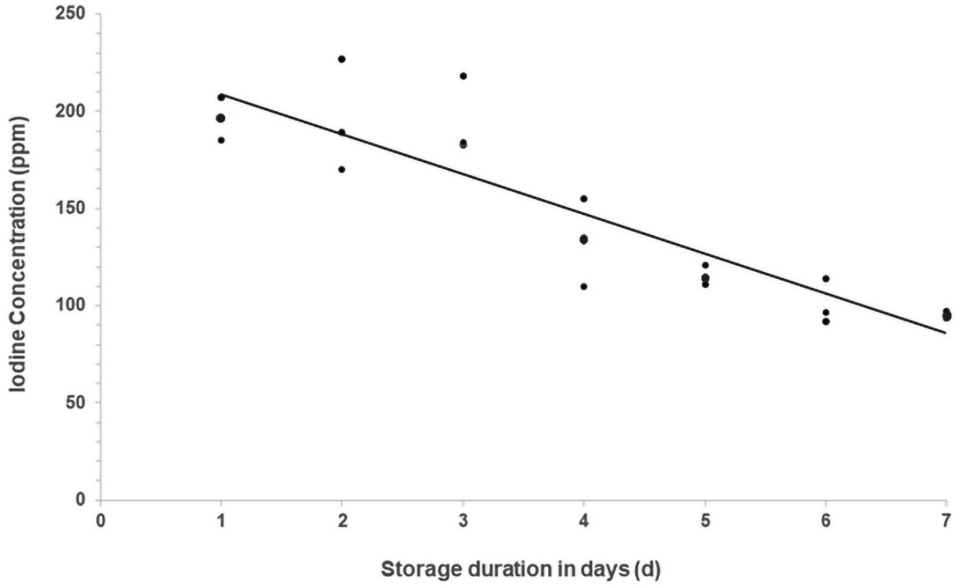

Figure 2. Changes in the iodine level of *C. racemosa* (by dry weight) over 1 wk of storage. Regression: y = –20.438x + 228.99; $R^2 = 0.8937$; $p = 0.001$; n = 21. Iodine concentrations shown are for every 1 g of dry weight of *C. racemosa*.

a significant linear decreasing trend in iodine levels of *C. racemosa* over time of storage is still clear (Figure 2; regression analysis: n = 21, $R^2 = 0.872$; $p = 0.001$). On Day 1, the samples had the highest mean Iodine concentration at 196 ppm for every 1 g dry weight of *C. racemosa*. By the 7th or last day of analysis, this dropped to 94.8 ppm. The samples by this time appeared considerably shrunk. The greatest decrease in iodine concentration was observed on the fourth day, with a 61.6 ppm difference in iodine concentration from the previous day. The regression result, with the slope of the linear regression line at b = –20.438, indicated an average decrease of 20 ppm of iodine per day of storage in *C. racemosa*.

The average dry weight calculated from 100 g wet weight of *C. racemosa* was equivalent to 9.56 g. This was rounded to 10 g.

DISCUSSION

As the results confirm, *C. racemosa* is best consumed when freshly picked to maximize its iodine content. It is apparent that levels of iodine in the seaweeds change when seaweeds are removed from their natural habitat. This observation is particularly relevant as many seaweeds are traded in the market for a few days after harvesting. Iodine level during the second and third day is still comparable to that of the first day though; thus, eating these after the second or third day after harvest under similar storage conditions should be alright. The present storage techniques done by market vendors are somehow effective, but this could be improved, noting that the greatest loss of iodine was just by the fourth day. As shown in the present study, *C. racemosa* iodine levels lowered gradually over time with an average rate of decrease of 20 ppm/d. The iodine level eventually dropped to 94.8 ppm on the seventh day of storage. This means that on the seventh day, for every 1 g of *C. racemosa* (dry weight), there is 94.8 µg of iodine. Conversely, the needed wet weight of *C. racemosa* to get 94.8 µg of iodine is 10 g.

The Food and Nutrition Research Institute of the Philippines (FNRI) has a recommended daily intake amount of iodine for each age group of adults, including pregnant and lactating women. Using the FNRI Philippine Dietary Reference Intake of 2015, adults aged 19 yr old and above need as much as 150 µg of iodine per day. Based on the lowest content of iodine found in the present study (94.8 ppm during the seventh day), an estimate of 15.82 g wet weight of *C. racemosa* from Babatngon, Leyte is enough to provide this amount. This also means that, although almost half of the iodine content in *C. racemosa* from the same locality would have been lost after maximum storage of 7 d in the local markets, it is still more than enough to supply adults with the recommended daily iodine intake. This amount, however, might not be true for *C. racemosa* harvested in other parts of the Philippines and in all seasons year-round, as chemical contents of seaweeds are known to vary with place and season of collection.

Seaweeds as a dietary source of iodine have been well known for centuries and levels of iodine have been examined in many studies. Some examples of these are listed in Table 1. In general, iodine levels tend to be highest among brown algae, ranging 2500–206 ppm/ g dry weight biomass, followed by the red algae at 1250–120 ppm. Green algae, *C. racemosa* included, have the lowest iodine values at 259–20 ppm.

Different amounts of iodine could be found, even in the same algal species but from different locations. For example, Mairh *et al.* (1989) found the iodine level of *C. racemosa* from Bhavnagar, India to be 88.3 ppm, lower than the initial iodine level measured in the present study (195.9 ppm). Grimm (1952) measured the iodine level of *C. racemosa* from Bermuda but yielded a negative result, *i.e.* iodine was not detected.

It is not clear why the same species of seaweeds from different localities would differ in their iodine content or why and how *C. racemosa* would lose its iodine content under storage. Most of the studies on the iodine mechanism in seaweed have been on brown algae. Iodide ions are released into the water by brown seaweeds in response to environmental or oxidative stress (Küpper 2008; Ball *et al*. 2010). Kundel *et al*. (2012) proposed that the slightest exposure to air stimulates the release of iodine compounds from the seaweeds, leading to a decrease in its stored iodine over time. Similarly, Nitschke *et al*. (2013) found that air exposure and prolonged degradation after removal from their natural habitat can result in a release of iodine in kelps. Air exposure leads to oxidative stress, making oxidation of iodine by H_2O_2 possible.

Aside from accumulating iodine for metabolism purposes, brown algae release iodine compounds that contribute to ozone depletion and new particle formation (Pirjola *et al*. 2005). These compounds are formed as by-products of the stress-induced production of hydrogen peroxide (H_2O_2) inside cells (Pedersen *et al*. 1996; Küpper *et al*. 1998; Colin *et al*. 2003; Leblanc *et al.* 2006). Giese *et al*. (1999) stated that although organoiodine compounds released by these macroalgae do not directly give any benefits to the cells, by not releasing them, the macroalgal cells may suffer oxidative degradation from peroxide.

Detailed analysis of the mechanism of iodine release is beyond the scope of this study, but the rate of emission is species-dependent (Ball *et al*. 2010). Kundell *et. al.* (2012) measured the iodine emissions of different seaweeds and

Table 1. Iodine content of various species of seaweeds reported from other works, in comparison with that reported in the present study. This list is not meant to be comprehensive.

Species tested	Location of collection	Iodine content (ppm)	Reference
Ochrophyta (Phaeophyceae, brown algae)			
Desmarestia aculeata (L.) J.V.Lamouroux	Woods Hole, Massachusetts, USA	2500	Grimm (1952)
Macrocystis integrifolia Bory	Cape Arago, Oregon, USA	1060	Grimm (1952)
Turbinaria ornata (Turner) J.Ag.	Mandaitivu, Sri Lanka	810	Mageswaran and Sivasubramaniam (1984)
Fucus vesiculosus L.	Helgoland, Germany	494	Kundel *et al.* (2012)
Levringia boergesenii Kylin	Bhavnagar, India	247	Mairh *et al.* (1989)
Sargassum cinereum J.Ag.	Gujarat Coast, India	206*	Vinogradova (1953)
Rhodophyta (red algae)			
Asparagopsis sanfordiana Harvey	Hawaii, USA	1250	Grimm (1952)
Gracilaria crassa Harvey ex J.Ag.	Mandaitivu, Sri Lanka	889	Mageswaran and Sivasubramaniam (1984)
Gelidiella acerosa (Forsskål) Feldmann & Hamel	Mandaitivu, Sri Lanka	524	Mageswaran and Sivasubramaniam (1984)
Chondus crispus Stackhouse	Helgoland, Germany	271	Kundel *et al.* (2012)
Sarconema filiforme (Sonder) Kylin	Bhavnagar, India	151	Mairh *et al.* (1989)
Galaxaura obtusata (J.Ellis & Solander) J.V.Lamouroux (= *Dichotomaria obtusata* (J.Ellis & Solander) Lamarck)	Bermuda	120	Grimm (1952)
Chlorophyta (green algae)			
Ulva reticulata Forsskål	Mandaitivu, India	259	Mairh *et al.* (1989)
***Caulerpa racemosa* (Forsskål) J.Ag.**	**Babatngon, Leyte, Philippines**	**196**	**This study**
Caulerpa racemosa (Forsskål) J.Ag.	Bhavnagar, India	88.3	Mairh *et al.* (1989)
Chaetomorpha sp.	Nainativu, Sri Lanka	44	Mageswaran and Sivasubramaniam (1984)
Enteromorpha flexuosa (Wulfen) J. Ag. (= *Ulva flexuosa* Wulfen)	Bhavnagar, India	38.1	Mairh *et al.* (1989)
Avrainvillea longicaulis (Kützing) G.Murray & Boodle	Bermuda	20	Grimm (1952)
Caulerpa chemnitzia (Esper) J.V.Lamouroux. (= *Caulerpa racemosa* var. *laetevirens* (Montagne) Weber Bosse)	Bermuda	0 (negative result)	Grimm (1952)

*Converted from µg/L

found the rate to range from 0.09 pmol min^{-1} gFw^{-1} or 1.64 x 10^{-5} ppm/d in the brown alga *Fucus vesiculosus* L. and 1.25 pmol min^{-1} gFw^{-1} or 2.28 x 10^{-4} ppm/d in *F. serratus* L. The manner of release of iodine also varies. The kelps *Laminaria* spp. were shown to emit iodine compounds temporally in multiple emission bursts (Dixneuf *et al.* 2008), whereas other brown algae like *Ascophyllum nodosum* (L.) Le Jolis, *F. serratus*, and *F. vesiculosus* showed a different emission rate that increased over an extended period of time (Kundel *et al*. 2012). Ball *et al*. (2010) found out that although the iodine concentration in *Laminaria digitata* (Hudson) J.V.Lamouroux is 7–30 times greater than that in *F. vesiculosus*, *i.e.* the I_2 emission rates from *L. digitata* surpassed that from *F. vesiculosus* by approximately three orders of magnitude. Similarly, in kelps, exposure to air during low tide was shown to induce the release of molecular iodine into the atmosphere (Nitschke *et al.* 2015).

As these studies on iodine release were done mostly using brown algae, we can only hypothesize that similar mechanisms may apply to green algae. To our knowledge, there are still no studies on the details of iodine emission from the green algae, including *C. racemosa.* It is not clear

which iodine species are retained or which are released into the atmosphere. Hence, further studies are necessary to answer these questions and to understand the iodine mechanism in green algae.

Nonetheless, as revealed in the present study, the lowering of *C. racemosa* iodine levels in storage followed a linear trend at an average decreasing rate of 20 ppm/d over 7 d. Upon closer examination, however, the dramatic decrease was observed only on the fourth day, with an almost constant rate that followed gradually thereafter. This may be attributed to the environmental stress causing an unpleasant odor and limp physical appearance of *C. racemosa* on the fourth day. On this day, *C. racemosa* cells may have experienced the greatest oxidative stress, as shown in their physical characteristics. This stress on seaweed samples may have caused an outburst of hydrogen peroxide in their tissues, leading to the oxidation of accumulated iodide ions and releasing them into the atmosphere as elemental iodine (Giese *et al*. 1999; Kundell *et al.* 2012). Additionally, we surmise that some cells of the algal samples might have died off on the fourth day due to cell disruption, bacterial activities, and/or lack of metabolic activities. This is evidenced by the slight foul smell of the seaweeds. This needs to be verified though, and additional microbial examination should be done in future studies.

Biological membranes such as in algal species are usually permeable to water and elemental forms of oxygen, nitrogen, carbon dioxide, and others. They are also semi-permeable to some ions, sugar, and other small molecules. Another contribution to the observed shrinking of the samples was the diffusion of elements out of the cell, such as iodine, when biological membranes are destroyed (Mageswaran and Sivasubramaniam 1984; Ghosh 2004; Zava and Zava 2011).

In summary, the iodine concentration of fresh *C. racemosa* from Babatngon, Leyte was measured using titration to be in the highest average of 196 ppm/ g dry weight of algal biomass. Changes in the concentration of iodine in *C. racemosa* were recorded daily for 7 d. From 196 ppm initial concentration of iodine, this dropped to 94.8 ppm on the seventh day with an average rate of decrease of 20 ppm/d. The highest iodine decrease was observed during the fourth day and may be related to the initiation of tissue decay. Nonetheless, iodine concentration on the seventh day of storage was still enough to meet the normal recommended daily amount of iodine intake for an average Filipino adult.

The iodine concentration of *C. racemosa* from other locations aside from Babatngon, Leyte should also be investigated. Different procedures in determining iodine concentrations could also be used. Any different results obtained will be useful for further iodine biogeochemistry studies. Also, hypotheses on the causes of the decline in iodine concentrations in the *C. racemosa* samples need to be evaluated and verified through further experiments.

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