Phytochemistry, Vol. 28, No. 12, pp. 3307–3310, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

SEASONAL VARIATION, BIOACCUMULATION AND PREVENTION OF LOSS OF IODINE IN SEAWEEDS

O. P. MAIRH, B. K. RAMAVAT, A. TEWARI, R. M. OZA and H. V. JOSHI

Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, India

(*Received in revised form* 17 *May* 1989)

Key Word Index—*Caulerpa, Enteromorpha, Sargassum, Levringia, Asparagopsis, Hypnea*; iodine; seaweed; determination of iodine; bioaccumulation; prevention of loss of iodine.

Abstract—The seasonal variation of iodine levels of 21 species of marine algae from Okha have been analysed. The levels in green seaweeds were highest (66.83 to 88.25 mg/100 g dry algae) in *Caulerpa racemosa* and lowest (30.26 to 38.07) in *Enteromorpha flexuosa*. The level increased from young to mature plant. Amongst the brown algae, the levels were highest (188.94 to 246.89) in *Levringia boergensenii* and lowest (27.92 to 72.05) in *Padina tetrastromatica*. In the red algae, the levels were highest (91 to 438.5) in *Asparagopsis taxiformis* and lowest (66.14 to 151.22) in *Sarconema filiformis*. The alcoholic-potassium hydroxide method generally gave a higher iodine level than the ionanalyser and Larsen methods. *Levringia boergensenii* grown in seawater enriched with potassium iodide and nutrients, absorbed 17.2% more iodine than the controls.

INTRODUCTION

Iodine is an essential element for many organisms. Its deficiency causes serious disorders such as goitre and cretinism in many parts of the world [1]. In India, over 170 million people are exposed to endemic goitre [1]. In view of the high demand for iodine for medicinal use, it is essential to search for feasible sources. At present, seaweeds are not used for the production of iodine, but in China, Japan, the U.S.A. and Canada, goitre is prevented by the use of seaweeds [2]. The seasonal variation of the iodine content of 11 spp. of seaweeds from Mandapam [3] and red alga *Asparagopsis taxiformis* (Delile) Collins et Harvey, from Okha [4] and Mandapam [5] has been studied. However, no other algae occurring on the Gujarat coast were examined. Therefore, an attempt has been made to study the above aspects in some selected macromarine algae from Okha.

Different methods [6–8] have been used for the determination of iodine in seaweeds, therefore, it is not possible to compare the results of different studies. In this study, all these methods were used for the determination of iodine in some selected seaweeds so that any variation in iodine content due to these methods could be determined.

The concentration of iodine by Indian seaweeds from iodine-rich media has not been studied previously, therefore, experiments were devised to find out the concentrating ability of *Levringia boergensenii* Kylin which is readily available and contains a high-level of cellular iodine so that in future it may be utilized to concentrate iodine from complex industrial and hospital wastes.

Iodovolatilization and liberation of iodine in algal extracts have been reported from some seaweeds [9]. No such report is available for tropical seaweeds including the Indian seaweeds. Hence, efforts were made to study iodovolatilization, liberation of iodine and prevention of its loss by chemical pretreatments.

RESULTS

Seasonal variation

Amongst the green algae the iodine content was highest in *Caulerpa racemosa* (Forssk.) Weber v. Bosse (66.83 to 88.25 mg iodine/100 g of dry alga; this unit is applicable throughout this paper) followed by *Ulva fasciata* Delile (32.78 to 76.10). It was lowest in *Enteromorpha flexuosa* (Wulf.) J. Ag. (30.26 to 38.07). The iodine content of *Caulerpa racemosa* was highest in October. Later, it gradually declined up to December. In *Caulerpa scalpelliformis* (R. Br.) Web. v. Bosse, the highest value (56) was recorded in December to March and then it declined to reach a minimum in April. In *Codium dwarkense* Boergs., the values increased slightly from January to March and then declined during April. In *Halimedia tuna* (Ell. et Sol.) Lamour, there was a continuous increase in iodine content from December to February. In *Ulva lactuca* Linn. high values were recorded in November and January and low values in December and by the end of February. In *Enteromorpha tubulosa* Kutz. the values increased from November to February. In *E. flexuosa* a similar trend was observed from November to January.

Amongst the brown algae, the values were highest in *Levringia boergensenii* (188.94 to 246.89), the next highest values were recorded in *Sargassum tenerrimum* J. Ag. (87.92 to 148.0) and the lowest in *Padina tetrastromatica* Hauck. (27.92 to 72.05). In these plants, the highest values were recorded during December and January when the plants reached their maximum size. They then declined when the plants started to degenerate in February. In

Cystoseira indica (Thivy et Doshi) Mairh, the highest values were found in January (88.71) and May (87.92), this corresponds to the two peak growth periods found in this alga [10]. In *Sargassum johnstonii* Setchell et Gardner, the lowest value (52.52) was recorded in September when the plants were small and young, and then the concentration of iodine increased continuously until a maximum (108) was reached in January when the plants attained maximum size. Thereafter, it declined slowly up to May. In *Iyengaria stellata* (Boergs.), the lowest value was recorded in December and then the iodine content increased continuously till March. In the case of *Levringia boergensenii*, however, the trend was just the opposite. In *Padina tetrastromatica*, the iodine content was lower in young than in mature plants. In *Spathoglossum asperum* J. Ag., the lowest value was observed in November and highest in December, later, the values fluctuated without showing much change.

Amongst the red algae, the iodine content was highest in *Asparagopsis taxiformis* (91 to 438.5) followed by *Hypnea musciformis* (Wulf.) Lamour. (170.8 to 251.30). The lowest values were obtained with *Sarconema filiformis* (Sond.) Kylin. (66.14 to 151.22). *Acanthophora spicifera* (Vahl.) Boergs. was also rich in iodine (180.0 to 186.71). These data show that iodine content is highest in red algae followed by the brown and green algae.

Variation of iodine content when determined by different methods

Generally the algae analysed by the alcoholic-potassium hydroxide method gave higher values compared to those analysed by either an ionanalyser or the Larsen method (Table 1).

Table 1. Variations in iodine levels determined by different methods

Alga	Iodine (mg/100 g of dry alga)		
	Alcoholic-KOH	Ionanalyser	Larsen
Green alga			
Enteromorpha flexuosa	79.37	68.25 (14.00)	65.00 (18.00)
Brown alga			
Spathoglossum asperum	111.40	118.13 (−7.00)	109.50 (0.82)
Red alga			
Asparagopsis taxiformis	525.00	515.80 (4.70)	500.00 (5.00)

Values in parenthesis indicate % reduction of iodine as compared to alcoholic-potassium hydroxide method.

Effect of different habitats on cellular content of iodine

Levringia boergensenii and *Sargassum johnstonii* occurring in submerged habitat showed a slightly higher iodine content (6.4 and 3.2% respectively) than those in exposed habitat.

Effect of nutrients on bioaccumulation of iodine

Levringia boergensenii grown in seawater enriched with nutrients [11] (without KI) absorbed 7.06% more iodine (215.2 mg 100/g of dry alga) than the control alga (201.0). The alga accumulated more iodine (17.26% more) than the control, when the concentration of iodine was increased (166.0 μg KI/l) in the medium.

Iodovolatilisation and iodine liberation studies

The seaweeds did not show any sign of iodovolatilization during exposure in a natural habitat or during drying in sun and shade. This was shown by a negative test for iodine. However, an exudate containing iodine

Table 2. Effect of chemical pretreatments on the prevention of loss of iodine during processing of *Asparagopsis* and *Levringia*

Algae	Iodine (mg/100 g of dry alga)						
	Formalin treatment for						
		2 sec		1 hr			
	Control plants dried 1	Plants dried 2	Plants kept in NaOH solution soaked filter paper envelope and dried 3	Plants dried 4	Plants kept in NaOH solution soaked filter paper envelope and dried 5	NaOH treatment for 2 sec. Plant dried on filter paper 6	Blank, filter paper only 7
Asparagopsis taxiformis	219.7	364.90	349.44	295.75	340.40	387.66	ND*
Asparagopsis taxiformis with filter paper	227.5	373.10 (64)	373.0 (64)	304.85 (34)	345.80 (52)	384.00 (70.4)	ND
Levringia boergensenii	121.0		128.0 (5.78)	—	—	136.0 (12.4)	ND

* = Not detected

Values in parenthesis indicate % increase over control.

was observed on the surface of *Asparagopsis taxiformis* and *Levringia boergensenii*, as a paper moistened with starch solution turned blue when brought in contact with the algae. The exudation of iodine in the former species was much more than in the latter species.

When *Asparagopsis* was dried in a specially designed apparatus (see Experimental), the majority of the liberated iodine remained within the flask in which plants were kept, while, very little iodine passed through the outlet tube into the starch solution.

Prevention of loss of iodine during processing

In *Asparagopsis taxiformis*, the highest iodine level (387.66 including that absorbed by filter paper) was found when the plants were dipped in 1% NaOH solution (for 2 sec) and then dried (Table 2). In these samples, a 70.4% increase in iodine was observed as compared to the control alga. The next best values were found in the plants which were pretreated with 4% formalin as well as formalin and sodium hydroxide for 2 sec. and then dried. Formalin treatment for 1 hr also prevented loss of iodine. But, in these samples 22.3% iodine was lost as compared to samples treated for 2 sec with formalin only. The lowest iodine value (227.5) was found in the control alga i.e. in the plants which were not pretreated with formalin or NaOH before drying. The Iodine content measured after the different treatments followed the trend (Table 2): $6>2$ and $3>5>4>1$. In *Levringia boergensenii*, 12.4% and 5.78% more iodine was observed after sodium hydroxide and formalin treatments respectively, as compared to the control alga. The results indicate that chemical pretreatments have to be standardized for each species.

DISCUSSION

The lowest, and least variable, iodine levels are found in ephemeral forms occurring in the upper littoral region, e.g. *Enteromorpha flexuosa* and *Ulva lactuca* (30.26 to 38.07). *Ulva lactuca*, a thin and flat thalloid form, contains less iodine than the ribbon shaped *Ulva fasciata*, found in the lower littoral region. Similarly, *Caulerpa racemosa* which is a multiaxial siphonus form and occurs in subtidal condition, accumulated comparatively more iodine amongst the green algae. Thus, in green algae the iodine levels appear to be related to habitat and morphology of the plant body.

Amongst brown alga, the highest iodine levels (188.94 to 246.89) are found in *Levringia boergensenii* in which the thallus is an aggregate of uniseriate filaments. The next best levels are found in *Sargassum tenerrimum* in which the plant body is thick and differentiated into holdfast, stem and leaves. The lowest levels (65.7 to 72.05) are found in *Padina tetrastromatica* which is a flat thalloid form, and are comparable to that reported earlier (61.2) from Goa [12]. By contrast, the same alga collected from Andaman and Nicobar [13] gave a very low value (5.0). The iodine content in *Sargassum johnstonii* increased from the young to adult stage (September–January) and declined after the plants attained maturity and started degenerating in February and March. The same trend is shown by *Cystoseira* in which the iodine levels ranged from 44.9 to 88.97. In this alga, the two peaks of iodine levels (70.5 and 87.92) correspond to the two peak growth periods (December to January and May to June) on the Gujarat coast [10]. In the Phaeophyceae, iodine is distributed throughout the plant body, the fronds are often richer than stipe [9].

Amongst the Indian red algae, the highest iodine level (740) is reported in *Chondrus* sp. (?) from the Goa coast [12], the next higher level is found in *Asparagopsis taxiformis* from Okha [14, 4] and the Mandapam region [5]. In *A. taxiformis*, the maximum iodine level is recorded in December when vegetative branches and erect system of the plant are predominant, thereafter, it continues to decline until a minimum is reached in April when the plants were decaying and rhizomatous branches predominate [4]. In this alga, the iodine is localized in iodine cells (*Ioduques*) [9], which are more abundant in the aerial parts than in the basal rhizomatous portion [4].

Other seaweeds rich in iodine are *Laminaria saccharina* Lamour. (0.45 to 0.76% iodine of dry weight) and *Ecklonia* (0.13 to 0.59%) from Japan and *Phyllophora* (0.27 to 0.58%) in Russia. In these algae also, the iodine level shows seasonal and morphological variation [15].

Out of the three different methods used for the determination of iodine, the alcoholic-potassium hydroxide method gave higher iodine levels than the Larsen and ionanalyser methods. This may be due to the strong and rapid binding of the potassium hydroxide in solution form in the alcoholic-potassium hydroxide method with the iodine in the alga, coupled with continuous refluxing in a closed system at a low temperature. In the Larsen and ionanalyser methods, the lower iodine levels are due to improper contact of the algal powder with the digestion mixture (in solid form) and subsequent use of a high temperature for complexing of the iodine. This resulted in escape of iodine to the atmosphere.

Loss of iodine was prevented by short-term (2 sec) pretreatment with formalin and sodium hydroxide. This may be due to inhibition of iodide oxidase which is responsible for loss of iodine from seaweeds [9]. Long pretreatment period by these chemicals did not prevent the loss of iodine, this may be due to their effects on the permeability of the cell membrane resulting in leaching of iodine. Certain seaweeds, e.g. *Ceramium*, *Ulva* and *Polysiphonia* can concentrate iodine from seawater or from artificial media [16]. Similar results were obtained for *Levringia boergensenii*.

EXPERIMENTAL

Fresh samples of algae were collected every month from Port Okha reef. The plants were washed with seawater to remove adhering impurities and epiphytes, rinsed in fresh water, and then dried immediately in the open-air in shade. The dried samples were powdered to 100 mesh, and then analysed for their I_2 contents by the alcoholic-KOH method [6]. Some selected algae were also analysed by the Larsen [7] and ionanalyser [8] methods. An ionanalyser (Orion model No. 901) and I_2-sensitive electrode (Orion 94-53) were used for analysing I_2 by the last method.

Effect of nutrients on uptake of I_2 from I_2-supplemented medium was studied using the commonly occurring I_2-rich *Levringia boergensenii*. The freshly collected live alga (100 g) was thoroughly washed with seawater and kept in 20 l of media: (a) enriched seawater [11], (b) enriched seawater + KI [11] and (c) seawater only. The experiment was performed under diffused sunlight (20 to 60 $\mu E.m^{-2}.s^{-1}$) for 12 hr. Thereafter, the plants were rinsed with seawater, followed by fresh water and dried in shade. I_2 was determined as described earlier [6].

The effect of desiccation on iodovolatilization of seaweeds *in situ* was studied by holding filter paper, moistened with starch soln (1%), very near to the seaweeds. Similar studies were made on the algae kept for drying on land near the sea. The algae were also spread on a herbarium paper and covered over with filter paper or cloth pieces moistened with 1% starch soln and dried under shade and sun. In another experiment, freshly collected *Asparagopsis* (10 g wet wt) was kept in a glass conical flask fitted with inlet and outlet tubes. The inlet tube reached upto the bottom of the flask and connected with an aerator. The outlet tube originated from the neck of the flask and passed through starch soln contained in another flask. The experiment was conducted under diffused sunlight. The liberated I_2 was collected in the first flask as well as in starch soln kept in another flask. I_2 was detected by the appearance of a blue colour in the starch soln.

Asparagopsis taxiformis and *Levringia boergensenii* were pretreated within an hr after collection by steeping them for 2 sec. and 1 hr in formalin (4%) and NaOH (1% soln) in six different combinations. The plants were then dried on filter paper in shade to 15% moisture within 48 hr. I_2 in different algae samples was determined (with and without filter papers) by the alcoholic-KOH method [6].

Acknowledgements—The authors wish to express their sincere gratitude to Prof. M. M. Taqui Khan (Director of this Institute) for his keen interest and encouragement, and to Dr E. R. R. Iyengar, for his helpful suggestions. We also thank M. R. Rajyaguru, S. G. Chauhan, H. M. Pandya and G. N. Pathak for their help during the field work.

REFERENCES

1. Nutrition foundation of India. (1983) *The National Goitre Control Programme, Scientific Report* Vol. 1, p. 10.
2. Arasaki, S. and Arasaki, T. (1983). *Low Caloric High Nutrion, Vegetables from the Sea, to Help You Look and Feel Better*. p. 46. Japan Publications.
3. Pillai, K. (1956) *Proc. Indian Acad. Sci.* **44**, 3.
4. Mairh, O. P. and Dave, H. M. (1982) *Salt Res. Ind.* **18**, 59.
5. Thomas, P. C., Ramavat, B. K., Rama Rao, K. and Subbaramaiah, K. (1987) *Phykos* **26**, 57.
6. Kappanna, A. N. and Rao, V. S. (1962) *J. Sci. Ind. Res.* **21**, 559.
7. Larsen, B. (1978) in *Handbook of Phycological Methods.* (Hellebust, J. A. and Craigie, J. S., eds), p. 185. Cambridge University Press, Cambridge.
8. Whyte, J. N. C. and Englar, J. R. (1976) *Analyst* **101**, 815.
9. Shaw, T. I. (1962) in *Physiology and Biochemistry of Algae* (Lewin, R. A., eds.), p. 247. Academic Press, New York.
10. Mairh, O. P. (1981) *Seaweed Res. Util.* **4**, 1.
11. von Stosch, H. A. (1964) in *Proc. 4th Int. Seaweed Symp.* (Virville De and Feldmann, J., eds), p. 142. Pergamon Press, Oxford.
12. Solimabi and Das, B. (1977) *Indian J. Mar. Sci.* 6, 180.
13. Solimabi, Das, B., Mittal, P. K. and Kamat, S. Y. (1981) *Indian J. Mar. Sci.* **10**, 310.
14. Dave, H. M., Rao, V. S and Tipnis, U. K. (1969) *Phykos* **8**, 68.
15. Chapman, V. J. and Chapman, D. J. (1980) *Seaweeds and their Uses*, 3rd Edn, p. 20. Chapman & Hall, London.
16. Roche, J. and Andre, S. (1968) *C. R. Seanc. Soc. Biol.* **156**, 71.