Original Article

Accumulation of manganese and zinc in growing Hijiki (*Sargassum fusiforme*) plants.

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Summary

Hijiki (*Sargassum fusiforme*) plants were collected during their growth period from the end of November through April at the sea coast of Kushimoto, Wakayama, Japan. The samples were brought back to the laboratory under ice-cold conditions and washed thoroughly with artificial sea-water and distilled water successively. The plants were cut into pieces of a 10 cm length along the stalk, and separated into leaves and stalks. The samples were lyophilized, and decomposed in a mixture of conc H_2SO_4 and conc HNO_3 (3 to 1, v/v) on an electric heater. Manganese (*Mn*) and zinc (*Zn*) in 1N HCl were determined with an atomic absorption spectrophotometer.

Mn and *Zn* accumulated in the respective tissues at concentrations of 10 to 50 μg/g dry weight of tissues at the beginning of growth, and their concentrations remained at similar levels until the beginning of April. These may, as discussed, suggest that the levels of *Mn* and *Zn* are their biochemically required concentrations in the tissues of Hijiki.

Keywords: Hijiki, *Sargassum fusiforme*; Manganese (*Mn*); zinc (*Zn*); growing Hijiki; atomic absorption spectrophotometry; seaweed.

Introduction

Brown algae, such as Hijiki (*Sargassum fusiforme*), grow on rocks at the sea-coast of Japan, bathed by the Kuroshio Current stream. Hijiki is familiar and used as a traditional food-stuff in daily Japanese dishes[1)]. Hijiki is rich in some nutritionally beneficial minerals[2-3)] and it has high contents of dietary fibers[4)].

We intended to elucidate accumulation processes of zinc and manganese during the growth of Hijiki plants.

Materials and Methods

1. Sampling of Hijiki plants

Hijiki, *Sargassum fusiforme*, (Harvey) Setchell*, were used. The embryos of Hijiki are fixed on rocks and germinate in summer[6-8)]. At the beginning of winter, they grow to the primary-leaf stage through the germlings in autumn. Early samples corresponding to this stage were collected in November. After then, Hijiki grows to adult plants. The samples were collected at the time of the lowest tide from the end of November through April on a fixed rock at the sea-coast of Kushimoto, Wakayama, Japan.

The harvested samples were immediately packed in an ice-cold box and brought back to the laboratory on the same day.

The sample plants were washed thoroughly with artificial sea water (three times) and distilled water[9)] (three times), successively. The samples, after blotted with filter paper to remove extra water, were cut to pieces of a 10 cm length from the lower end to the top, and each piece was designated as a', b', c', etc. They were separated to stalks and leaves (Fig. 1), and placed in small polyethylene bags, stored frozen under −30℃, and lyophilized.

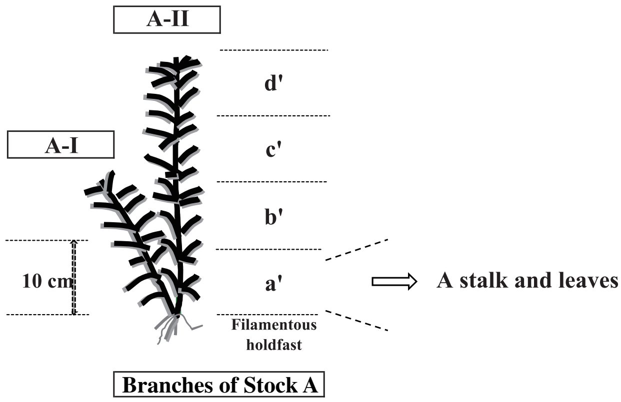

Fig. 1 Fractionation of Hijiki plants.

After washing, the sample palnts were cut into pieces of 10 cm length, and separated to respective tissues, a stalk and leaves. Their respective sections were designated as a', b', c', etc from the lower end to the upper end.

* Newly proposed taxonomic name of *Hizikia fusiforme* Okam.[5)]

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2. Ashing the samples

A portion of each sample was decomposed in a mixture of conc H_2SO_4 and conc HNO_3 (3 to 1, v/v) on an electric heater for a few hr.

The decomposed samples were dissolved in 1 N HCl, and *Mn* and *Zn* were determined with an atomic absorption spectrophotometer (Shimadzu AA-6200, Japan).

3. Atomic absorption spectrophotometry

The samples in 1 N HCl were suctioned up with compressed air and burnt with acetylene gas, *Mn* and *Zn* were determined using the Hollow-Cathode lamps for manganese and zinc (Hamamatsu Photonics Co. ltd.)

4. Reagents

The reagents were of the JIS Special Grade or its equivalent.

Manganese Standard Solution (JCSS) contained *Mn*, 1,000 mg/L as $Mn(NO_3)_2$ in 0.1 N HNO_3. Zinc Standard Solution (JCSS) contained *Zn*, 1,000 mg/L as $Zn(NO_3)_2$ in 0.1 N HNO_3.

Results

1. Growth of the Hijiki plants

1–1. The Hijiki plants, harvested at the end of November, were composed of 6 to 13 leaves in a stock (5 samples, Stock A to K in Table 1), the leaves show no visual stalks, corresponding to the primary leaf stage. Their lengths of the leaves were 2.13 ± 1.13 cm. The water contents in the fresh leaves were mostly 88%. These plants of the primary leaf stage contained several percent-less water than those of the samples harvested in February, suggesting that they were in a rather mature state.

1–2. In February, the Hijiki stalks grew to 12–17 cm in length, showing the shape of an adult plant. The water contents in the respective sections were 85 to 88% of the stalk weight and 90 to 91% of the leaf weight. These trends were observed in the samples harvested in March and April (Table 1). The leaves of the upper sections had higher contents of water than those of the lower sections, reflecting their maturity. The water contents of the leaves were higher than those of the stalks in the same sections, suggesting that immature leaves co-exist among these leaves, especially in the upper sections.

2. Accumulation of *Mn* (Table 2)

2–1. In the Hijiki plants of the primary-leaf stage harvested in November, the manganese contents were 7

Table 1 Water contents in the Hijiki plants during growth.

Date of harvest	Samples*	Tissues**	Number of leaves	Wet weight (g)	Dry weight (g)	Water contents (g water/g wet weight of tissues)
11/30/2008	A	Leaves	13	1.6187	0.1759	0.891
	B		9	1.0672	0.1174	0.890
	E		7	0.4927	0.0612	0.876
	F		12	0.5906	0.0734	0.876
	K		6	0.4844	0.0543	0.888

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water/g wet weight of tissues)
2/8/2009	A–1	Stalks	c'	0.0688	0.0104	0.849
			b'	0.6070	0.0850	0.860
			a'	0.4863	0.0738	0.848
	A–2	Stalks	b'	0.1865	0.0212	0.886
			a'	0.3741	0.0485	0.870
	A–3	Stalks	b'	0.2837	0.0342	0.880
			a'	0.4080	0.0500	0.877
	A–4	Stalks	b'	0.1139	0.0134	0.883
			a'	0.3808	0.0466	0.878
	A–1	Leaves	c'	1.1991	0.1146	0.904
			b'	2.1814	0.2354	0.892
			a'	1.0568	0.1047	0.901
	A–2	Leaves	b'	1.1957	0.1142	0.904
			a'	0.6428	0.0606	0.906
	A–3	Leaves	b'	1.1504	0.1095	0.905
			a'	0.6423	0.0588	0.908
	A–4	Leaves	b'	0.5573	0.0525	0.906
			a'	0.5837	0.0552	0.905

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water/g wet weight of tissues)
3/11/2009	A–1	Stalks	d'	0.5369	0.0801	0.851
			c'	0.7266	0.1213	0.833
			b'	0.7363	0.1282	0.826
			a'	0.5749	0.1050	0.817
	A–2	Stalks	d'	0.4166	0.0587	0.859
			c'	0.7172	0.1130	0.842
			b'	0.7184	0.1219	0.830
			a'	0.5897	0.0992	0.832
	A–3	Stalks	d'	0.0742	0.0102	0.863
			c'	0.6088	0.0883	0.855
			b'	0.6382	0.0942	0.852
			a'	0.5203	0.0845	0.838
	A–1	Leaves	d'	4.5252	0.4458	0.901
			c'	2.7554	0.3028	0.890
			b'	2.4778	0.2658	0.893
			a'	0.7324	0.0766	0.895
	A–2	Leaves	d'	3.6957	0.3680	0.900
			c'	2.5382	0.2788	0.890
			b'	2.5854	0.2720	0.895
			a'	0.2301	0.0264	0.885
	A–3	Leaves	d'	0.7658	0.0699	0.909
			c'	2.7152	0.2766	0.898
			b'	1.6423	0.1652	0.899
			a'	0.6545	0.0702	0.893

Date of harvest	Samples*	Tissues	Sections***	Wet weight (g)	Dry weight (g)	Water contents (g water/g wet weight of tissues)
4/12/2009	A–1	Stalks	e'	0.7400	0.0980	0.868
			d'	1.0452	0.1534	0.853
			c'	1.0735	0.1858	0.827
			b'	1.9837	0.1923	0.903
			a'	0.7316	0.1353	0.815
	A–2	Stalks	d'	0.7298	0.1033	0.859
			c'	0.9725	0.1522	0.844
			b'	0.8293	0.1495	0.820
			a'	0.6894	0.1178	0.829
	A–3	Stalks	c'	0.0996	0.0147	0.853
			b'	0.6523	0.0954	0.854
			a'	0.5625	0.0899	0.840
	A–1	Leaves	e'	5.4560	0.5161	0.905
			d'	4.5284	0.4521	0.900
			c'	4.0549	0.4266	0.895
			b'	1.9361	0.2163	0.888
			a'****	—	—	—
	A–2	Leaves	d'	5.1128	0.4923	0.904
			c'	3.9871	0.4216	0.894
			b'	1.7084	0.1765	0.897
			a'	0.1075	0.0112	0.896
	A–3	Leaves	c'	0.9063	0.0847	0.907
			b'	1.8905	0.1990	0.895
			a'	0.6173	0.0571	0.907

* A,B, E etc are designated to the respective stocks.
** No stalks were recognized for the sample harvested on 2008-11-30.
*** a' is designized to the bottom section (lower sectin of the stalk), and the value-columns were arranged in the order from the top section to the bottom section of the stalks.
**** No leaves.

Table 2 Manganese concentrations in the Hijiki plants during growth.

Date of harvest	Samples*	Tissues**	Number of leaves	μg *Mn*/g dry weight
11/30/2008	A	Leaves	13	6.863
	B		9	8.782
	E		7	7.114
	F		12	13.259
	K		6	15.961

Date of harvest	Samples*	Tissues	Sections***	μg *Mn*/g dry weight
2/8/2009	A-1	Stalks	c'	20.630
			b'	9.998
			a'	8.840
	A-2	Stalks	b'	25.170
			a'	12.635
	A-3	Stalks	b'	—
			a'	10.700
	A-4	Stalks	b'	42.117
			a'	12.854
	A-1	Leaves	c'	12.894
			b'	5.215
			a'	8.055
	A-2	Leaves	b'	6.223
			a'	—
	A-3	Leaves	b'	14.332
			a'	18.067
	A-4	Leaves	b'	20.429
			a'	27.943

Date of harvest	Samples*	Tissues	Sections***	μg *Mn*/g dry weight
3/11/2009	A-1	Stalks	d'	26.014
			c'	5.704
			b'	8.220
			a'	7.785
	A-2	Stalks	d'	29.587
			c'	11.832
			b'	15.139
			a'	16.984
	A-3	Stalks	d'	26.471
			c'	8.283
			b'	6.187
			a'	23.406
	A-1	Leaves	d'	10.915
			c'	14.054
			b'	11.725
			a'	17.797
	A-2	Leaves	d'	5.620
			c'	7.763
			b'	9.285
			a'	36.208
	A-3	Leaves	d'	22.570
			c'	9.203
			b'	14.855
			a'	26.802

Date of harvest	Samples*	Tissues	Sections***	μg *Mn*/g dry weight
4/12/2009	A-1	Stalks	e'	5.469
			d'	10.804
			c'	17.381
			b'	10.692
			a'	17.946
	A-2	Stalks	d'	11.750
			c'	9.552
			b'	7.480
			a'	10.541
	A-3	Stalks	c'	47.347
			b'	8.993
			a'	11.548
	A-1	Leaves	e'	2.500
			d'	2.642
			c'	4.695
			b'	5.108
			a' ****	—
	A-2	Leaves	d'	5.361
			c'	7.601
			b'	14.383
			a'	93.346
	A-3	Leaves	c'	14.883
			b'	8.082
			a'	25.325

* A,B, E etc are designated to the respective stocks.
** No stalks were recognized for the sample harvested on 2008-11-30.
*** a' is desigized to the bottom section (lower sectin of the stalk) and the value-column was arranged from the top section to the bottom section of the stalks.
**** No leaves.

Table 3 Zinc concentrations in the Hijiki plants during growth.

Dates of harvest	Samples*	Tissues**	Number of leaves	μg *Zn*/g dry weight*
11/30/2008	A	Leaves	13	27.873
	B		9	62.700
	E		7	34.062
	F		12	32.784
	K		6	28.159

Date of harvest	Samples*	Tissues	Sections***	μg *Zn*/g dry weight*
2/8/2009	A-1	Stalks	c'	—
			b'	4.942
			a'	5.085
	A-2	Stalks	b'	3.081
			a'	1.617
	A-3	Stalks	b'	—
			a'	2.132
	A-4	Stalks	b'	3.222
			a'	4.189
	A-1	Leaves	c'	10.411
			b'	8.183
			a'	5.899
	A-2	Leaves	b'	4.739
			a'	—
	A-3	Leaves	b'	5.241
			a'	9.124
	A-4	Leaves	b'	6.543
			a'	6.354

Date of harvest	Samples*	Tissues	Sections***	μg *Zn*/g dry weight*
3/11/2009	A-1	Stalks	d'	12.466
			c'	4.835
			b'	8.255
			a'	17.314
	A-2	Stalks	d'	29.028
			c'	10.923
			b'	12.616
			a'	8.408
	A-3	Stalks	d'	74.740
			c'	13.013
			b'	11.944
			a'	14.773
	A-1	Leaves	d'	8.699
			c'	9.439
			b'	10.839
			a'	12.645
	A-2	Leaves	d'	5.873
			c'	9.314
			b'	11.856
			a'	41.554
	A-3	Leaves	d'	15.670
			c'	7.757
			b'	13.869
			a'	29.454

Date of harvest	Samples*	Tissues	Sections***	μg *Zn*/g dry weight*
4/12/2009	A-1	Stalks	e'	6.637
			d'	4.763
			c'	5.248
			b'	6.239
			a'	9.869
	A-2	Stalks	d'	5.097
			c'	4.955
			b'	8.173
			a'	12.686
	A-3	Stalks	c'	29.116
			b'	6.087
			a'	8.241
	A-1	Leaves	e'	4.366
			d'	5.018
			c'	5.811
			b'	7.835
			a' ****	—
	A-2	Leaves	d'	4.135
			c'	6.656
			b'	9.716
			a'	46.198
	A-3	Leaves	c'	1.283
			b'	6.710
			a'	9.131

*, **, ***, **** Same as described in the legends of Table 2.

to 16 μg *Mn*/g dry weight, corresponding to 0.12 to 0.29 microgram-atom/g dry weight of the tissues.

2–2. The leaves harvested in February showed lower manganese contents than those harvested in March and April. In most branches, the leaves of the lowest sections, designated as **a'** in Table 2, showed highest manganese concentrations in a branch. These may indicate that a longer time of accumulation resulted in a higher concentration of manganese in the respective sections.

2–3. In the stalks, the highest concentration of manganese was often found in the highest sections designated as **c'** or **d'**, in contrast to the leaves.

3. Accumulation of *Zn* (Table 3)

3–1. In the Hijiki plants harvested in November, the zinc contents were 30 μg/g dry weight in most stocks, except one which contained more than 60 μg *Zn*/g dry weight. These values correspond to 0.43 to 0.96 microgram-atom *Zn*/g dry weight of tissues.

3–2. In the leaves harvested in February, the zinc contents were lower than those harvested in March and April. During the growth of the tissues, zinc is to be accumulated more in the mature tissues. In the leaves of the lowest section, designated as **a'** in Table 3, the highest concentration of zinc in the branch was often found.

3–3. In the stalks, the zinc content was also higher in the samples harvested in March and April.

The zinc concentrations in the lowest section designated as **a'** in Table 3 in the respective stalks as well as those in the highest sections designated as **d'** often showed greater levels than those of the middle sections.

Discussion

During the accumulation of zinc in the tissues, some properties of manganese, which is replaceable for zinc in the biochemical reactions of zinc-containing enzymes, may not be dispensed with, and this may affect somewhat the accumulating processes of these elements. In the samples of November, the levels of zinc as well as manganese are not uniform; but when these concentrations are expressed in gram-atom and those of both elements are summed up, the summed values are more uniform, 0.55 to 1.11 microgram-atom/g dry matter, than the individual concentrations.

In the adult type plants harvested after February, the leaves of the lower sections of the branches showed higher concentrations of manganese than the stalk fraction. If these elements are viewed as enzymatic active sites in polysaccharide biosynthesis, the higher accumulation of manganese (Table 2) in the lower sections is interesting. The higher manganese accumulations in the uppermost sections may also indicate greater necessity for enzymatic activities in polysaccharide biosynthesis and photosynthesis.

The zinc accumulation may be related to carbonate dehydratase activity in the CO_2 assimilating process, since it is known that, under the higher CO_2 utilizing conditions, greater activities of this enzyme occurred transiently[10]. It is our subject to investigate the reason why the most upper sections including the apex accumulated highest concentrations of zinc (more than 29 μg/g dry matter) in a branch, and why some samples, such as those harvested in March and April, accumulated higher zinc concentrations in the leaves in the lowest sections (Table 3).

Out of several brown algae used as food stuffs, Hijiki is an alga (seaweed) remarkably rich in nutritionally beneficial elements[2, 3]. This will be an attractive property of Hijiki as a foodstuff to contribute to the longevity[1] of Japanese. On the other hand, Hijiki often accumulates large concentrations of arsenic[2, 11–14]. However, water-soaking, as a pre-cooking process of dried Hijiki, could be effectively employed to remove most of the arsenic levels[15–17] in Hijiki.

Moreover, Hijiki has been found to have a remarkable preventive effect on colon carcinogenesis[18]. Thus, Hijiki will deserve recommendation as a daily usable foodstuff, by practicing appropriate pre-cooking processes.

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