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Original Article

Behaviors of minerals in Hijiki plants becoming chlorotic

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Summary

Hijiki (*Sargassum fusiforme*, a brown algae) plants were harvested on the Hime Coast, Kushimoto, Wakayama, Japan, in early April, 2016. The harvested Hijiki plants were classified to "grade A" when normal in appearance and "grade B" when rather discolored.

The sample plants were cut at a length of 10cm along the stalk, and the stalks and leaves were separately lyophilized. Respective samples were ashed in conc HNO_3-conc $HClO_4$ on an electric heater for a few hours. The concentrations of arsenic (*As*), calcium (*Ca*), iron (*Fe*), magnesium (*Mg*), manganese (*Mn*), potassium (*K*) and zinc (*Zn*) in the tissue*s* were determined by atomic absorption spectrophotometry. The correlative coefficient values of some pairs of elements in grade B plants were higher than those in grade A plants, and the number of pairs of a higher correlative coefficient in grade B plants was more than in grade A plants. The grade B plants becoming chlorotic contained less amount of *chlorophyll a* and more homogenous concentrations of various elements. The growing conditions of the Hijiki plants and accumulation patterns of those elements were discussed in relation to the warming ocean sea water.

Keywords: Hijiki (*Sargassum fusiforme) plants*; arsenic (*As*); calcium (*Ca*); Iron (*Fe*); magnesium (*Mg*); manganese (*Mn*); potassium (*K*); zinc (*Zn*); chlorophylls; chlorosis.

Introduction

Out of the coastal fishery products, seaweeds such as brown algae groups are consumed[1)] mostly beause of their "Umami" taste. Hijiki, *Sargassum fusiforme*, a family of brown algae, *Phaeophyta*, is rich in nutritionally beneficial minerals[2)] as well as in dietary fiber[3)]. Hijiki is used as a traditional Japanese foodstuff for a long time.

The HIjiki plants, harvested on the Coast of Kushimoto, Wakayama Prefecture, Japan, showed a unique process[4)] of accumulating arsenic (*As*)[5)], calcium (*Ca*)[5)], iron (*Fe*)[6)] magnesium (*Mg*)[6)], manganese (*Mn*)[7)], potassium (*K*) and zinc (*Zn*)[7)] during their growth. Out of these elements, a strong correlation was found between the accumulation processes[4)] of *Mg* and *Mn* as well as between those of *Mn* and *Zn*. The *Ca* concentrations became constant during the growth of Hijiki, although *Mn* and *Zn* continued to increase in their accumulation[4)].

The warming-up of ocean sea water is becoming to affect the physiology of seaweeds as well as corals, as feared from the chlorosis of corals. On the coast line of Kushimoto, Wakayama Pref., the normal growth of Hijiki plants are partly changing since about ten year's ago[***]. The Hijiki plants growing with some change in pigments would make it difficult to maintain their commercial values on their market because of their change in color from the traditional black. From view points of nutritional science as well as plant physiology, we were interested to study some alteration of mineral components in discolored Hijiki plants.

Foot note: [***]Personal communication from Ms. Hori, Chief of Hime Hijiki-Harvesting Fishermen's Union Association.

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Materials and Methods

1. Sampling of Hijiki plants

Hijiki, *Sargassum fusiforme*, plants were harvested on 9th April, 2016, on the Hime coast of Kushimoto District, Wakayama Prefecture, Japan, at the time of the lowest tide, during their growing period[4-7] as indicated in the data. The fresh plants were brought back in an ice-cold box to our laboratory[4].

2. Preparation of Hijiki plants[4]

The fresh plants were washed thoroughly with artificial sea-water three times and then with purified distilled water[5] three more times, being blotted each time with filter paper. The sample plants harvested in April 2016 were cut at a length of 10 cm along the stalk from the bottom to the top of the plants, each section designated as a', b', c', and so on. The leaves and stalks were separated, weighed and stored at −40℃ until freeze-dried.

The individual plants normal in appearance were designated as grade A and those discolored were designated as grade B.

3. Determination of arsenic (*As*), calcium (*Ca*), Iron (*Fe*), magnesium (*Mg*), manganese (*Mn*), potassium (*K*) and zinc (*Zn*)

The samples were ashed in conc HNO_3- conc $HClO_4$ (5:1,v/v) on an electric heater for a few hours, and made up to a constant volume with 1N HCl. *As, Fe, Mn* and *Zn* in HCl solution were determined by the flame-less method on a pyro-coated graphite tube connected to an atomic absorption spectrophotometer (Shimadzu AA-7000 with ASC-7000 and GFA-7000). For *As* determination, 10 mg/L of palladium nitrate was added.

Ca, Mg and *K* were determined in acetylene gas flame with an atomic absorption spectrophotometer (Shimadzu AA-7000 and ASC-7000).

The elements in the specimens were determined in duplicate or triplicate and expressed in the average values.

4. Chlorophyll and carotenoid contents

The fresh tissues were mixed in 80% acetone for overnight at 4℃ with a small amount of L-ascorbic acid. The chlorophyll and carotenoid spectra of the extracts were determined[8] in 80% acetone with a spectrophotometer, and the contents were calculated using the factors[8] proposed for the brown algae.

Also a portion of each extract was developed on a thin layer chromatograph, by the ascending process with petroleum ether and acetone (7:3 v/v). The respective spots were confirmed under a UV lamp to detect the spots of the respective chlorophyll and carotenoids.

5. Reagents

Reagents of the JIS special grade and/or JCSS grade were used. Water was MilliQ or a distilled water of high grade[9].

6. Statistic treatment

The respective values were expressed as average ± standard deviations, with numbers (n) in parenthesis. The correlation between the minerals' accumulation was expressed as Pearson's correlation coefficients two-tailed test, with *p* values.

The statistic calculations were made by a built-in-function in Microsoft Excel 2011 (Mac version) and Statcel 3 (OMS Publishing Co., Japan), add-in forms on Excel.

Results

1. Water contents in Hijiki plants

The average values of water contents in the individual plants were 83.7 ± 3.1% (n = 14) in grade B plants, and 86.5 ± 3.0% (n = 22) in grade A plants. However, the average value of the leaves of grade B plants was 87.3 ± 0.4% (n = 6) and that of grade A plants, 89.2 ± 0.5% (n = 11) (Table 1). The lesser amount of water contents in grade B plants than that in grade A plants may suggest a more matured tendency in grade B plants (Table 1). The maturation of the plants of this time increased more than in the plants of the previous samples[4].

2. Determination of *K*

The average values of the *K* concentrations in leaves and stalks were less in grade B plants (86.18 mg *K*/g dry weight, n = 14) than in grade A plants (108.88 mg *K*/g dry weight, n = 22) (Table 2). In the samples of April 2013[4], the *K* concentrations were 110 mg *K*/g dry weight (n = 48).

3. Determination of *As*

The average values of the *As* concentrations were higher in the leaves than those in the stalks at the same sections, and the average concentrations of summed *As* values of the stalks and leaves were less in grade B plants (132.85 μg *As*/g dry weight) than those in grade A plants (117.79 μg *As*/g dry weight). (Table 3). The *As* concentrations in samples of leaves and stalks harvested on April 2013[4] were 97.6 μg *As*/g dry weight.

Table 1 Water (H_2O) contents in Hijiki plants, expressed as g H_2O/g wet weight of tissues

Samples*	Tissues	Sections**	*g H_2O/g wet weight of tissues*
A-Ⅰ	Stalks	f'	0.860
		e'	0.861
		d'	0.851
		c'	0.839
		b'	0.833
		a'	0.835
	Leaves	f'	0.899
		e'	0.892
		d'	0.887
		c'	0.886
		b'	0.886
		a'	0.888
A-Ⅱ	Stalks	e'	0.860
		d'	0.847
		c'	0.825
		b'	0.821
		a'	0.799
	Leaves	e'	0.891
		d'	0.901
		c'	0.891
		b'	0.892
		a'	0.895
B-Ⅰ	Stalks	d'	0.798
		c'	0.810
		b'	0.814
		a'	0.811
	Leaves	d'	0.875
		c'	0.872
		b'	0.880
		a'	— ***
B-Ⅱ	Stalks	d'	0.808
		c'	0.813
		b'	0.808
		a'	0.819
	Leaves	d'	0.868
		c'	0.867
		b'	0.874
		a'	— ***

* Plants of sample A are regular type designated as *grade A* and sample B, irregular type designated as *grade B.*
** Samples were cut at 10 cm from the bottom side (filamentous holdfast side) to the top side of the plants, designated as a', b', c' etc.
*** No leaves existed.

Table 2 Pottasium (K) contents in the Hijiki plants, expressed as mg K/g dry weight of tissues

Samples*	Tissues	Sections**	*mg K/g dry weight of tissues*
A-Ⅰ	Stalks	f'	62.59
		e'	75.90
		d'	80.34
		c'	68.58
		b'	67.16
		a'	61.55
	Leaves	f'	149.73
		e'	147.98
		d'	149.10
		c'	143.30
		b'	135.75
		a'	129.93
A-Ⅱ	Stalks	e'	96.09
		d'	7.81
		c'	77.10
		b'	52.85
		a'	46.86
	Leaves	e'	161.09
		d'	181.09
		c'	164.72
		b'	174.39
		a'	161.51
B-Ⅰ	Stalks	d'	50.74
		c'	61.19
		b'	56.93
		a'	46.00
	Leaves	d'	141.18
		c'	119.15
		b'	53.22
		a'	— ***
B-Ⅱ	Stalks	d'	62.56
		c'	65.32
		b'	65.18
		a'	56.70
	Leaves	d'	149.90
		c'	134.07
		b'	144.32
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 3 Arsenic (*As*) contents in the Hijiki plants, expressed as μg *As*/g dry weight of tissues

Samples*	Tissues	Sections**	*μg As/g dry weight of tissues*
A-Ⅰ	Stalks	f'	42.84
		e'	52.63
		d'	50.74
		c'	56.65
		b'	95.69
		a'	126.57
	Leaves	f'	73.88
		e'	170.83
		d'	20.56
		c'	204.35
		b'	311.04
		a'	260.77
A-Ⅱ	Stalks	e'	68.19
		d'	67.94
		c'	78.85
		b'	75.03
		a'	142.37
	Leaves	e'	150.62
		d'	101.31
		c'	139.56
		b'	128.29
		a'	172.62

Samples*	Tissues	Sections**	*μg As/g dry weight of tissues*
B-Ⅰ	Stalks	d'	64.52
		c'	100.49
		b'	132.05
		a'	88.06
	Leaves	d'	138.87
		c'	168.36
		b'	157.09
		a'	— ***
B-Ⅱ	Stalks	d'	66.23
		c'	113.47
		b'	122.06
		a'	109.09
	Leaves	d'	163.08
		c'	208.82
		b'	227.65
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 4 Calcium (*Ca*) contents in the Hijiki plants, expressed as mg *Ca*/g dry weight of tissues

Samples*	Tissues	Sections**	*mg Ca/g dry weight of tissues*
A-Ⅰ	Stalks	f'	11.46
		e'	11.89
		d'	12.43
		c'	12.52
		b'	13.88
		a'	14.48
	Leaves	f'	9.86
		e'	12.66
		d'	11.72
		c'	12.25
		b'	12.27
		a'	12.77
A-Ⅱ	Stalks	e'	11.39
		d'	12.35
		c'	12.43
		b'	11.35
		a'	13.11
	Leaves	e'	11.63
		d'	10.86
		c'	10.86
		b'	11.07
		a'	10.61

Samples*	Tissues	Sections**	*mg Ca/g dry weight of tissues*
B-Ⅰ	Stalks	d'	13.17
		c'	13.73
		b'	13.59
		a'	13.29
	Leaves	d'	11.13
		c'	10.37
		b'	7.88
		a'	— ***
B-Ⅱ	Stalks	d'	13.19
		c'	12.88
		b'	13.14
		a'	12.83
	Leaves	d'	10.97
		c'	11.09
		b'	10.82
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 5 Iron (*Fe*) contents in the Hijiki plants, expressed as *μg Fe*/g dry weight of tissues

Samples*	Tissues	Sections**	*μg Fe/g dry weight of tissues*
A-Ⅰ	Stalks	f'	68.61
		e'	25.39
		d'	25.61
		c'	9.37
		b'	28.58
		a'	40.09
	Leaves	f'	11.28
		e'	46.68
		d'	27.56
		c'	24.46
		b'	65.82
		a'	42.59
A-Ⅱ	Stalks	e'	20.72
		d'	19.04
		c'	28.43
		b'	15.05
		a'	20.17
	Leaves	e'	111.68
		d'	58.14
		c'	15.69
		b'	35.31
		a'	13.89
B-Ⅰ	Stalks	d'	14.27
		c'	11.71
		b'	23.10
		a'	68.25
	Leaves	d'	27.22
		c'	23.05
		b'	152.64
		a'	— ***
B-Ⅱ	Stalks	d'	10.69
		c'	16.10
		b'	29.25
		a'	76.29
	Leaves	d'	21.59
		c'	24.37
		b'	32.09
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 6 Magnesium (*Mg*) contents in the Hijiki plants, expressed as mg *Mg*/g dry weight of tissues

Samples*	Tissues	Sections**	*mg Mg/g dry weight of tissues*
A-Ⅰ	Stalks	f'	5.90
		e'	6.38
		d'	6.72
		c'	7.21
		b'	7.33
		a'	6.97
	Leaves	f'	5.31
		e'	6.02
		d'	6.15
		c'	6.37
		b'	6.23
		a'	5.21
A-Ⅱ	Stalks	e'	6.65
		d'	6.83
		c'	7.31
		b'	5.96
		a'	7.50
	Leaves	e'	6.06
		d'	7.08
		c'	6.22
		b'	6.32
		a'	6.71
B-Ⅰ	Stalks	d'	7.41
		c'	6.97
		b'	6.84
		a'	6.49
	Leaves	d'	5.86
		c'	5.68
		b'	5.00
		a'	— ***
B-Ⅱ	Stalks	d'	6.96
		c'	6.38
		b'	6.59
		a'	6.02
	Leaves	d'	5.55
		c'	5.78
		b'	5.67
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 7 Manganese (*Mn*) contents in the Hijiki plants, expressed as μg *Mn*/g dry weight of tissues

Samples*	Tissues	Sections**	*μg Mn/g dry weight of tissues*
A- I	Stalks	f'	3.72
		e'	3.44
		d'	2.97
		c'	5.33
		b'	4.76
		a'	7.42
	Leaves	f'	4.63
		e'	7.94
		d'	2.01
		c'	7.84
		b'	12.17
		a'	13.50
A- II	Stalks	e'	2.94
		d'	3.45
		c'	2.32
		b'	2.46
		a'	3.77
	Leaves	e'	9.08
		d'	5.38
		c'	6.91
		b'	6.01
		a'	9.68

Samples*	Tissues	Sections**	*μg Mn/g dry weight of tissues*
B- I	Stalks	d'	4.34
		c'	4.00
		b'	7.63
		a'	13.12
	Leaves	d'	7.73
		c'	5.55
		b'	9.49
		a'	— ***
B- II	Stalks	d'	3.61
		c'	4.26
		b'	5.24
		a'	11.74
	Leaves	d'	6.25
		c'	7.24
		b'	8.70
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 8 Zinc (*Zn*) contents in the Hijiki plants, expressed as μg *Zn*/g dry weight of tissues

Samples*	Tissues	Sections**	*μg Zn/g dry weight of tissues*
A- I	Stalks	f'	51.14
		e'	19.75
		d'	2.22
		c'	3.29
		b'	1.58
		a'	2.73
	Leaves	f'	5.62
		e'	12.43
		d'	10.03
		c'	6.93
		b'	27.70
		a'	71.45
A- II	Stalks	e'	4.80
		d'	10.13
		c'	6.72
		b'	1.13
		a'	6.92
	Leaves	e'	15.07
		d'	8.15
		c'	31.13
		b'	5.45
		a'	8.62

Samples*	Tissues	Sections**	*μg Zn/g dry weight of tissues*
B- I	Stalks	d'	0.98
		c'	2.94
		b'	0.76
		a'	9.83
	Leaves	d'	21.23
		c'	18.25
		b'	57.20
		a'	— ***
B- II	Stalks	d'	5.95
		c'	9.46
		b'	5.44
		a'	108.58
	Leaves	d'	22.18
		c'	29.90
		b'	17.17
		a'	— ***

*, **, *** The explanations are as described in Table 1.

Table 9 Correlative coefficients between the concentrations of two kinds of elements or H_2O in the grade A or grade B Hijiki plants.

	Grade A plants							
Elements and H_2O	***H2O***	***Zn***	***Mn***	***Mg***	***K***	***Fe***	***Ca***	***As***
As	0.353	0.405	**0.908** *	−0.245	0.403	0.319	0.154	
Ca	**−0.582** ***	−0.038	0.095	0.450	**−0.542** ***	0.057		**−0.660** ***
Fe	0.319	0.348	0.405	−0.224	0.292		−0.587	0.109
K	**0.880** *	0.126	0.487	−0.412		−0.297	−0.476	**0.766** **
Mg	**−0.601** ***	**−0.573** ***	−0.363		−0.570	−0.563	**0.894** *	**−0.758** ***
Mn	0.516	0.513		−0.425	−0.056	**0.663** ***	−0.219	0.195
Zn	0.305		0.581	−0.524	−0.029	0.632	−0.356	0.159
H2O		0.267	0.218	**−0.900** *	**0.784** **	0.301	**−0.898** *	**0.830** **
	Grade B plants							

The concentrations of the respective elements or H_2O in the stalks and leaves were compared.
The values upper area of the diagonal line are of the grade A plants, and the values under area of the diagonal line are of the grade B plants.
The correlation between the elements in the row and those in the column was expressed as Pearson's correlation coefficients two-tailed test.
The p values of the correlation coefficients were as follows, * $p < 0.0001$, ** $p < 0.001$, *** $p < 0.01$.
The sample numbers of the grade A plants, leaves and stalks, were 22 (n), and those of the grade B plants were, 14 (n).
The elements analyses and the designations of grade A and grade B plants were described in the text.

Table 10 Chlolophyll-a and carotenoides contents (μg/g of wet weight of tissues)

	Grade A plants	Grade B plants
Chlorophyll *a*	71.4	58.7
Carotenoides	23.0	22.4

Chlorophyll *a* and carotenoid were calculated with the equations in the reference 8.

4. Determination of *Ca*

The concentrations of *Ca* in leaves and stalks of grade A plants were 11.99±1.05 mg *Ca*/g dry weight, and those of grade B plants were 12.01±1.61 mg/g dry weight (Table 4), which are comparable to the values of the samples harvested in April 2013[4] (11.8±1.3 mg *Ca*/g dry weight).

5. Determination of *Fe*

The average values of the *Fe* concentrations were higher in grade B plants (average, 37.9 μg *Fe*/g dry weight) than in grade A plants (average, 34.3 μg *Fe*/g dry weight) (Table 5). However, these values were much less than the values in the samples of April 2013[4] (114.6 μg *Fe*/g dry weight in average).

6. Determination of *Mg*

The *Mg* concentrations were similar or less in grade B plants (average, 6.15 mg *Mg*/g dry weight) than those in grade A plants (average, 6.49 mg *Mg*/g dry weight) (Table 6), which are less than the values in the April 2013[4] samples[4] (8.13±1.72 μg *Mg*/g dry weight in average).

7. Determination of *Mn*

The *Mn* concentrations were higher in grade B plants (average, 7.11 μg *Mn*/g dry weight) than those in grade A plants (average, 5.76 μg *Mn*/g dry weight) (Table 7). These values are higher than the *Mn* concentration in the April 2013 samples[4] (4.87 μg *Mn*/g dry weight).

8. Determination of *Zn*

The *Zn* concentrations were much higher in grade B plants (average, 22.82 μg *Zn*/g dry weight) than those in grade A plants (average, 13.86 μg *Zn*/g dry weight). The higher contents of *Zn* in the leaves of grade B plants contributed to the high values (Table 8). The *Zn* values in April 2013 samples[4] were 11.5 μg *Zn*/g dry weight.

9. Correlation coefficients between pairs of elements

In grade A plants, the correlation coefficients (r), higher than 0.5 ($p < 0.01$) in two pairs of elements were found between *As* and *Mn* ($r = 0.91$), *Ca* and *K* ($r = -0.54$), *K* and H_2O ($r = 0.88$), *Ca* and H_2O ($r = -0.58$), *Mg* and H_2O ($r = -0.60$), and between *Mg* and *Zn* ($r = -0.57$). In grade B plants, however, correlation coefficients (r) higher than 0.5 ($p < 0.01$) were found in 9 pairs as follows: *As* and *Ca*

($r = -0.66$), *As* and *K* ($r = 0.77$), *As* and *Mg* ($r = -0.76$), *As* and H_2O ($r = 0.83$), *Ca* and *Mg* ($r = 0.89$), *Ca* and H_2O ($r = -0.90$), *Fe* and *Mn* ($r = 0.66$), *K* and H_2O ($r = 0.78$), and *Mg* and H_2O ($r = -0.90$). These will be a particular characteristics of the grade B plants. In Table 9, the correlation coefficients between every pairs of the elements were described with the *p* values (< 0.01).

10. Chlorophyll-a and *carotenoid* contents

The chlorophyll *a* contents in the leaves of Hijiki of grade A plants were 71 *μg /g* wet weight of leaves and those of grade B plants were 59 *μg /g* wet weight of leaves. The carotenoid contents in grade A plants were similar to those in grade B plants (Table 10).

Discussion

Irregular changes occurring in Hijiki plants: Warming of the earth atmosphere, bringing a warm-up of ocean sea water seems to affect the growth of seaweeds, such as brown algae, Hijiki, *Sargassum fusiforme*. The brown color of Hijiki on the coast became purple, and its commercial products became to have a color different from the typical black color* (* Refer to the foot note in the previous page), resulting in quality deterioration of the dried products as commercial products. These changes may be partially similar to chlorosis observed in terrestrial plants.

Chlorophyll *a* and carotenoid contents: In grade B samples, the amount of chlorophyll *a* on the wet weight basis decreased by 18 % and the carotenoid contents were similar level. These may suggest an occurrence of irregularity in photosynthesis.

Mineral concentrations: The concentrations of minerals such as *K* and *Mg* in grade B plants' leaves decreased by several % compared to those of grade A plants (Table 2~8). From the data of water contents (Table 1), grade B plants seem to be in a more matured or more irregular stage than grade A plants. However, the average *Zn* contents of grade B plants are greater than those of grade A plants. Interestingly, some enzymes such as dehydrogenases and carbonic anhydrase have *Zn* at their active site, although the existing forms of excess *Zn* in Hijiki plants are to be investigated. The concentration of *As* in the April 2013 samples (100 *μ*g/g dry weight of tissues) increased by about 20 to 30% in the April 2016 samples, but the *K* concentrations were similar or less. In the plants of April 2016, grade A plants had 12.0 mg *Ca*/g dry weight of tissues and grade B plants had 11.8 mg *Ca*/g dry weight of tissues. The concentrations of *Ca* in March 2009 and April 2009 samples gradually arrived at the constantly higher values of 11.8 mg *Ca*/g dry weight of tissues in April 2013[4].

On the assumption that the samples of April 2016 are aged near to or more than the April 2013 samples, these values may be a maximum in the Hijiki plants.

The accumulated concentrations of *As* in the samples of April 2016 had a bigger dispersion than those of the April 2013 samples, although the correlation coefficient between *As* and H_2O had a strong correlation by grade B plants of April 2016 (Table 9).

Correlation of respective minerals: The correlation coefficients between the *K* and *As* concentrations in grade B plants of the April 2016 samples ($r = 0.77$) were higher than that in grade A plants ($r = 0.40$). Between *Zn* and *Mn*, the correlation coefficients were 0.51 in grade A plants, and 0.58 in grade B plants. The correlation between *Ca* and *Mg* was also stronger in grade B plants ($r = 0.89$, $p < 0.0001$; Table 9) than that in grade A plants ($r = 0.45$; Table 9).

The numbers of pairs showing greater correlation coefficients (whether positive or negative, with $p < 0.01$) increased in grade B plants in comparison to grade A plants. Out of them, a strong correlation coefficient between *As* and *Mn,* in grade A plants was lost in grade B plants. Moreover, the value between *As* and *Ca*, as well as that between *As* and *Mg* became strong in minus values in grade B plants.

In relation to this, the correlation coefficients between the water contents and the contents of several elements in grade B plants were higher than those in grade A plants, and the numbers of pairs showing a greater coefficient value increased in grade B plants (Table 9).

For the conservation of the regularity of metabolism in Hijiki plants: The upper critical temperature for regular growth of Hijiki plants has been shown to be 32℃[10], and thus the Hijiki plants on rocks under sunshine during the lower tide are apt to be exposed to an untolerable high temperature. The irregularity of mineral accumulations in the Hijiki plants may reflect more or less non-regulation of normal Hijiki metabolism. Although the ratio of physiological irregularity in normal regular Hijiki plants is not yet confirmed at present, it seems to be important to regain regularity for Hijiki growth. At least, global-warming-up should be reduced and/or stopped at an earliest time. This leads to a confirmation that the energy source on the whole earth should be limited to natural energy sources only, eliminating non-natural energy sources such as fossil-fuel energy and nuclear-fuel energy.

Conclusion

The "purple colored" (fishermen's description) plants, designated as grade B plants, contained lower levels of chlorophyll-a than the grade A plants, and concentrations of the respective elements are generally becoming homogeneous, and thus the entropy level of grade B plants is becoming higher. This will be a characteristic of grade B plants, becoming chlorotic.

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