Trace Nutrients Research 30 : 52－57 (2013)

Original Article

Accumulation of arsenic and calcium during the growth of Hijiki plants.

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Summary

In an attempt to study the accumulation processes of nutritionally important as well as ecologically unavoidable minerals in growing Hijiki (*Sargassum fusiforme* (Harvey) Setchell**) plants, tissue concentrations of calcium and arsenic were determined. Hijiki plants were harvested in several stages of their growth from November through April. Fresh plants were washed thoroughly and cut at a length of 10 cm along the stalk from the bottom to the top of the plants. The leaves and stalks were separated, weighed and stored at －40℃ until freeze-dried. Arsenic was determined by HPLC-ICP-MS analysis after extraction of the samples with a HNO_3 solution, and calcium was determined by atomic absorption spectrophotometry in a 1M HCl solution containing strontium chloride, after ashing the samples with a mixture of conc. H_2SO_4-conc. HNO_3 on an electric furnace. The distribution of arsenic concentrations showed a correlation with those of calcium during growth of Hijiki plants, in contrast to the correlations between arsenic and zinc, which correlated only in the earlier stages of growth, although these correlations were not so evident as found between manganese and zinc concentrations.

Keywords: arsenic (*As*); calcium (*Ca*); Hijiki (*Sargassum fusiforme*); correlation; growing Hijiki; atomic absorption spectrophotometry; HPLC-ICP-MS analysis; seaweed.

Introduction

Hijiki (*Sargassum fusiforme* **) is a brown algae, familiar to Japanese as a foodstuff[2]. The behaviors of nutritionally important minerals during their growing periods of Hijiki[3, 4] are worthy of investigation in comparison with the behavior of the ecologically unavoidable element, arsenic (*As*), in the same periods[5-8]. In the previous reports, the concentrations of iron (*Fe*)[4], magnesium (*Mg*)[4], manganese (*Mn*)[3], and zinc (*Zn*)[3] in Hijiki plants were determined. Although magnesium was accumulated in 10 to 100-fold higher concentrations than iron in the tissues[4], their accumulation patterns looked similar to each other. In order to find a practicable way to regulate the arsenic level in fresh Hijiki plants, the accumulation patterns of some other metal elements may provide some useful information. In this report, the accumulation behavior of calcium (*Ca*) in comparison with that of arsenic (*As*) is presented.

Materials and Methods

1. Sampling of Hijiki plants

Hijiki, *Sargassum fusiforme*, plants were harvested on the Hime coast of Kushimoto District, Wakayama Prefecture, at the time of the lowest tide, during their growing period[3, 4] as indicated in the data. The fresh plants were brought back in an ice cold box to the 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[9] three more times, being blotted each time with filter paper. The samples harvested in November 2008 were in the stage of *Primary Leaf*[10-12], having no stalks detectable. The sample plants harvested after February were cut at a length of 10 cm along the stalk from the bottom to the top of the plants, designated as a', b', c', and so on. The leaves and stalks were separated, weighed and stored at －40℃ until freeze-dried.

3. Determination of calcium and arsenic

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

a) *Ca*: The specimens were ashed with a mixture of conc. H_2SO_4-HNO_3 on an electric furnace, and the residues were dissolved in a defined amount of 1 M HCl[4]. For

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** Newly proposed taxonomic name of *Hizikia fusiforme* Okam.[1]

the determination of calcium, 0.3% of strontium coexisted in the solution of the specimens to be applied to the atomic absorption spectrophotometry using acetylene flame. The instrument was an atomic absorption spectrophotometer, Shimadzu AA6200, Japan, equipped with a Hollow-Cathode lamp for *Ca* (Hamamatsu Photonics, Co. Ltd, Japan).

b) *As*: The respective portions of the Hijiki samples were heated in 0.3 M HNO_3, and the soluble fractions were applied to the HPLC-ICP-MS spectrometry instrument (Agilent 1200ce and Agilent 7500ce, Agilent Technology Co. Ltd).

4. Reagents

Reagents of the JIS special grade and/or JCSS grade were used.

5. Statistic treatment

The correlation coefficients were calculated by a built-in-function of CORREL in Microsoft Excel 2011 (Mac version).

Results

1. Accumulation of arsenic

When harvested in November, arsenic accumulation in the tissues of the *primary leaf* stage was at the level of 11.5 μg *As*/g dry weight of tissues. In February, the arsenic levels in the leaves and stalks were 3.2 μg and 16.3 μg *As*/g dry weight of tissues, respectively. In March, the arsenic levels in the leaves and stalks were 33.7 μg and 7.97 μg *As*/g dry weight of tissues, and in April the values were 25.6 μg and 10.0 μg *As*/g dry weight of tissues, respectively. The whole plants harvested in February, March, and April gave arsenic concentrations of 7.1 μg, 26.1 μg and 22.5 μg *As*/g dry weight of tissues, respectively. (Table 1).

2. Accumulation of calcium

In the *primary leaf* stage, calcium accumulated already at an average level of 7,062 μg *Ca*/g dry weight of tissues. Levels of ca.6,000 μg *Ca*/g dry weight of tissues were maintained throughout the whole harvesting period of Hijiki. Out of the whole tissues, the calcium level in the stalks harvested in February was lowest, although the level was already 4,500 μg *Ca*/g dry weight of tissues. In April, the calcium levels of all tissues attained constant values higher than 6,000 μg *Ca*/g dry weight. (Table 2).

Table 1 Accumulation of Arsenic (As)

Date of harvest	Samples *	Tissues **	Number of leaves	μg *As*/g Dry weight
11/30/2008	A	Leaves	13	12.90
	B		9	13.60
	E		7	9.48
	F		12	10.20
	K		6	6.44

Date of harvest	Samples *	Tissues **	Sections ***	μg *As*/g Dry weight
2/8/2009	A-1	Stalks	c'	8.11
			b'	24.70
			a'	21.30
	A-2	Stalks	b'	14.10
			a'	13.40
	A-3	Stalks	b'	10.50
			a'	7.38
	A-4	Stalks	b'	5.30
			a'	15.70
	A-1	Leaves	c'	0.54
			b'	4.64
			a'	6.14
	A-2	Leaves	b'	3.26
			a'	5.08
	A-3	Leaves	b'	—
			a'	3.27
	A-4	leaves	b'	0.81
			a'	3.90

Date of harvest	Samples *	Tissues **	Sections ***	μg *As*/g Dry weight
3/11/2009	A-1	Stalks	d'	5.09
			c'	7.76
			b'	8.33
			a'	11.30
	A-2	Stalks	d'	3.98
			c'	7.93
			b'	8.60
			a'	10.30
	A-3	Stalks	d'	0.44
			c'	5.83
			b'	6.90
			a'	9.77
	A-1	Leaves	d'	32.30
			c'	33.60
			b'	45.10
			a'	20.10
	A-2	Leaves	d'	31.00
			c'	34.00
			b'	44.50
			a'	6.10
	A-3	Leaves	d'	5.42
			c'	37.00
			b'	29.80
			a'	21.40

Date of harvest	Samples *	Tissues **	Sections ***	μg *As*/g Dry weight
4/12/2009	A-1	Stalks	e'	4.58
			d'	9.32
			c'	12.20
			b'	18.60
			a'	15.70
	A-2	Stalks	d'	4.62
			c'	6.91
			b'	8.42
			a'	9.77
	A-3	Stalks	c'	0.58
			b'	5.45
			a'	6.53
	A-1	Leaves	e'	21.60
			d'	29.20
			c'	35.90
			b'	32.30
			a'	****
	A-2	Leaves	d'	18.30
			c'	35.10
			b'	24.20
			a'	1.62
	A-3	Leaves	c'	3.40
			b'	13.40
			a'	6.77

* The respective stocks were designated as A, B, E, etc.
** No stalks were recognized for the sample harvsted on 2008-11-30, corresponding to *Primary leaf* stage.
*** The bottom sections (lower section of the stalk) was designated as a', and the data columns were arranged in the order of the top to the bottom sections of the stalks.
**** No leaves.

Table 2 Accumulation of calcium (Ca)

Date of harvest	Samples *	Tissues **	Number of leaves	mg *Ca*/g Dry weight
11/30/2008	A	Leaves ***	13	7.349
	B		9	9.101
	E		7	6.180
	F		12	5.317
	K		6	5.077

Date of harvest	Samples *	Tissues **	Sections ***	mg *Ca*/g Dry weight
2/8/2009	A-1	Stalks	c'	2.879
			b'	5.835
			a'	5.517
	A-2	Stalks	b'	3.569
			a'	4.150
	A-3	Stalks	b'	—
			a'	5.141
	A-4	Stalks	b'	2.861
			a'	5.204
	A-1	Leaves	c'	4.722
			b'	7.039
			a'	5.570
	A-2	Leaves	b'	5.370
			a'	—
	A-3	Leaves	b'	5.843
			a'	8.035
	A-4	leaves	b'	5.876
			a'	5.278

Date of harvest	Samples *	Tissues **	Sections ***	mg *Ca*/g Dry weight
3/11/2009	A-1	Stalks	d'	4.931
			c'	6.094
			b'	7.622
			a'	6.509
	A-2	Stalks	d'	4.997
			c'	6.124
			b'	6.715
			a'	6.150
	A-3	Stalks	d'	5.234
			c'	5.045
			b'	7.216
			a'	5.980
	A-1	Leaves	d'	5.709
			c'	5.875
			b'	6.162
			a'	5.056
	A-2	Leaves	d'	7.382
			c'	5.450
			b'	6.540
			a'	3.551
	A-3	Leaves	d'	5.163
			c'	7.890
			b'	7.297
			a'	4.420

Date of harvest	Samples *	Tissues **	Sections ***	mg *Ca*/g Dry weight
4/12/2009	A-1	Stalks	e'	6.708
			d'	7.027
			c'	7.118
			b'	6.756
			a'	7.361
	A-2	Stalks	d'	5.951
			c'	7.933
			b'	7.169
			a'	8.207
	A-3	Stalks	c'	3.041
			b'	6.199
			a'	7.349
	A-1	Leaves	e'	7.064
			d'	7.235
			c'	8.331
			b'	6.930
			a'	****
	A-2	Leaves	d'	6.018
			c'	11.351
			b'	7.579
			a'	6.328
	A-3	Leaves	c'	7.284
			b'	6.919
			a'	6.049

* The respective stocks were designated as A, B, E, etc.
** No stalks were recognized for the sample harvsted on 2008-11-30, corresponding to *Primary leaf* stage.
*** The bottom sections (lower section of the stalk) was designated as a', and the data columns were arranged in the order of the top to the bottom sections of the stalks.
**** No leaves.

3. Correlations of arsenic and calcium accumulation patterns

In April, the calcium concentrations became rather constant, although the correlative coefficients were higher in younger plants (2008-Nov. and stalks of 2009-Feb samples). The correlative coefficients were similar, 0.5 to 0.6 (Table 3 and Fig. 1),

Table 3 Correlation coefficients of *As* and *Ca* concentrations *

Date of harvest	Tissues	Sample size	Coefficient
2008-Nov	Leaves **	5	0.8704
2009-Feb	Stalks	8	0.7459
	Leaves	7	0.2552
2009-Mar	Stalks	12	0.5860
	Leaves	12	0.6687
2009-April	Stalks	12	0.5331
	Leaves	11	0.6049

* Concentrations of *As* and *Ca* on the basis of g dry weight of the tissues were compared.
** Corresonding to *Primary leaf* stage.

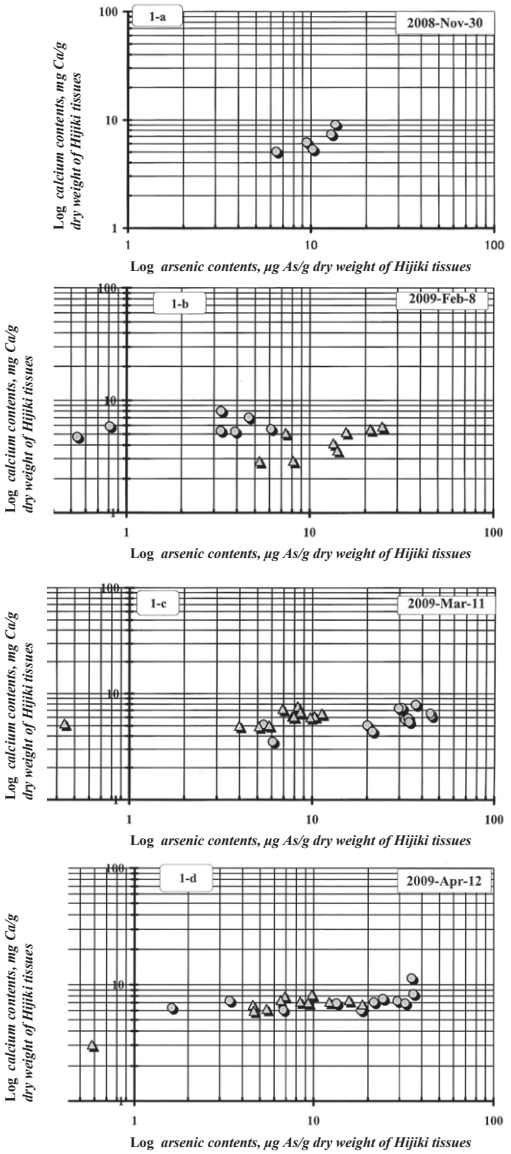

Fig. 1 Comparison between arsenic (*As*) and calcium (*Ca*) accumulation patterns during the growth of Hijiki. Concentrations of both elements in the stalks (△) and leaves (●) were plotted in the log scale.

from Mar. through April, and those in less mature plant tissues (leaves of the 2009-Feb samples, Table 1 of Ref 4) were lowest.

Discussion

1. Correlation of the concentrations of accumulated arsenic and calcium

The concentrations of accumulated arsenic in the fresh tissues of Hijiki plants were often not uniform along the stalks[5-8]. It is noticed that accumulation of calcium became constant, and that the correlating coefficients with that of arsenic were similar (Table 3). The concentrations of calcium seem to have become constant faster than those of arsenic (Fig. 1). The arsenic concentrations of the March samples were lower in the stalks than in the leaves, and the values in the stalks caught up with the values in the leaves in the April samples. The calcium accumulation pattern does not seem to be similar to that of iron, reaching a plateau faster than iron accumulation[4]. These patterns may reflect regulated metabolism of these elements in the tissues.

2. Correlation of the concentrations of accumulated arsenic and zinc

Accumulation of zinc correlated with arsenic accumulation in younger leaf tissues, when harvested before March (Table 4). As the plants grew up, the zinc concentrations in the stalks increased, and reached the levels in the leaves. This is also interesting in view of the fact that zinc occupies the active site of carbonic anhydrase, which is important in carbon dioxide metabolism, and that carbonic anhydrase activities in lettuce leaves were inhibited typically by inorganic arsenic (Ⅲ and Ⅴ) compounds***. So, it would be interesting to investigate whether there may be differences in the states of arsenic compounds in the younger and older tissues of Hijiki plants.

3. Correlation of the concentrations of accumulated manganese and zinc[3]

Out of the above several elements, manganese and zinc[3] showed relatively high correlation coefficients in their accumulation (Table 5), the values increasing with the growth of the plants (Fig. 3).

Table 4 Correlation coefficients of *As* and *Zn* concentrations*

Date of harvest	Tissues	Sample size	Coefficient
2008-Nov	Leaves**	5	0.5853
2009-Feb	Stalks	8	0.7181
	Leaves	7	−0.6451
2009-Mar	Stalks	12	−0.7568
	Leaves	12	−0.6653
2009-April	Stalks	12	−0.4058
	Leaves	11	−0.4376

* Concentrations of *As* and *Zn*[2] on the basis of g dry weight of the tissues were compared.

** Corresonding to *Primary leaf* stage.

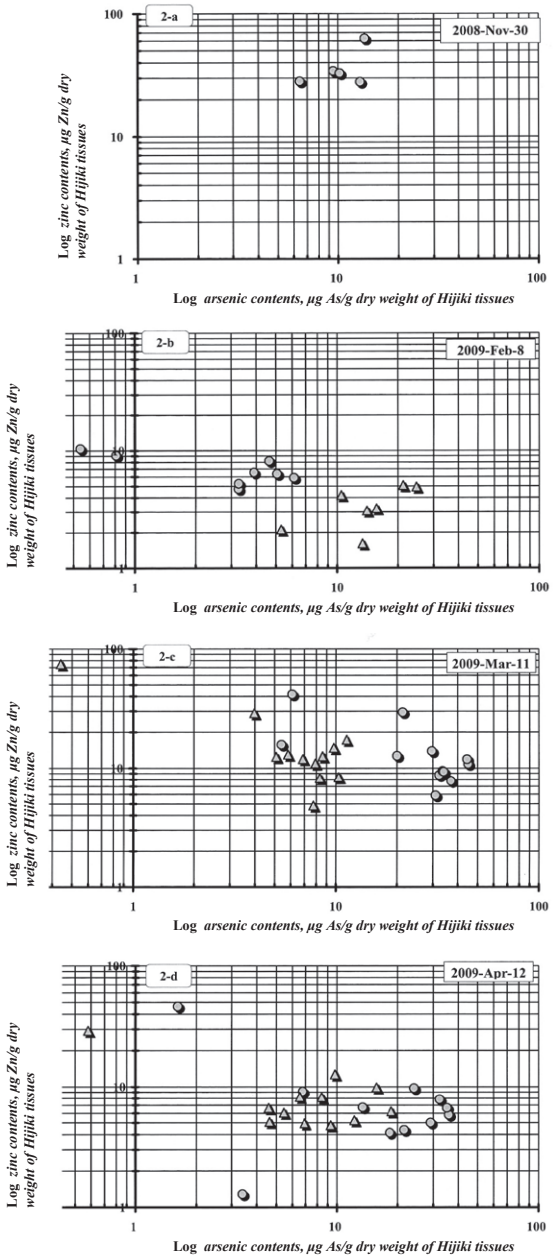

Fig. 2 Comparison between arsenic (*As*) and zinc(*Zn*) accumulation patterns during the growth of Hijiki.
Concentrations of both elements in the stalks (△) and leaves (●) were plotted in the log scale.

*** In preparation for publication; Katayama M, Inhibition patterns by inorganic arsenic compounds on carbonic anhydrase activities in lettuce leaves.

Table 5 Correlation coefficients of *Mn* and *Zn* concentrations *

Date of harvest	Tissues	Sample size	Coefficient
2008-Nov	Leaves **	5	−0.2591
2009-Feb	Stalks	8	−0.1982
	Leaves	7	0.0554
2009-Mar	Stalks	12	0.5519
	Leaves	12	0.9420
2009-April	Stalks	12	0.8985
	Leaves	11	0.9659

* Concentrations of *Mn*[2] and *Zn*[2] on the basis of g dry weight of the tissues were compared.

** Corresonding to *Primary leaf* stage.

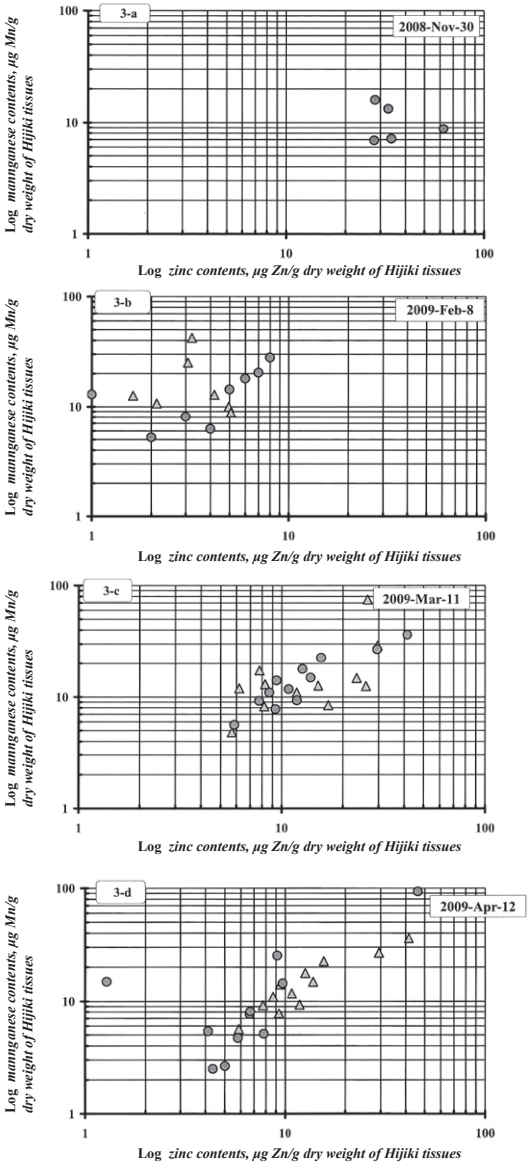

Fig. 3 Comparison between manganese (*Mn*) and zinc (*Zn*) accumulation patterns during the growth of Hijiki. Concentrations of both elements in the stalks (△) and leaves (●) were plotted in the log scale.

4. Comparison of arsenic concentrations in Hijiki plants with those in animal organs

Hijiki belongs to a family of brown algae, (*Phaeophyceae* Class). Brown algae generally contain higher concentrations of arsenic than members of other various classes of seaweeds[13], although some exceptions are found. It should also be stated that, in the case of Hijiki plants, the arsenic level individually varies greatly as indicated in the previous paper[14]. When rats were administered a great amount of arsenate as much as 40% of its LD_{50} in total in 2 days, the blood cells accumulated ten times higher concentration levels of arsenic than other rat organs[15], which accumulated several μg *As*/g dry weight of tissues. A high amount of arsenic in the blood cells has been shown to be detoxicated by combining to CYS93 in the hemoglobin[16].

Conclusion

From the viewpoint of cultivation of Hijiki plants, we might be able to develop ways to regulate arsenic levels in living plants by controlling the accumulating processes of other elements.

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