Distribution of Accumulated Arsenic in the Seaweed Hijiki, *Hizikia fusiforme* Okam. (1)

Masayuki KATAYAMA[1)], Chie SAKIYAMA[2)], Yukihiro NAKANO[3)] and Yohko SUGAWA-KATAYAMA*[2)]

[1)] *Osaka Prefectural University***,

[2)] *Department of Human Health Science, Graduate School of Human Environment Sciences, Fukuoka Women's University****,

[3)] *Research Reactor Institute, Kyoto University*****.

Summary

To investigate the distribution of the arsenic accumulated by Hijiki plants, we collected Hijiki plants along the seashores of Tushima Archipelago, Japan in May. Hijiki plants were separated into several parts such as leaves, stems and roots. After washing them with distilled water, the samples were lyophilized. Arsenic was determined by neutron activation analysis in the nuclear reactor of Research Reactor Institute, Kyoto University. The arsenic localization in individual plants along their stems was not uniform, when expressed in μg arsenic per g of wet or dry weight (ppm) as well as μg arsenic per unit length of the stem. This may suggest that the accumulating process of arsenic changes during their growth, reflecting at least partially the variable environmental conditions of the ocean.

Keywords : Arsenic, *Hizikia fusiforme* Okam., Seaweeds, Tsushima Archipelago, Neutron activation analysis.

The seaweed Hijiki (*Hizikia fusiforme* Okam.) has been used as a traditional Japanese food, and it has been recognized as a good source for minerals as well as for beneficial dietary fibers[1,2)]. On the other hand, arsenic is found at relatively higher levels in all kinds of seafood. Thus, in the urine of those ingesting a higher ratio of seafood, rather higher levels of metabolized arsenic are detected[3,4)]. Results from experiments with rats fed a diet containing Hijiki suggested that the Hijiki diet stimulated arsenic detoxification metabolism in rats[5,6)]. Hijiki accumulates a large amount of minerals including arsenic from the environmental ocean seawater[7)].

In the present study, we intended to investigate the distribution of the arsenic accumulated by Hijiki plants.

Experimentals

Hijiki, *Hizikia fusiforme* Okam.

We collected Hijiki, *Hizikia fusiforme* Okam.[8)], in May, 2000 along the seashores of Tsushima Archipelago, Japan. Samples of mature Hijiki plants were stored in an icebox until we bring them back to our laboratory.

Preparation of the samples

Samples of Hijiki plants were washed with artificial sea water three times and washed more than twice with distilled water. Excessive water on the samples was removed by blotting on filter paper. Hijiki plants were cut into

* : To whom the correspondence should be sent.

** Address : Professor Emeritus (2 - 33 - 10 Shiroyamadai, Hashimoto city, 648 - 0054, Japan)

*** Address : 1 - 1 - 1 Kasumigaoka, Higashi-ku, Fukuoka 813 - 8529, Japan

**** Address : Noda, Kumatori-cho, Sen-nan-gun, Osaka 590 - 0490

pieces of 10 cm in length along their main branches from the top (shoot apex) down to the filamentous holdfast, and separated into leaves and stalks. The shoot apexes of the respective main branches and filamentous holdfast (radicle like) were separated. After washing them with distilled water, the samples were separately lyophilized in small polyethylene bags.

Arsenic determination

The amount of arsenic of each sample was determined in duplicate in comparison with the arsenic standard. The dried samples were mixed well and separately packaged in small polyethylene bags. To determine the arsenic concentration in the samples, 40 of those bags were put together in a polyethylene Neuma-Capsule, with 10 bags of various amounts of a standard arsenic compound ; two of the standard specimens were arranged for every 8 specimens of Hijiki.

Thermal neutron activation analysis

The samples in the Neuma-capsules were irradiated by a flux of 10^{13} neutrons $\cdot$ cm^{-2} $\cdot$ sec^{-1} for 20 min in the center position of the nuclear reactor of the Research Reactor Institute, Kyoto University. After the cooling time of 72 hr, the arsenic content in the samples was determined by the gamma radiation from ^{76}As using a Ge/Li gamma-detector at 559.1 keV. Energy levels of ^{60}Co and ^{137}Cs were used for the calibration.

Results

Growing state of Hijiki

Among the coasts where Hijiki was harvested, the Waniura district gave Hijiki plants with main branches of about 80cm in length and those from the Kinn district were longer than 100cm. Those from other districts were shorter. The weight of a Hijiki plant was measured after it was cut into 10cm pieces from the top (apex) to the filamentous holdfast (Table 1.). The main branche samples, A－1, A－2, A－3, and A－4, from the Waniura district, connected one common stem and filamentous holdfast as shown in Table 1. The wet weight of the stalk was largest in the middle, but the dry weight showed little difference. On the other hand, the weight of the leaves differed along the main branches, giving larger values at two positions. The wet weight of the leaves differed depending on their wet volume. However, the dry weight differed more than expected from the value of the wet weight. These may suggest that different types of leaves or tissues may exist.

The total weight of the leaves was about 80% of the total tissues. Other samples harvested at the Waniura district as well as those from the Kinn district also gave a similar trend. The filamentous holdfast was shared by all the main branches, giving about 3.5g in the wet weight and 0.7g in the dry weight (Table 1, column A－1).

Arsenic contents in the individual tissues

The arsenic contents along the main branches in the respective sections (10cm in length) were 10μg to 0.07μg in the leaves, and 1.7μg to 0.01μg in the stalks. The arsenic distribution in individual main branches along their stalks was not uniform. This suggests that the arsenic changes during their growth may reflect the variable environmental conditions of the ocean, or the physiological differences along their branches.

Arsenic concentrations in the tissues on the wet weight basis

The distribution patterns of the arsenic concentration expressed on the wet weight (g) basis were different along their main branches (Table 2).

The filamentous holdfast gave a high level of arsenic : In the main branch, A－1, the filamentous holdfast gave the highest value of arsenic, and the other branches had highest distribution in the lower part of the stalks. In general, the lower part of the main branches gave higher concentrations of arsenic than the upper part, and in the apexes of

the branches, the arsenic content was the lowest or lower than the leaves.

Arsenic concentrations in the tissues on the dry weight basis

The distribution patterns of the arsenic concentration expressed on the dry weight (g) basis were different along their main branches (Table 3), showing patterns different from those on the wet weight basis. The differences of these distribution patterns were noticeable in the A - 3 and A - 4.

Table 1. Growing state of Hijiki plants, harvested at Waniura district.

Samples*	Tissues	Sections**	Wet weight (g)	Dry weight (g)
A - 1	Apex		1.50	0.1483
	Stalk	a	0.63	0.0796
		b	0.80	0.1147
		c	0.87	0.1584
		d	0.90	0.1651
	Leaf	a	5.05	0.5010
		b	5.78	0.6245
		c	5.51	0.6176
		d	3.50	0.3896
	Filamentous holdfast		3.45	0.6489

Samples*	Tissues	Sections**	Wet weight (g)	Dry weight (g)
A - 2	Apex		1.96	0.1931
	Stalk	a	0.69	0.1021
		b	0.72	0.1130
		c	1.02	0.1716
		d	0.98	0.1733
		e	0.87	0.1630
		f	0.84	0.1974
		g	0.66	0.1761
		h	0.75	0.1437
	Leaf	a	4.21	0.4516
		b	3.96	0.4067
		c	5.73	0.6606
		d	3.17	0.3624
		e	3.85	0.3687
		f	4.24	0.5666
		g	7.25	0.5667
		h	2.21	0.1275
	Filamentous holdfast		3.45	0.6489

Samples*	Tissues	Sections**	Wet weight (g)	Dry weight (g)
A - 3	Apex		0.62	0.0615
	Stalk	a	0.54	0.0749
		b	0.76	0.1102
		c	0.75	0.1223
		d	0.88	0.1580
		e	0.86	0.1772
		f	0.89	0.1496
		g	0.74	0.1661
		h	0.61	0.1791
	Leaf	a	3.59	0.3600
		b	2.35	0.2562
		c	0.75	0.3095
		d	1.93	0.2497
		e	3.23	0.3729
		f	4.86	0.0934
		g	4.80	0.8292
		h	1.04	0.2634
	Filamentous holdfast		3.45	0.6489

Samples*	Tissues	Sections**	Wet weight (g)	Dry weight (g)
A - 4	Apex		1.06	0.1773
	Stalk	a	0.56	0.0829
		b	0.86	0.1497
		c	0.74	0.1362
		d	0.53	0.1158
		e	0.84	0.1798
		f	0.66	0.1981
	Leaf	a	2.06	0.2374
		b	2.23	0.2581
		c	0.74	0.0785
		d	0.46	0.0526
		e	3.24	0.4561
		f	1.02	0.4943
	Filamentous holdfast		3.45	0.6489

* The samples, A - 1, A - 2, A - 3, and A - 4 mean the main branches, connected to one common filamentous holdfast (the same as expressed in A - 1 column).

** The alphabets, a, b, c, etc mean the sections from the top down to the filamentous holdfast, sectioned at 10cm length.

Table 2. Arsenic concentration in Hijiki plants, harvested at Waniura district, expressed on the basis of wet weight.

Samples*	Tissues	Sections**	μg As/g wet weight	Percentage ratio (%***)
A - 1	Apex		0.0090	0.89
	Stalk	a	0.0083	0.82
		b	0.0995	9.77
		c	0.0184	1.81
		d	0.9115	89.50
	Leaf	a	0.0129	1.27
		b	0.3102	30.46
		c	0.0144	1.41
		d	0.0184	1.81
	Filamentous holdfast		1.0184	100.00

Samples*	Tissues	Sections**	μg As/g wet weight	Percentage ratio (%***)
A - 2	Apex		0.0091	0.54
	Stalk	a	0.0176	1.05
		b	0.0140	0.83
		c	0.0153	0.91
		d	0.4317	25.69
		e	0.9452	56.24
		f	1.0661	63.44
		g	1.6805	100.00
		h	1.2193	72.55
	Leaf	a	0.3595	21.39
		b	0.2611	15.54
		c	0.0120	0.72
		d	0.0194	1.16
		e	0.3540	21.07
		f	1.1093	66.01
		g	0.7172	42.68
		h	0.4305	25.61
	Filamentous holdfast		1.0184	60.60

*, **: The same as described in the Table 1.
***: The highest value was taken as100%.

Samples*	Tissues	Sections**	μg As/g wet weight	Percentage ratio (%***)
A - 3	Apex		0.6215	17.56
	Stalk	a	0.6122	17.30
		b	0.5537	15.65
		c	0.8082	22.84
		d	0.9429	26.65
		e	1.4159	40.02
		f	1.2144	34.32
		g	1.4850	41.97
		h	2.2725	64.23
	Leaf	a	0.7274	20.56
		b	0.7175	20.28
		c	0.9344	26.41
		d	0.9298	26.28
		e	1.8082	51.10
		f	0.3350	9.47
		g	2.0677	58.44
		h	3.5383	100.00
	Filamentous holdfast		1.0184	28.78

Samples*	Tissues	Sections**	μg As/g wet weight	Percentage ratio (%***)
A - 4	Apex		0.0321	0.56
	Stalk	a	0.5082	8.81
		b	0.8623	14.94
		c	1.9527	33.84
		d	3.1949	55.37
		e	1.8692	32.40
		f	2.7972	48.48
	Leaf	a	1.0951	18.98
		b	1.7980	31.16
		c	2.0890	36.21
		d	1.8157	31.47
		e	1.4116	24.47
		f	5.7698	100.00
	Filamentous holdfast		1.0184	17.65

Table 3. Arsenic concentration in the Hijiki plants, harvested in Waniura district, on the basis of dry weight.

Samples*	Tissues	Sections**	μg As/g dry weight	Percentage ratio (%***)
A - 1	Apex		0.0914	1.69
	Stalk	a	0.0662	1.22
		b	0.0796	1.47
		c	0.1012	1.87
		d	4.9687	91.77
	Leaf	a	0.1304	2.41
		b	2.8710	53.02
		c	0.1283	2.37
		d	0.1656	3.06
	Filamentous holdfast		5.4146	100.00

Samples*	Tissues	Sections**	μg As/g dry weight	Percentage ratio (%***)
A - 2	Apex		0.0925	1.01
	Stalk	a	0.1192	1.30
		b	0.0894	0.97
		c	0.0907	0.99
		d	2.4412	26.61
		e	5.0448	54.98
		f	4.5365	49.44
		g	6.2984	68.65
		h	6.3636	69.36
	Leaf	a	3.3517	36.53
		b	2.5427	27.71
		c	0.1044	1.14
		d	0.1699	1.85
		e	3.6965	40.29
		f	8.3009	90.47
		g	9.1752	100.00
		h	7.4612	81.32
	Filamentous holdfast		5.4146	59.01

*, **: The same as described in the Table 1.
***: The same as described in the Table 2.

Samples*	Tissues	Sections**	μg As/g dry weight	Percentage ratio (%***)
A - 3	Apex		6.2652	35.94
	Stalk	a	4.4137	25.32
		b	3.8184	21.90
		c	4.9560	28.43
		d	5.2515	30.13
		e	6.8716	39.42
		f	7.2248	41.45
		g	6.6160	37.95
		h	7.7401	44.40
	Leaf	a	7.2536	41.61
		b	6.5810	37.76
		c	2.2643	12.99
		d	7.1863	41.23
		e	15.6626	89.85
		f	17.4317	100.00
		g	11.9695	68.67
		h	13.9706	80.14
	Filamentous holdfast		5.4146	31.06

Samples*	Tissues	Sections**	μg As/g dry weight	Percentage ratio (%***)
A - 4	Apex		0.2905	1.48
	Stalk	a	3.4336	17.44
		b	4.9535	25.15
		c	10.6096	53.88
		d	14.6227	74.26
		e	8.7326	44.35
		f	9.3194	47.33
	Leaf	a	9.5025	48.25
		b	15.5349	78.89
		c	19.6922	100.00
		d	15.8783	80.63
		e	10.0279	50.92
		f	11.9060	60.46
	Filamentous holdfast		5.4146	27.50

Discussion

The concentration of total arsenic in these samples harvested in the Tsushima Archipelago was somewhat lower than those of other specimens hitherto investigated in our laboratory. These data may suggest the changeability of the arsenic level partially according to the environmental conditions. This is supported by data in this report, indicating the non-uniform levels of arsenic along the branch length.

Analysis of the chemical state of the arsenic compounds in these Hijiki samples may elucidate the phenomena. As for the arsenic accumulation, the report on some 'Giant clams' (*Tridacna maxima* and *T. derasa*) of Lizard Island and Davies Reef, concentrating extraordinarily high levels of arsenic[9] may be referred as reflecting the ocean conditions involving algae.

The arsenic metabolism is affected by the concentration of the environmental phosphate level, and this may also have occurred in the Hijiki plants of this study in Tsushima Archipelago.

The non-uniform arsenic accumulation in individual Hijiki plants may suggest two possibilities : one is some sea-

sonal changes of nutritional conditions in the coastal ocean water occurring during the growing seasons of Hijiki plants, and another is the possible existence of some physiological compartments of arsenic metabolism in the Hijiki plants. These phenomena interest us to elucidate more by analysis of the type of the arsenic compounds in Hijiki plants. These areas of investigation may also shed light on more details of the arsenic behavior in the ocean biosphere.

Acknowledgement

To the members of Nagasaki Prefectural Tsushima Fisheries Extension Advisory Center, the authors express their appreciation for the collaborative arrangement to the observation and the collection of growing Hijiki on the seashores of the Tsushima Archipelago. Also, the authors express their thanks to Ms. Inoue, Y. and Ms. Tanaka, R. for their assistance in the preparation of the samples of Hijiki.

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