

# Vanadium levels in marine organisms of Onagawa Bay in Japan

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**Abstract** Vanadium in marine organisms from Onagawa Bay in Miyagi, Japan, was determined by an instrumental neutron activation analysis (INAA) method using anti-coincidence gamma-ray spectrometry at the Dalhousie University SLOWPOKE-2 Reactor (DUSR) facility in Canada. Seaweeds, cultivated oysters, plankton, and four different species of sea squirt were collected from Onagawa Bay during 2005–2008. Vanadium levels around  $20 \mu\text{g g}^{-1}$  (dry weight) were found in Japanese tangle and hijiki seaweeds. One species of sea squirt (*Ciona savignyi*) contained 160–500 ppm of V and it was highest among the four species of sea squirts studied. Protein-bound V species were separated by gel permeation chromatography (GPC) and the element determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

**Keywords** Vanadium levels · Neutron activation analysis · Seaweeds · Sea squirts · Bivalves

## Introduction

Vanadium is naturally present in various kinds of organism as a trace constituent. Until now two types of vanadium-

containing enzymes have been identified, namely vanadium-nitrogenases and vanadium-dependent haloperoxidases [1]. Also, it is known that vanadyl peroxo complexes, vanadyl cysteine methyl ester complex, and similar compounds reduce blood glucose level [2, 3]. Some species of brown seaweeds, mushrooms, and sea squirts are known to accumulate V [4]. Seaweeds, planktons, oysters, and sea squirts are important components of an ecosystem in a shallow sea. It is of interest to determine the V levels in these organisms to understand the role and transfer mechanism of this element in food chain. In Japan, Michibata and coworkers have extensively studied the V accumulation in and clearance mechanisms of sea squirts [5–7]. Analyses for V in marine samples have also been done to study the effects of contamination by oil. Murphy et al. analyzed several elements including V in ships' ballast water by inductively coupled plasma mass spectrometry (ICP-MS) [8]. Amiard et al. analyzed V and Ni metallothioneins in mussel by electrothermal atomic absorption spectrometry (ET-AAS) to investigate the role of V in this organism [9]. Odate et al. investigated the role of V in sea squirts by flame atomic absorption spectrometry (AAS) [10]. In the present study V levels in marine organisms from Onagawa Bay, Miyagi, Japan, were measured by an instrumental neutron activation analysis (INAA) method.

## Experimental

### Samples

Two different species of brown seaweeds (Japanese tangle and sea mustard) were collected from Onagawa Bay. Hijiki, collected from the same bay, was purchased in dried state from a local market. Several other edible and dried

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seaweeds were also obtained from the same market for comparison purposes. Plankton was collected with a plankton net of 0.11 mm mesh at Takenoura, Onagawa Bay, in 2008 May and September. After the collection, the plankton was filtered sequentially through 10, 2, and 0.45  $\mu\text{m}$  membrane filters, and washed with small amount of distilled water. Four different species of sea squirt, namely *Halocynthia roretzi*, *Styela clava*, *Ciona savignyi*, and *Halocynthia bispida*, were collected from Onagawa Bay. *Styela clava* and *Ciona savignyi* were collected in 2008 every month from May through September. Cultivated oysters were collected in 2005 October. These were separated into gills, mantles, muscles, and hepatopancreas, and kept frozen until analysis. Mussel was collected from Onagawa Bay, washed with tap water followed by distilled water. Then the soft tissue was removed from the shell. All samples were cut into pieces, freeze dried, and pulverized by mill.

### INAA

All samples, comparator standards and reference materials were irradiated at the DUSR facility in a neutron flux of  $2.5 \times 10^{11} \text{ cm}^{-2} \text{ s}^{-1}$ . Some samples were irradiated in the reactor neutron flux for 3 s to 1 min depending on their activity. On the other hand, samples with high activity had to be irradiated with epithermal neutrons in the Cd-site for 1–5 min. After appropriate decay times, gamma-ray spectra were recorded using a Compton suppression system. This system consisted of an EG&G ORTEC HPGe p-type coaxial detector with a crystal diameter of 51.2 mm and a length of 65.2 mm, a peak-to-Compton ratio of 93:1, a relative efficiency of 25% with respect to a standard NaI(Tl) detector, and a resolution of 1.8 keV at the 1332-keV photopeak of  $^{60}\text{Co}$ . The guard detector was a  $10'' \times 10''$  NaI(Tl) annulus with 5 photomultiplier tubes (PMT) supplied by Harshaw and a  $3'' \times 3''$  NaI(Tl) plug with one PMT supplied by Teledyne. The peak-to-Compton plateau ratio of this system was 582:1 at the 662-keV  $\gamma$ -ray of  $^{137}\text{Cs}$  using the IEEE convention of the number of counts per channel in the Compton plateau (358–382 keV) [11].

### Separation of protein-bound vanadium

In order to estimate the amount of metallothionein in sea squirt, protein-bound V was separated following the procedures described by Amiard et al. [9] and Erk et al. [12]. Briefly, samples were homogenized in 100 mM Tris buffer with 0.15 M NaCl and 1 mM 2-mercaptoethanol, and centrifuged at 10,000 rpm for 10 min at 4 °C. Supernatant (S1) was heated to 70 °C for 10 min, cooled immediately in an ice bath, then centrifuged at 15,000 rpm for 20 min at

4 °C. The supernatant (S2) obtained from this centrifugation was used for separating proteins by GPC. Proteins in S2 were separated in a Sephadex G-150 column ( $d = 12 \text{ mm}$ , 45 cm long) using 100 mM Tris buffer with 0.15 M NaCl and 1 mM 2-mercaptoethanol at a flow rate of  $0.85 \text{ mL min}^{-1}$ . Protein separation profile was obtained by following the absorbance at 280, 254, and 230 nm of each fraction. The V levels in the protein fractions were measured by ICP-AES.

## Results and discussion

### INAA

Sensitivities obtained from standard solutions of V and the detection limits for NIST SRM 1566b Oyster Tissue are shown in Table 1. Although the best sensitivity was obtained using an irradiation time ( $t_i$ ), decay time ( $t_d$ ), counting time ( $t_c$ ) of 1, 1, and 10 min, respectively, shorter irradiation times were chosen in this work since marine samples contained high levels of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$  ions, etc. causing high background activity. Samples were generally irradiated for 3 s (and in rare cases for 5 min) at the DUSR facility except several seaweeds which were irradiated in the epithermal neutron flux of  $1.09 \times 10^{12} \text{ cm}^{-2} \text{ s}^{-1}$  of the Kyoto University Reactor, Japan. After appropriate decay times, gamma-ray spectra were recorded using the Compton suppression system at DUSR facility. Decay times were chosen depending mainly on the activities of  $^{28}\text{Al}$  (2.24 min),  $^{80}\text{Br}$  (17.68 min), and  $^{38}\text{Cl}$  (37.24 min). The V levels in samples were measured using the 1434-keV gamma-ray of  $^{52}\text{V}$  (3.75 min) using a comparator standard.

### Reference materials

In order to evaluate the accuracy of measurement, NIST SRM 1566b Oyster Tissue and two different RMs, namely NIES No. 9 Sargasso and BCR CRM No. 279 Sea Lettuce, were analyzed. Our results were:  $0.62 \pm 0.06 \mu\text{g g}^{-1}$  for Oyster Tissue ( $n = 3$ ),  $0.84 \pm 0.01 \mu\text{g g}^{-1}$  for Sargasso

**Table 1** Sensitivity for comparator standard solutions and detection limits (DL) for NIST SRM 1566b Oyster Tissue using INAA-AC

$t_i:t_d:t_c$	Sensitivity (counts $\mu\text{g}^{-1}$ )	DL ( $\mu\text{g}$ )
INAA-AC		
12:20:60 s	133	0.753
0.5:1:10 min	1402	0.097
1:1:10 min	2659	0.078
EINAA-AC		
1:3:10 min	69	0.34

( $n = 2$ ), and  $3.35 \pm 0.11 \mu\text{g g}^{-1}$  for Sea Lettuce ( $n = 3$ ). These values agreed well with the certified values of  $0.577 \pm 0.023 \mu\text{g g}^{-1}$  for Oyster Tissue and  $1.0 \pm 0.1 \mu\text{g g}^{-1}$  for Sargasso, and the indicative value of  $3.54 \pm 0.08 \mu\text{g g}^{-1}$  for Sea Lettuce.

#### Vanadium levels in seaweeds

Vanadium levels in sea mustards at different growing stages showing rather high levels of V are presented in Fig. 1. Although the statistical errors are rather high for most of the values, they still indicate the trend of V levels with respect to growing stage. Odate et al. investigated the role of V in a species of sea squirt, and concluded that V complexes act as a chemical defense against bacterial infection [10]. There is not much information on how photosynthesis is controlled in seaweeds. Photosynthesis performs well at the upper part of seaweeds. It may be said that root works as a reservoir of V during the initial stages of growth. Then the photosynthesis spot moves from the bottom part to the upper part of the stem as it grows.

#### Vanadium content of other edible seaweeds

Only Japanese tangle and hijiki were irradiated in the Kyoto University Reactor in an epithermal neutron flux for 1 min. The samples were allowed to decay for 5 min and then counted for 5 min. The V levels were 22.2 and  $19.7 \mu\text{g g}^{-1}$  for Japanese tangle and hijiki, respectively (Table 2). Compared to the V levels in other edible

seaweeds, such as laver, glue plant, sea lettuce and hornwort, the levels of Japanese tangle and hijiki were high.

#### Vanadium levels in plankton and invertebrates

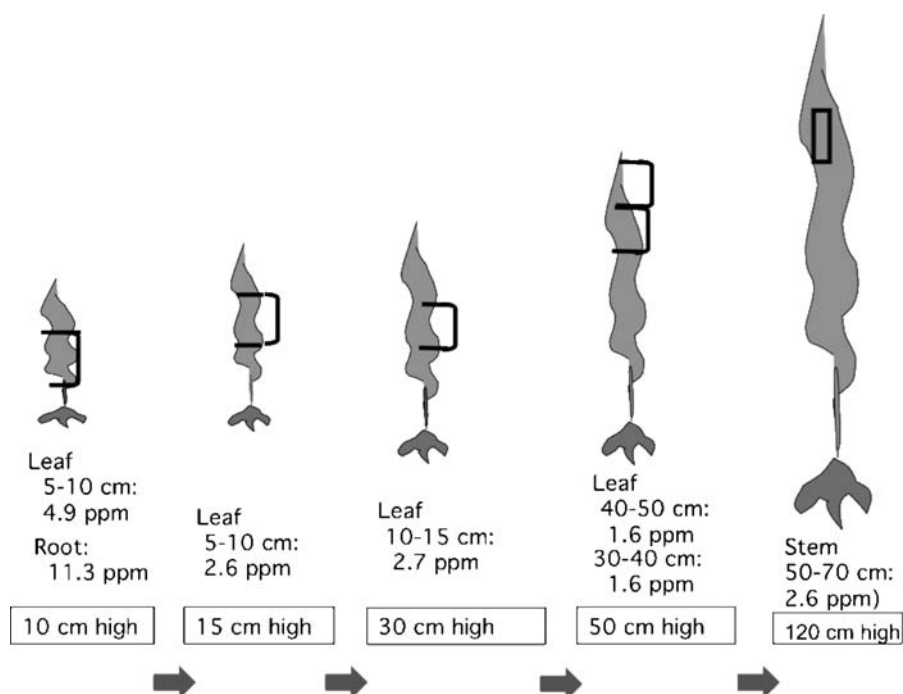
The levels are shown in Table 2 with counting errors. Levels in both oysters from Onagawa Bay and mussels from three different bays were not high. Mussel is used as an indicator for monitoring toxic element levels in marine environment. Although mussel has the tendency for accumulating several trace elements in its soft tissues, it may not accumulate V efficiently.

It is well known that sea squirts accumulate V highly in their tunics and soft tissues. Among the four species analyzed here, *Ciona savignyi* showed extremely high levels of V. Seasonal variation of V levels is shown in Fig. 2 for *Styela clava* and *Ciona savignyi*. It can be seen that the levels are high in the summer time, particularly in July and August.

#### Estimation of protein-bound vanadium

As mentioned above, V content of *Ciona savignyi* was extremely high. In some species of sea squirt, V is accumulated highly and stored in particular cells called vanadocytes and vanabins which is a vanadium-binding protein. There are five types of these proteins, namely vanabin 1-4 and vanabin-p [5-7]. Moreover, metallothionein is known to be induced in mussels [3, 8, 10] and mice exposed to V. For estimating the amount of V metallothionein, protein

**Fig. 1** Highest levels of vanadium in sea mustards at different growing stages



**Table 2** Vanadium levels ( $\mu\text{g g}^{-1}$ ) in marine samples

Species	Sampling month	Organ	NAA conditions $t_i$ , $t_d$ , $t_c$ (min)	Vanadium level
<b>Seaweed</b>				
Hijiki <sup>a</sup>	Purchased		EINAA 1· 5· 5	19.7 ± 1.4
Raw laver	Purchased		EINAA 5· 3· 5	0.74 ± 1.18
Glue plant	Purchased		EINAA 5· 3· 5	0.68 ± 0.11
Sea lettuce	Purchased		EINAA 5· 3· 5	2.13 ± 0.27
Hornwort	Purchased		EINAA 5· 3· 5	ND
Japanese tangle <sup>a</sup>	Purchased		EINAA 1· 5· 5	22.2 ± 5.1
Japanese tangle	2004 May	Root	EINAA 5· 3· 5	1.19 ± 0.18
Sea mustard	2005 Dec–2006 Mar	Root, leaf, stem	EINAA 1· 3· 10	See Fig. 1
<b>Oyster</b>				
	2005 Oct	Gill	0.5· 1· 10	0.02 ± 0.02
		Hepatopancreas	1· 1· 10	0.58 ± 0.04
		Mantle	0.5· 1· 10	0.02 ± 0.02
		Muscle	1· 1· 10	0.05 ± 0.05
<b>Plankton</b>				
>10 $\mu\text{m}$	May 2008		0.05· 8· 1	11.1 ± 2.8
>1.2 $\mu\text{m}$			0.2· 0.25· 2	1.26 ± 0.25
>0.45 $\mu\text{m}$			0.2· 2· 1	0.68 ± 0.21
>10 $\mu\text{m}$	Sep 2008		0.05· 1· 2	38.1 ± 4.3
>0.45 $\mu\text{m}$			0.2· 2· 1	0.55 ± 0.18
<b>Mussel</b>				
<i>Mytilus coruscus</i>	May 2008		0.2· 0.25· 2	0.12 ± 0.01
<i>Mytilus galloprovincialis</i> <sup>b</sup>	Aug 2005		0.05· 1· 2	0.20 ± 0.30
<i>Mytilus galloprovincialis</i> <sup>c</sup>	Nov 2004		0.05· 1· 2	0.62 ± 0.20
<b>Sea squirt</b>				
<i>Halocynthia roretzi</i>	May 2008	Hepatopancreas	0.2· 0.25· 1	0.70 ± 0.10
	Sep 2008	Muscle	0.2· 0.25· 2	ND
		Tunic	0.2· 0.25· 2	0.40 ± 0.10
		Gonad	0.2· 0.25· 2	0.04 ± 0.10
		Hepatopancreas	0.2· 0.25· 2	2.10 ± 0.20
<i>Styela clava</i>	May 2008		0.05· 1· 2	3.80 ± 0.60
	Jun 2008		0.05· 1· 2	5.40 ± 1.0
	Jul 2008		0.05· 1· 2	17 ± 0.9
	Aug 2008		0.05· 1· 2	26.4 ± 0.9
	Sep 2008		0.05· 1· 2	8.6 ± 0.8
<i>Ciona savignyi</i>	May 2008		0.05· 8· 1	193 ± 11
	Jun 2008		0.05· 8· 1	316 ± 15
	Jul 2008		0.05· 8· 1	516 ± 18
	Aug 2008		0.05· 8· 1	199 ± 8
	Sep 2008		0.05· 8· 1	290 ± 15
<i>Halocynthia bispida</i>	Sep 2008	Soft tissue	0.2· 0.25· 2	4.2 ± 0.3
			0.2· 0.25· 2	1.1 ± 0.1

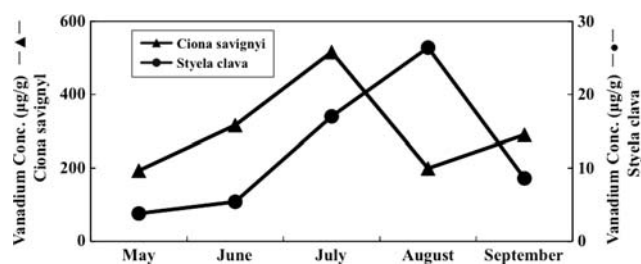
<sup>a</sup> EINAA at Kyoto University Reactor, Osaka

<sup>b</sup> Collected from Tanesashi Bay, Aomori Prefecture

<sup>c</sup> Collected from Tona Bay, Miyagi Prefecture

was extracted from *Ciona savignyi* and separated by GPC. From the dry powders of *Ciona savignyi* collected in 2008 June and September, V was extracted and found to be 99

and 93  $\mu\text{g g}^{-1}$ , respectively. The gel permeation column was calibrated by standard proteins. Six peaks were identified in the profile of the protein extract and their V levels



**Fig. 2** Seasonal variation of vanadium levels in two different species of sea squirt

were calculated. The peaks had molecular weights (MW) of 50, 22, 15, 12, 9, and 7 kDa. Ueki et al. [7] identified MW of 55 kDa as a vanabin and the MW of 7 kDa as the monomer of metallothionein [8]. From these information it can be postulated that the protein peaks of MW 22, 15 and 9 kDa are the trimer, dimer and monomer of metallothionein, and the peak with MW 50 kDa could be vanabin-interacting protein 1.

## Conclusions

Several species of seaweeds, plankton, shellfish, and sea squirts from Onagawa Bay were analyzed by INAA for V with good precision, accuracy, and sensitivity. Among the seaweeds investigated, Japanese tangle and hijiki were found to contain high amounts of V. High levels of V were also observed in two different species of sea squirts; moreover, these levels showed seasonal variation. Protein-bound V in sea squirts was separated and found to contain 6 proteins of MW 50, 22, 15, 12, 9, and 7 kDa which may very

well correspond to vanabin-interacting protein 1 and trimer, dimer and monomer of metallothionein, respectively.

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