

**ELEMENTAL STUDIES ON MARINE ALGAE BY
X-RAY ANALYTICAL MICROSCOPE
PART II . MAPPING OF ELEMENTS IN SPOROPHYLL
OF *UNDARIA PINNATIFIDA* SURINGAR ***

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INTRODUCTION

Seaweeds can efficiently accumulate almost all the elements in seawater, and serve as good experimental material for elemental analysis. *Undaria pinnatifida* Suringar, a favorite edible alga, can form distinctive reproductive organs, i. e. sporophylls (Chihara, 1975; Chapman and Chapman, 1973). Sporophyll of *Undaria pinnatifida* is believed to have great potential for enhancing human health and regarded as potent folklore medicine (Nisizawa, 1989). Some experimental data proved that sporophyll of *Undaria pinnatifida* is beneficial as cancer-preventive and cardiovascular protective agents (Furusawa and Furusawa, 1985; Oishi, 1993). Elements accumulated in the sporophyll can explain the physiological bioactivities of the sporophyll. So it is interesting to know the special elements and their localization in the sporophyll.

X-ray analytical microscopy, a rapid and visual method for analysis of elemental composition, can provide an X-ray transmission image of a sample and fluorescent X-ray analysis of elemental mapping at the same time (Hosokawa, 1995). Using such a non-destructive technique, the distribution of certain elements can be observed from the formed imaged directly if the element is high enough to show a clear image. Here, the authors report the results of their study on the location of major elements such as potassium and calcium in the sporophyll of *Undaria pinnatifida*.

MATERIAL AND METHODS

Cultured *Undaria pinnatifida* Suringar was collected in early February, 1997 from the coastal area of Karatsu shi, Saga-ken, Japan. Sporophyll was cut off, covered with soft papers, and deeply frozen (− 20℃) immediately until further treatment. Before scanning with X-ray microscope, the "leaf-like" part of the sporophyll was cut away, and one "leaf" cut and unfolded (like a circle with di-

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ameter of about 5 cm) was put on soft paper and dried using an electric hair dryer during thawing, and dried again in air for about 3 hours. Then, the test sample was put on a sample plate covered with polyethylene membrane for analysis.

X-ray analytical microscope model XGT-2000V (made by Horiba Co., Ltd., Japan) was used. The experimental conditions were as follows: spot size of X-ray beam, $100\mu\text{m}$; X-ray tube voltage, 30keV, tube current, 1 mA. For elemental image, the whole sample was scanned, pulse time of P1; number of pixels for mapping 256×256 ; integrated time of the pixels, 4000 sec. For spectrum measurement, pulse time, P4; scanning time 240 seconds; point analysis technique was used. Because the scanned area was a square, the upper-left point was designated as (0,0), and lower-right point as (255,255); three points (127,127), (211,85) and (244,93) were chosen to represent center, mid-part and edge.

RESULTS

Elemental characteristics in the spectrum In order to understand the overall characteristic elements in the sporophyll, the results of fluorescent spectrum obtained from different points were compared to the common characteristics. The element species was determined from the peak of the fluorescent line. From Fig. 1a, 2a and 3a, spectra look very similar. Potassium is the most prominent element, which gives the strongest peak, followed by calcium and sulfur. In Fig. 1b, 2b and 3b, which show clear peaks of potassium, calcium and sulfur. Sulfur gives the strongest peak in Fig. 2b of point (211,85), while the strongest peak appears in Fig. 3b. The spectra of Fig. 1c, 2c and 3c show that iron, zinc, bromine and strontium also have distinctive peaks. The peak intensity varies from one spectrum to the other, which indicate the uneven distribution of these elements. For example, the peak of bromine in Fig. 1c and Fig. 2c is stronger than that in Fig. 3c. On the other hand, some other elements such as cobalt and selenium cannot give distinctive peaks as strong as that of arsenic and vanadium. The sensitivity of magnesium is too low to give distinctive peaks.

Localization of potassium, calcium, sulfur and iodine The content of each element in the elemental image corresponds to the intensity of the fluorescent X-ray of the element, the brighter the spot, the more the content. The brightness and shadow distributed in the whole image give information on the localization of certain elements such as potassium, sulfur, calcium and iodine in the whole sporophyll. Plate I-1, I-2, I-3 are the images of potassium, calcium and sulfur, respectively. Although lacking in quantitative value, a general description can be made as follows: potassium is richer in the central part of sporophyll; calcium richer at the edge side; sulfur is located in the middle part. The color synthesis technique was used to form a new image (Plate II, where red represents sulfur, green represents potassium, and blue represents calcium).

Although iodine is not as rich as the above elements, the image of iodine (Plate I-4) clearly shows that iodine is located mainly on the edge of the sporophyll. Compared with the spectra of Fig. 1b, 2b and 3b, it also clearly shows that iodine is richer in the edge and gives a distinctive peak

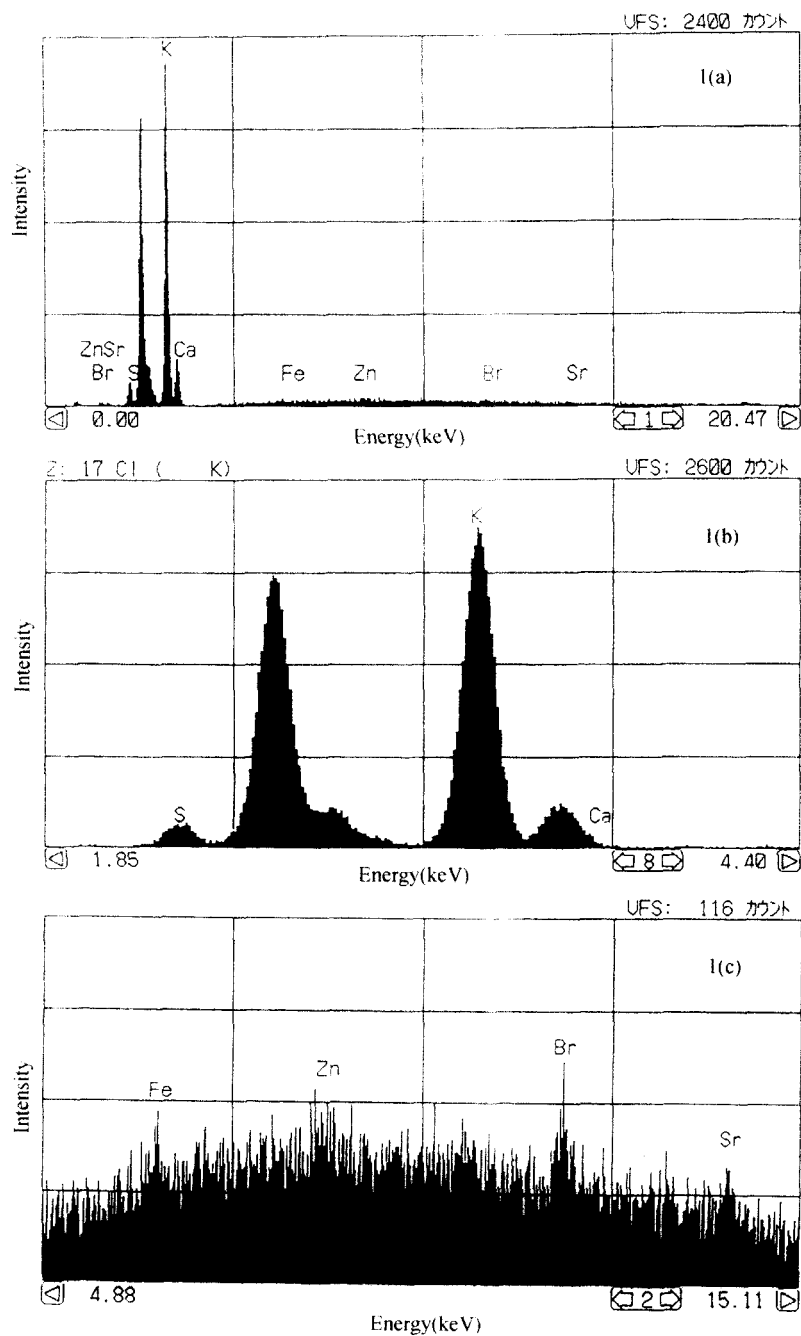


Fig. 1 Fluorescent X-ray elemental spectrum of point (127,127)

a. Fluorescent X-ray elemental spectrum of Zn, Sr, Br, S, K, Ca, Fe; b. Fluorescent X-ray elemental spectrum of S, K, Ca; c. Fluorescent X-ray elemental spectrum of Fe, Zn, Br, Sr

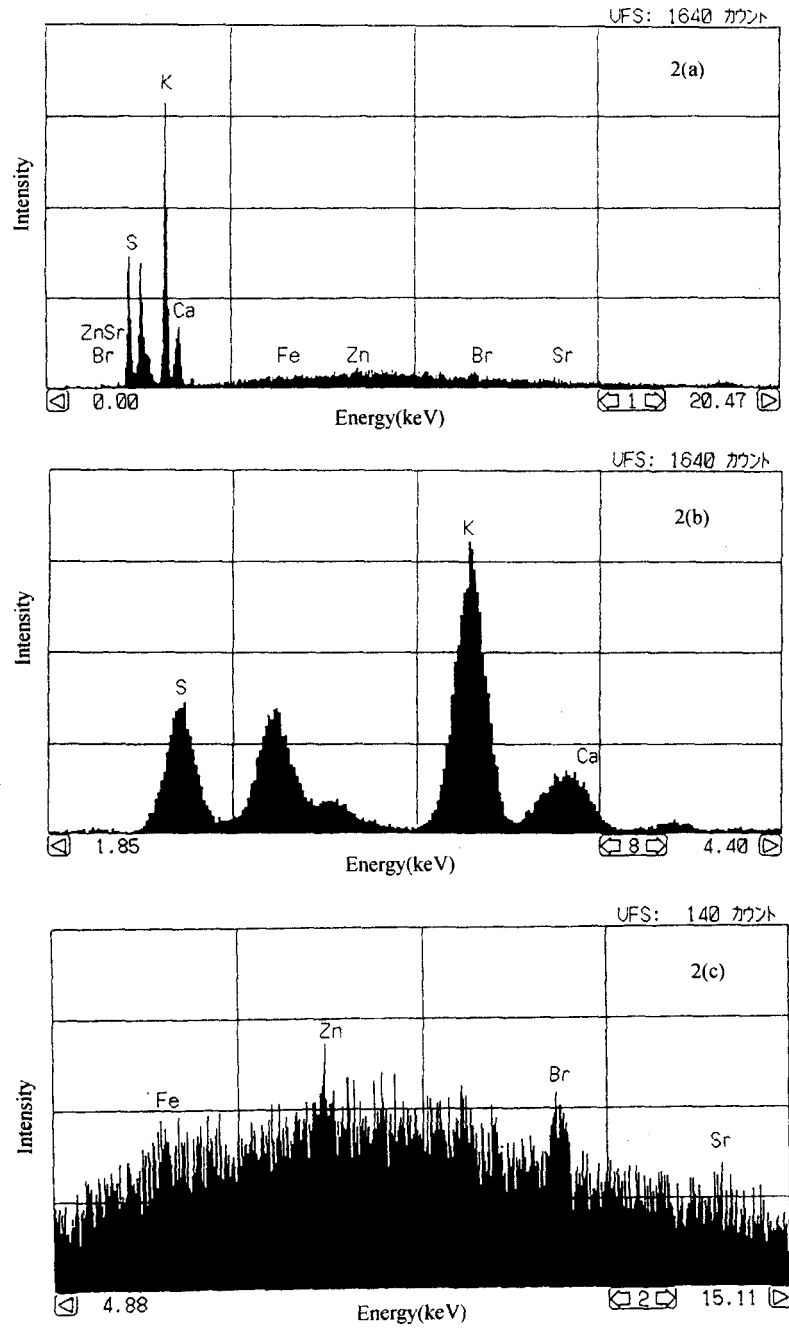


Fig. 2 Fluorescent X-ray elemental spectrum of point (211,85)

a. Fluorescent X-ray elemental spectrum of Zn, Sr, Br, S, K, Ca, Fe; b. Fluorescent X-ray elemental spectrum of S, K, Ca; c. Fluorescent X-ray elemental spectrum of Fe, Zn, Br, Sr

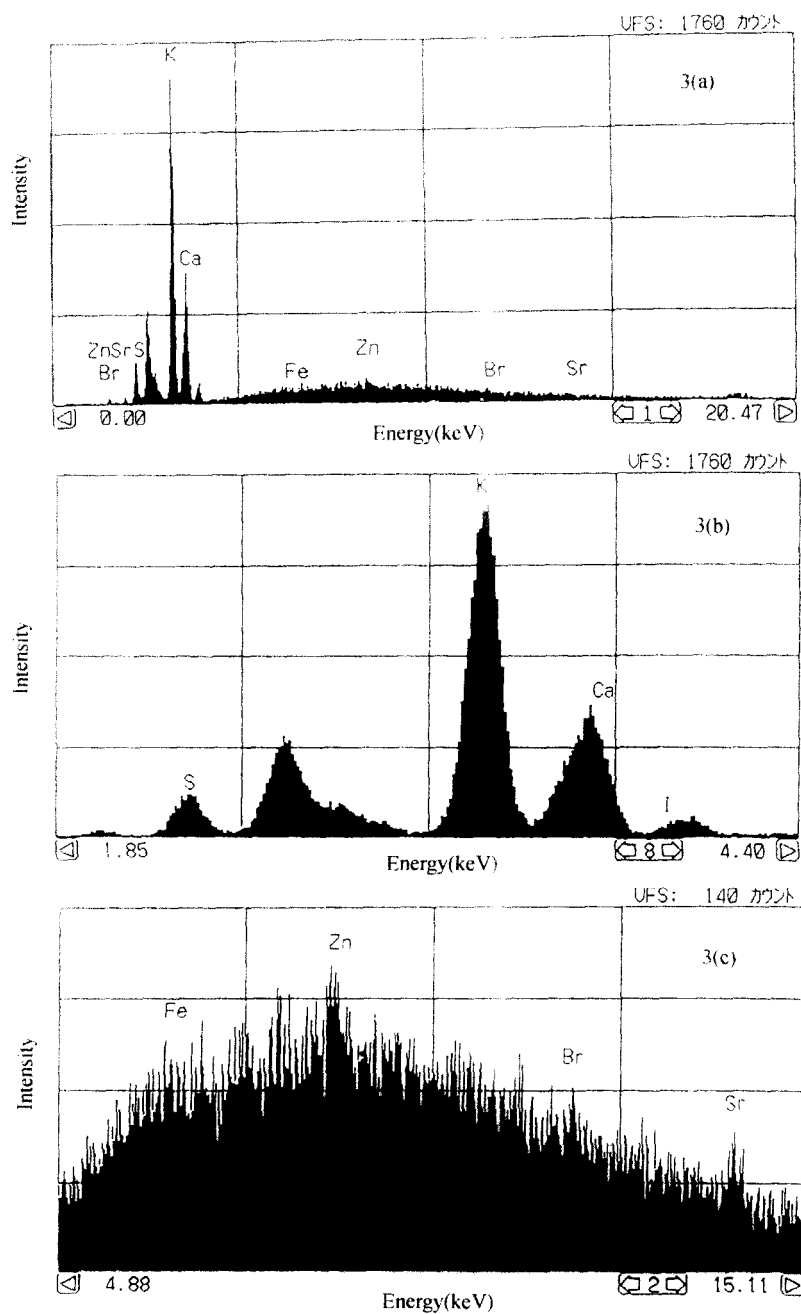


Fig. 3 Fluorescent X-ray elemental spectrum of point (244,93)
 a. Fluorescent X-ray elemental spectrum of Zn, Sr, Br, S, K, Ca, Fe; b. Fluorescent X-ray
 elemental spectrum of S, K, Ca; c. Fluorescent X-ray elemental spectrum of Fe, Zn, Br, Sr

(Fig. 3b) while Fig. 1b has no iodine peak at all, which means iodine is scarce in the central part. This result indicates that absorption of iodine differs in the different tissue. Other elements such as bromine, zinc and iron were also tried, but no meaningful images (data not show here) were obtained.

DISCUSSIONS

Seaweeds contain almost all the elements found in the sea, where sodium, magnesium, chloride, potassium, calcium, phosphors and sulfur are the major elements; and can accumulate iodine, bromine, and trace elements such as zinc, copper, nickel, cobalt, etc. They are commonly considered as valuable supplier of elements beneficial to human health. The elemental composition in *Undaria pinnatifida* has been studied by some researchers. For example, Ito et al. (1987, 1988) determined the seasonal variation and bioavailability of calcium, phosphors, iodine, iron and magnesium in *Undaria pinnatifida*. In the book "Science of Seaweeds", edited by Oishi (1993), the contents of calcium, phosphors, iron, sodium, magnesium, manganese, zinc, sulfur and iodine were listed which showed that there were distinctive differences in elemental contents between adult blade and sporophyll. But the distribution of elements in the sporophyll was never fully studied.

X-ray fluorescence images give new information on some elements. For instance, calcium in brown algae is mainly attached to algin, which is the anionic cell wall polysaccharide. The image clearly shows that more calcium is attached to the edge part of sporophyll. Sulfur in sporophyll is mainly from fucoidan, which has various bioactivities such as anticancer (Riou, 1996, Ito et al., 1993). There are many unmaturred zoospores covered with fucoidan in the sporophyll of *Undaria pinnatifida*. The image indicates that sulfur is mainly located in the middle part of sporophyll. For another example, the image of iodine shows that *Undaria pinnatifida* sporophyll's apex may be its most active part to absorb iodine in seawater. Iodine absorption is through the activity of iodo-peroxidase, so it is deduced, only at the edge of sporophyll where the iodo-peroxidase is metabolically active. So the physiology and ecology as well as nutritional aspects of these elements have important correlation with the whole sporophyll.

In summary, this is the first time that potassium, calcium, sulfur and iodine are localized in the sporophyll of *Undaria pinnatifida*, they distributed unevenly in the whole tissue. Potassium is rich in the central part of sporophyll, calcium and iodine are richer at the edge. The results given here indicated that attention should be paid to the anatomy when considering the importance of sporophyll.

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用 X 射线分析显微镜对海藻元素的研究 II . 裙带菜孢子叶的元素定位*

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摘 要

X 射线分析显微镜是一种快速的非破坏分析方法,可以用来对海藻中特定的元素在组织水平进行定位。本实验采用 X 射线分析显微镜对 1997 年 2 月在日本佐贺县唐津市海岸采集的养殖裙带菜 (*Undaria pinnatifida* Suringar) 的孢子叶进行了硫、钾、钙、碘的组织定位研究,对孢子叶的整体进行 X 射线扫描及特征元素次级荧光的图像分析,并选取孢子叶的边缘部、中部及中心各一点进行定点的 X 射线荧光光谱测定。X 射线荧光光谱的结果表明,钾是裙带菜孢子叶的最显著元素,具有最大的 X 射线荧光强度。定位分析表明,钾在孢子叶的中心部位分布较高,钙与碘则在边缘部分布较高,硫多位于孢子叶的中间部分,在中心部位及边缘部的分布均较低。除了钾、钙、硫以外,其他元素如铁、溴、锌也能够在 X 射线荧光光谱中给出谱峰。作者得到的 X 射线特征元素荧光图像首次报道了裙带菜孢子叶中钾、钙、硫等元素在组织水平的定位。应用 RGB 图像彩色合成技术获得的硫、钙、钾三元素的彩色合成图像也与定点分析结果相一致。

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