

ELEMENTAL STUDIES ON MARINE ALGAE BY X-RAY ANALYTICAL MICROSCOPE PART I. X-RAY FLUORESCENCE SPECTRA OF MARINE ALGAE*

Yan Xiaojun, Fan Xiao

(*Institute of Oceanology, Chinese Academy of Sciences*)

Tadahiro Nagata

(*National Food Research Institute, Tsukuba, Japan*)

INTRODUCTION

As marine algae can very efficiently accumulate almost all of the elements (especially trace elements such as iron, zinc, and iodine), studies on their element contents have a long history. Many analytical techniques including AES (Fan, *et al.*, 1988, 1995), AAS (Ito, *et al.*, 1987, 1988; Rao *et al.*, 1989; Fan, *et al.*, 1993) and NAA (Kawashima, *et al.*, 1983) have been tried to determine the content and seasonal variations of inorganic elements in marine algae. Recently, more attention has been paid to fast and non-destructive analytical techniques, which can deepen the studies on chemical state analysis of elements (such as arsenic and iodine) and on the bioavailability of iron and calcium, sulfur or bromine containing organic compounds like fucoidans and bromophenols (Nisizawa, *et al.*, 1979; Nisizawa, 1989; Oishi, 1993). In this work, the authors tried a new non-destructive and fast analytical method to study the element composition in 22 species of marine algae.

INSTRUMENT

Scheme 1 (Hosokawa, 1995) is the set-up of the instrument X-ray fluorescence (XRF) analytical microscope (XGT-2000V, Horiba Co., Japan), developed in 1993. It was composed of three parts: primary X-ray excitation equipment, detector of secondary X-ray, data recording and processing computer.

Primary X-ray excitation: The primary X-rays were excited by the X-ray tube, composed of rhodium target, tungsten filament and beryllium window. When high voltage was applied to the X-ray tube, heated electrons were produced between the tungsten filament and rhodium target. After impacting the target, the heated electrons were changed into X-rays which passed out through the beryllium window (Scheme 2).

Primary X-ray microfocus beam formation: Because the excited primary X-rays are dispersed,

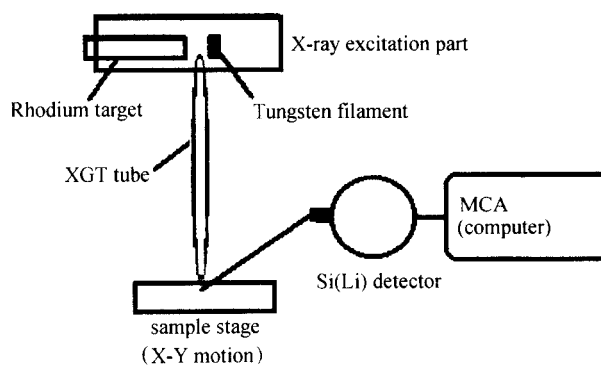
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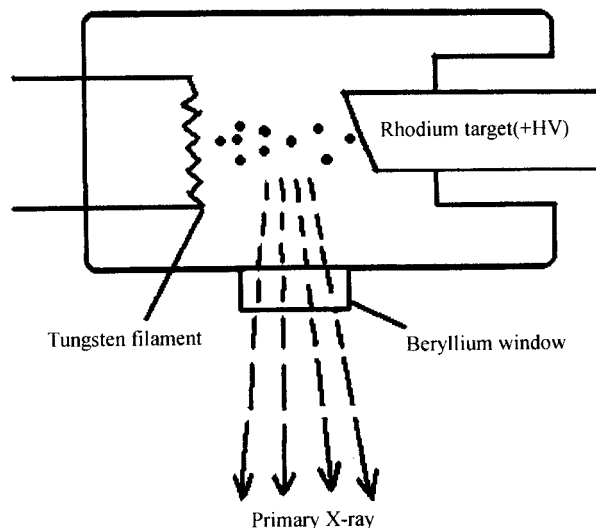
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they should be passed through a glass capillary, with inner parabolic surface to focus them into an intensely bright microbeam. This optical capillary is called X-ray guided tube (XGT), which can facilitate microscopic X-ray analysis of 1 mm to 100mm samples.

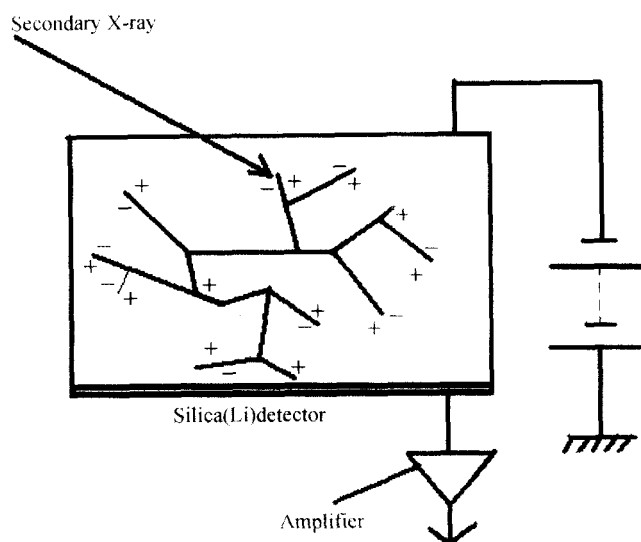
Secondary X-ray production: Secondary X-rays are produced after primary X-rays are irradiated on the surface of a sample. There are two kinds of secondary X-rays. One is called diffraction X-ray and is caused by Compton and Railey diffraction. The other is called X-ray fluorescence (XRF), which is closely related with the element in the sample, because it is produced when the outer electron enters into the inner electron orbit after the electron in the inner orbit (K or L) is driven off by the primary X-ray. The energy value of X-ray fluorescence can be used to identify various elements ranging from sodium to uranium, while the strength of X-ray fluorescence can be used for semi-quantitative determination.



Scheme 1 Set-up of X-ray analytical microscope



Scheme 2 Set-up of primary X-ray excitation



Scheme 3 Set-up of silica detector of X-ray

Detection of secondary X-ray: The secondary X-ray is detected by an Si(Li) detector widely used in XRF laboratories. It is with liquid nitrogen is used to enhance its capability to detect light elements (such as Na, Mg, Al). The basic working principle of the silica detector is that X-rays entering the detector can change the frequency and amplitude of the electron pulses. The strength and energy value of X-rays are related to the variations of the frequency and amplitude electron pulses. These variations can be transformed into a secondary X-ray graph by multi-channel wave analyzer (MCA). (Scheme 3)

The usage of X-ray analytical microscope includes element mapping and semi-quantitative determination of X-ray fluorescence spectrum.

MATERIALS AND METHODS

Collection of algae Twenty-two species of marine algae were collected in April and May, 1997 (Table 1). After removing foreign matter such as epiphytes and sand, they were washed gently with clean seawater and air dried in shaded place.

X-ray fluorescence spectrum measurement About 5 grams of seaweed sample put in the center of a plastic sample stage was carefully covered with a transparent polyethylene membrane, and measured by X-ray analytical microscope. The X-ray analytical microscope parameters were as follows: X-ray tube voltage, 50 keV; tube current, 1.0 mA; X-ray beam spot size, 100 micrometer; silica detector pulse, Pl. Semi-quantitative calculation was done by the fundamental parameter (FP) method.

Table 1 Species of collected marine algae

No	alga name	taxonomy	collection date and site
1	<i>Enteromorpha intestinalis</i>	Ch. , Ulvales, Ulvaceae	97/04/24, Weihai, Shandong
2	<i>Ulva pertusa</i>	Ch. , Ulvales, Ulvaceae	97/05/05, Weihai, Shandong
3	<i>Ulva lactuca</i>	Ch. , Ulvales, Ulvaceae	97/04/23, Weihai, Shandong
4	<i>Leathesia diffformes</i>	Ph. , Chordariales, Leathesiaceae	97/05/09, Weihai, Shandong
5	<i>Scytosiphon lomentarias</i>	Ph. , Scytosiphonales, Scytosiphonaceae	97/04/22, Weihai, Shandong
6	<i>Sargassum thunbergii</i>	Ph. , Fucales, Sargassaceae	97/04/22, Weihai, Shandong
7	<i>Sargassum kjellmanianum</i>	Ph. , Fucales, Sargassaceae	97/05/09, Weihai, Shandong
8	<i>Undaria pinnatifida</i>	Ph. , Laminariales, Alariaceae	97/05/03, Weihai, Shandong
9	<i>Dictyopteris divaricata</i>	Ph. , Dictyotales, Dictyotaceae	97/04/06, Weihai, Shandong
10	<i>Colpomenia sinuosa</i>	Ph. , Scytosiphonales, Scytosiphonaceae	97/04/24, Weihai, Shandong
11	<i>Desmarestia viridis</i>	Ph. , Desmarestiales, Desmarestiaceae	97/05/07, Weihai, Shandong
12	<i>Punctaria plantaginea</i>	Ph. , Dictyosiphonales, Punctariaceae	97/05/03, Weihai, Shandong
13	<i>Myelophycus simplex</i>	Ph. , Dictyosiphonales, Asperococcaceae	97/05/05, Weihai, Shandong
14	<i>Polysiphonia urceolata</i>	Rh. , Ceramiales, Rhodomelaceae	97/04/23, Weihai, Shandong
15	<i>Hyalosiphonia caespitosa</i>	Rh. , Cryptonemiales, Dumontiaceae	97/04/23, Weihai, Shandong
16	<i>Gelidium amansii</i>	Rh. , Gelidiales, Gelidiaceae	97/04/07, Weihai, Shandong
17	<i>Laurensia surculigera</i>	Rh. , Ceramiales, Rhodomelaceae	97/05/07, Weihai, Shandong
18	<i>Ceramium boydenii</i>	Rh. , Ceramiales, Ceramiaceae	97/04/07, Weihai, Shandong
19	<i>Gloiosiphonia capillaris</i>	Rh. , Cryptonemiales, Gloiosiphoniaceae	97/05/08, Weihai, Shandong
20	<i>Rhodomela confervoides</i>	Rh. , Ceramiales, Rhodomelaceae	97/04/24, Weihai, Shandong
21	<i>Ceramium kondoi</i>	Rh. , Ceramiales, Ceramiaceae	97/04/07, Weihai, Shandong
22	<i>Corallina pilulifera</i>	Rh. , Cryptonemiales, Corallinaceae	97/04/23, Weihai, Shandong

* Ch. 9 Chlorophyta; Ph. , Phaeophyta; Rh. , Rhodophyta.

RESULTS AND DISCUSSIONS

X-ray fluorescence spectra The energy values of X-ray fluorescence of some elements are listed in Table 2, element species can be identified by X-ray fluorescence peak. However, light elements, such as sodium, magnesium, did not show visible peaks because of their insensibility in X-ray fluorescence spectrum.

Table 2 Energy value of fluorescence X-rays of some elements

Element	Fluorescence line	Energy value (keV)	Element	Fluorescence line	Energy value (keV)
Na	K α 1	1.04	Mg	K α 1	1.25
Al	K α 1	1.49	P	K α 1	2.01
S	K α 1, β 1	2.31, 2.47	Cl	K α 1, β 1	2.62, 2.82
K	K α 1, β 1	3.31, 3.59	Ca	K α 1, β 1	3.69, 4.01
Mn	K α 1, β 1	5.89, 6.49	Fe	K α 1, β 1	6.40, 7.06
Zn	K α 1, β 1	8.63, 9.57	As	K α 1, β 1	10.53, 11.72
Br	K α 1, β 1	11.91, 13.29	Sr	K α 1, β 1	14.14, 15.83
Rh	L α 1, β 1	2.70, 2.83	I	L α 1, β 1	3.94, 4.22

Fig. 1 (sample 1—3) are spectra of green algae, with distinctive peaks attributed to potassium, calcium, and sulfur. It was found that polysaccharides in green algae had high sulfate content (Lai, *et al.*, 1994). Iron was abundant in *Ulva pertusa* and bromine in *Enteromorpha intestinalis*.

Fig. 1 to Fig. 2 (sample 4—13) are spectra of brown algae. The peaks of strontium was distinctively in all brown algae except *Undaria pinnatifida* and *Colpomenia sinuosa*. Sulfur was abundant in these algae and *Desmarestia viridis* for its high content of sulfuric acid as chemical defense substances. However, the sulfur in other species may probably be attributed to fucoidan which contains sulfate groups, and strontium should be bound to the guluronic acid block in algin, according to the egg-box theoretical model. So it was interesting to deduce that there may be some relationship of contents between guluronic acid and fucoidan in brown algae. *Sargassum thunbergii*, *Desmarestia viridis* and *Myelophycus simplex* also showed a significant peak of iron.

Fig. 2 to Fig. 3 (sample 14—22) are spectra of red algae. Apparently, Rhodomelaceae such as *Polysiphonia urceolata*, *Laurencia surculigera* and *Rhodomela confervoides* contain much amount of bromine, some organic bromine compounds (Nisizawa, *et al.*, 1979, Weinstein, *et al.*, 1975). But another species, *Gelidium amansii*, not notable previously for high bromine content, unexpectedly also contained a large amount of bromine. Some algae, *Polysiphonia urceolata*, *Hyalosiphonia caespitosa*, *Ceramium kondai*, *Rhodomela confervoides*, *Ceramium boydenii*, can accumulate iron to high amount. Algae such as Corallinaceae, *Corallina pilulifera*, only showed a calcium peak in the spectrum.

An important finding in this studies was that zinc can form a visible peak in spectra of algae such as *Enteromorpha intestinalis*, *Sargassum thunbergii*, *Desmarestia viridis*, *Polysiphonia urceolata*, *Hyalosiphonia caespitosa* and *Ceramium kondoi*.

Semi-quantitative results The Table 3 results were obtained from semi-quantitative calculation using the fundamental parameter method (FP), which can only be used for preliminary assessment, because it is accurate only if the total number of elements in the sample is limited and if the sample

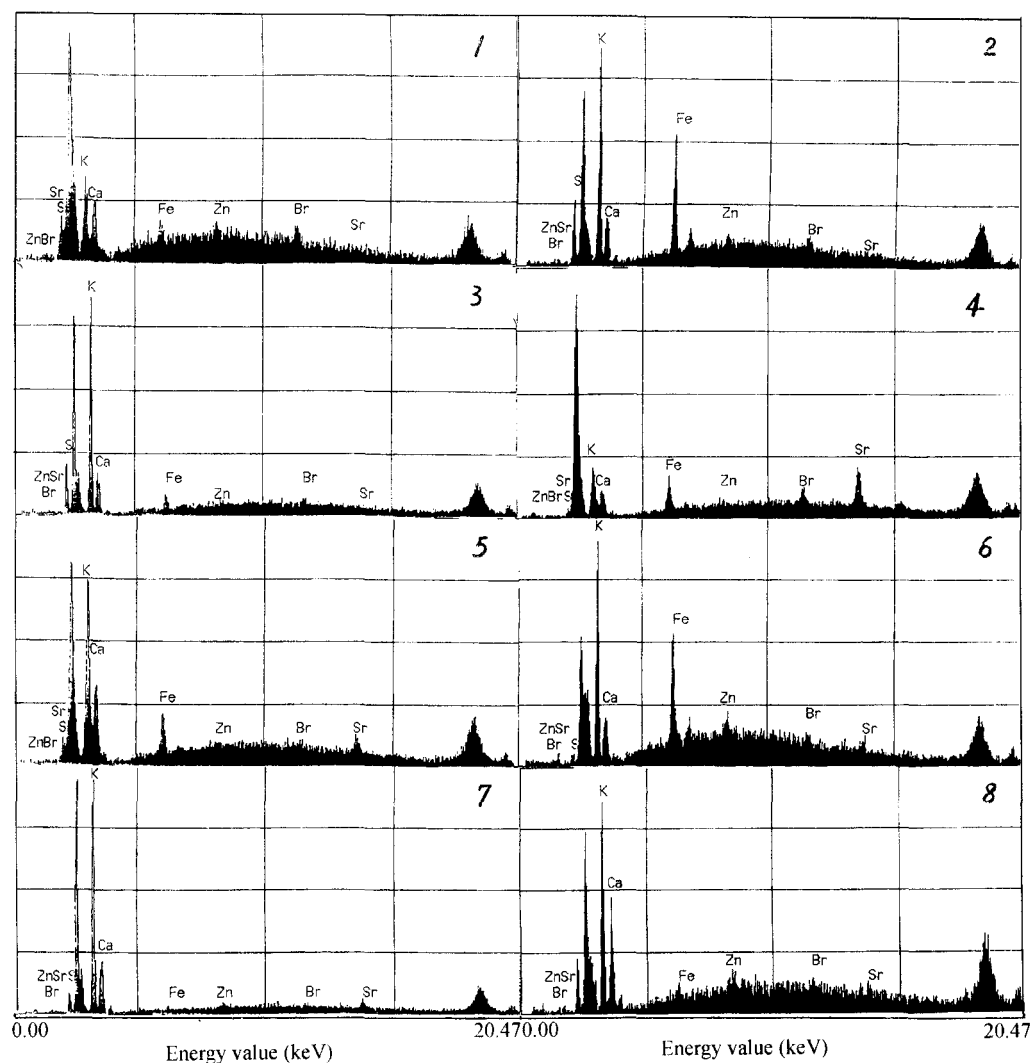


Fig. 1 X-ray fluorescence spectra of marine algae (sample 1—8)

surface is very flat(to reduce the diffraction background). In the case of marine algae, the element composition in the biological material is very complex, and the algae surface is also uneven. In spite of these unfavorable conditions, the data shown in Table 3 were compatible with the spectra given in Fig. 1—3. The sulfur contents shown in Table 3 indicated that some red algae (*Hyalosiphonia caespitosa*, *Laurencia surculigera*, *Gloisiphonia capillaris*) had high content of sulfur. It has been demonstrated that some sulfated polysaccharides in red algae had specific bioactivities (Nairn, *et al.*, 1996). If so, the above red algae can be good experimental materials for such purpose.

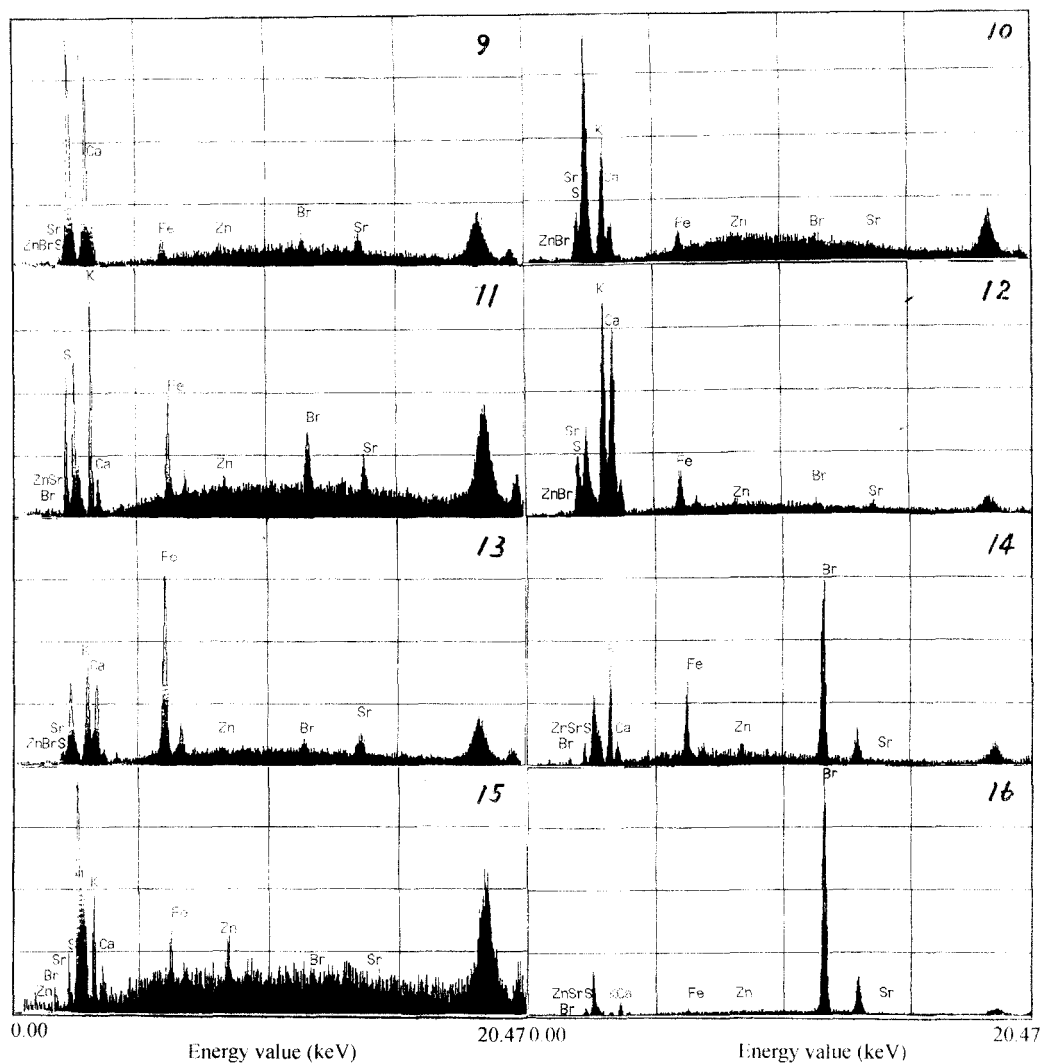


Fig. 2 X-ray fluorescence spectra of marine algae (sample 9—16)

Table 3 Semi-quantitative results from X-ray spectra (relative %)

alga name	S	K	Ca	Fe	Zn	Br	Sr
<i>Enteromorpha intestinalis</i>	14.19	37.27	40.52	3.48	2.45	1.80	0.29
<i>Ulva pertusa</i>	18.74	56.65	13.41	10.11	0.51	0.53	0.06
<i>Ulva lactuca</i>	19.67	67.35	11.08	1.34	0.29	0.26	0.00
<i>Leathesia difformes</i>	0.00 *	39.41	35.81	7.56	1.51	3.68	12.03
<i>Scytosiphon lomentarias</i>	4.24	46.65	38.92	6.93	0.83	0.74	1.69
<i>Sargassum thunbergii</i>	3.86	63.23	16.29	13.67	1.41	0.70	0.84
<i>Sargassum kjellmanianum</i>	5.48	65.34	28.06	0.42	0.27	0.09	0.33
<i>Undaria pinnatifida</i>	13.58	51.29	33.05	0.97	0.38	0.33	0.41
<i>Dictyopteris divaricata</i>	0.00	75.55	14.64	4.94	0.79	1.43	2.65
<i>Colpomenia sinuosa</i>	14.97	52.80	27.35	3.65	0.47	0.46	0.29

continued

alga name	S	K	Ca	Fe	Zn	Br	Sr
<i>Desmarestia viridis</i>	34.18	56.10	0.41	5.91	0.46	1.98	0.96
<i>Punctaria plantaginea</i>	15.53	36.63	43.11	3.78	0.36	0.25	0.34
<i>Myelophycus simplex</i>	4.25	31.34	34.57	24.80	0.71	1.57	2.75
<i>Polysiphonia urceolata</i>	11.84	55.74	6.74	8.52	0.92	16.20	0.04
<i>Hyalosiphonia caespitosa</i>	19.89	52.97	14.74	7.04	4.05	1.31	0.00
<i>Gelidium amansii</i>	22.76	8.13	15.70	0.68	0.34	52.28	0.12
<i>Laurencia surculigera</i>	18.14	29.51	25.98	2.62	1.05	22.70	0.01
<i>Ceramium boydenii</i>	10.61	13.71	64.37	9.87	0.18	1.09	0.17
<i>Gloiosiphonia capillaris</i>	25.15	69.29	2.66	2.04	0.44	0.38	0.05
<i>Rhodomela confervoides</i>	7.20	19.99	24.30	11.73	0.38	35.19	1.07
<i>Ceramium kondoi</i>	15.20	48.67	11.09	21.11	3.80	0.11	0.01
<i>Corallina pilulifera</i>	1.40	2.43	91.67	3.55	0.24	0.17	0.55

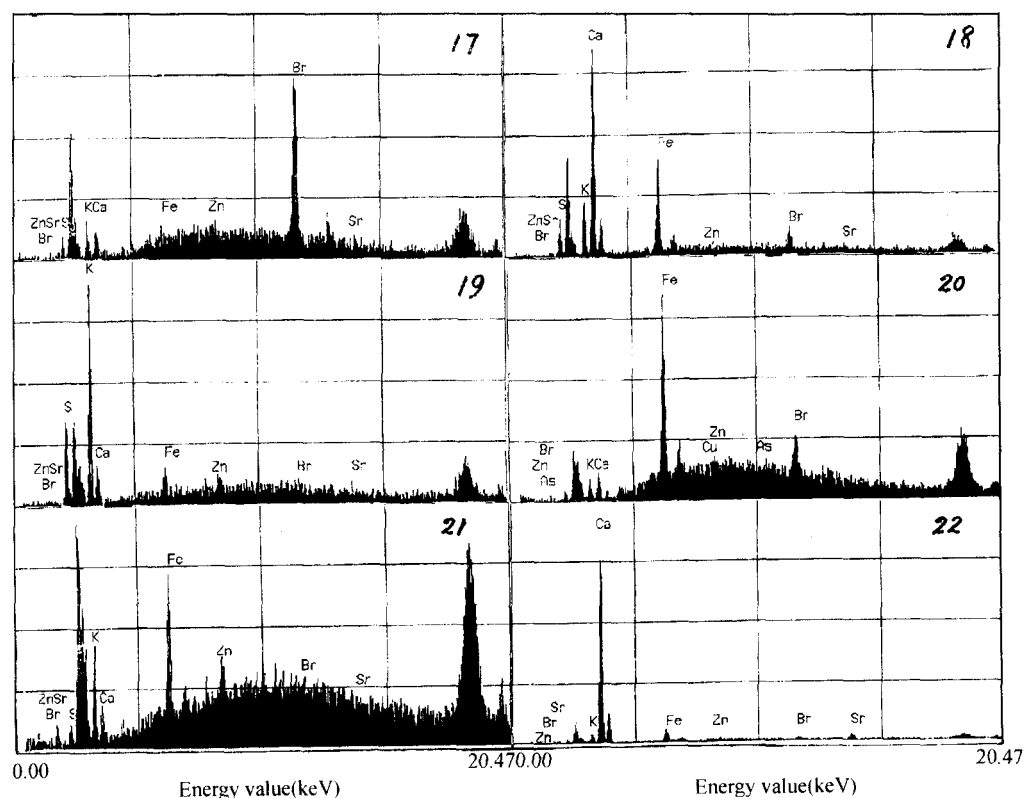


Fig. 3 X-ray fluorescence spectra of marine algae (sample 17—22)

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用 X 射线分析显微镜对海藻元素的研究 I. 海藻的 X 射线荧光光谱*

严小军 范 晓
(中国科学院海洋研究所)
永田忠博
(日本国立食品研究所)

摘 要

X 射线分析显微镜(XGT-2000V, Horiba Co., Japan, 1993 年制造)是一种新颖、快速的元素分析仪

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器,可以用来分析海藻中元素的组成特点。本研究对 1997 年 4,5 月在山东省威海市采集的 22 种海藻进行了研究,测定了海藻的 X 射线荧光光谱(XRF),并根据 X 射线特性荧光的能量分析海藻的主要元素组成。然后采用基本参数法(FP 法)对每种海藻的主要元素进行了半定量分析。研究表明,X 射线分析显微镜可以快速观测海藻中的主要元素如钾、硫、钙、铁、锌、溴、锶等,是研究元素组成与化学种态的有效的非破坏分析手段。从 X 射线荧光光谱可以看出,绿藻的特征元素是钾、钙、硫,孔石莼(*Ulva pertusa*)含有较多的铁元素,而浒苔则含有较多的溴元素。褐藻的特征元素除钾、钙、硫之外,还含有较多的锶元素。另外,酸藻(*Desmarestia viridis*)含有大量的硫及溴元素,鼠尾藻(*Sargassum thunbergii*)、单条髓藻(*Myelophycus simplex*)及酸藻(*Desmarestia viridis*)中尚含有较多的铁元素。红藻的特征是多种红藻均含有大量的溴元素,例如波登仙菜(*Ceramium kondai*)、松节藻(*Rhodomela confervoides*)、多管藻(*Polysiphonia urceolata*)、石花菜(*Gelidium amansii*)及亮管藻(*Hyalosiphonia caespitosa*)。另外,珊瑚藻(*Corallina pilulifera*)中含有大量的钙质。

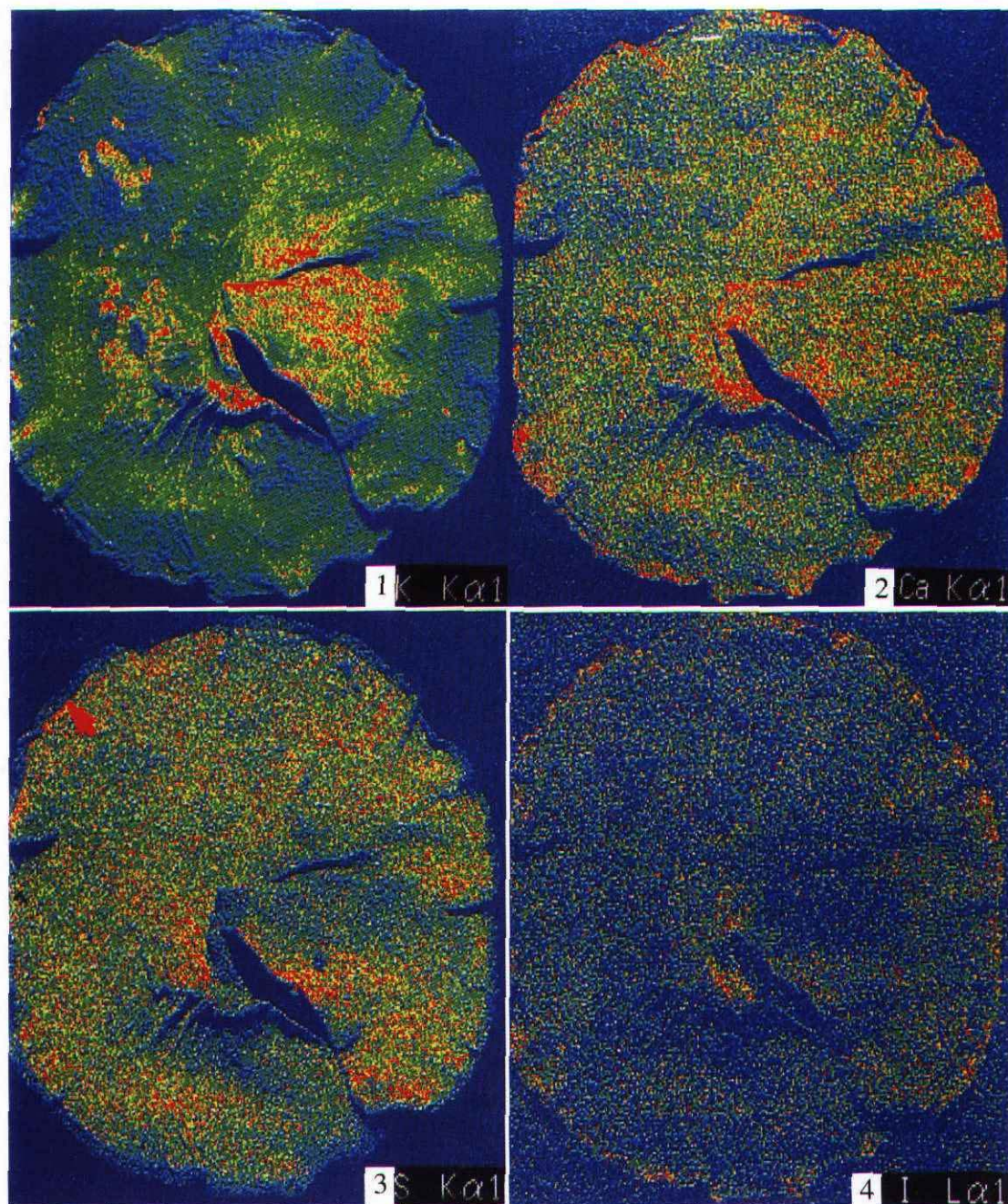


Plate I Elemental mapping of potassium, calcium
sulfur and iodine on sporophyll of *Undaria pinnatifida*

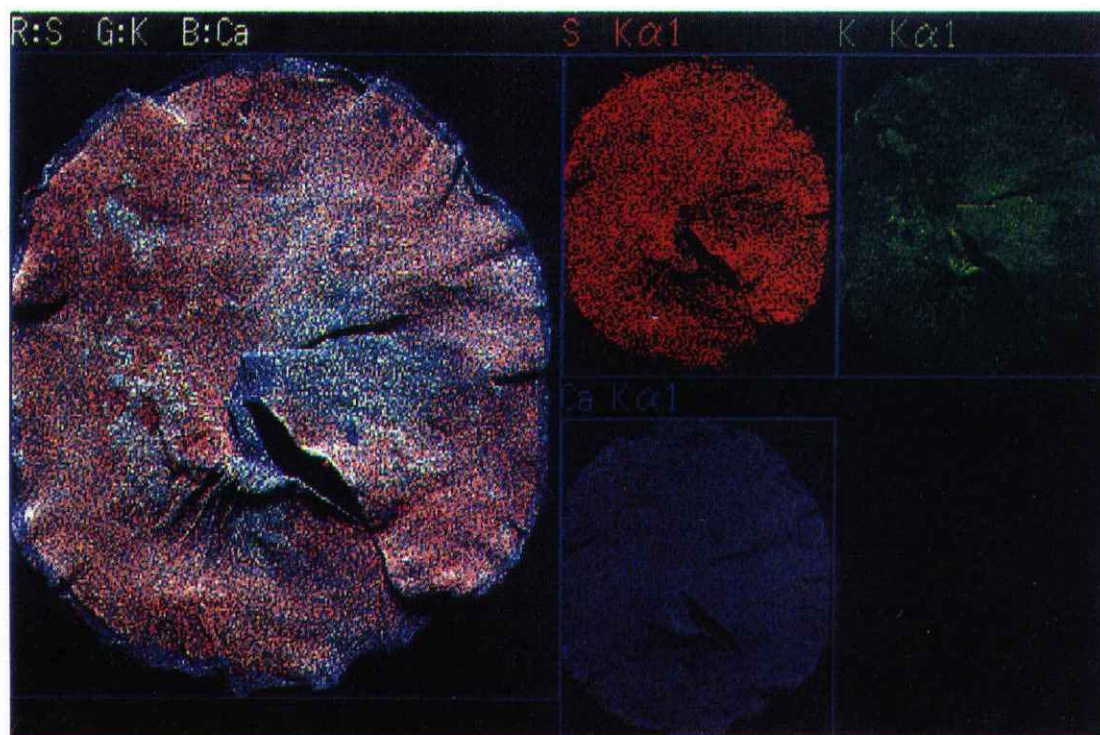


Plate II Sporophyll image synthesis of fluorescent X ray
of potassium, calcium and sulfur