

**Content and Uptake of Trace Metals in Benthic Algae,  
*Enteromorpha* and *Porphyra*. I.  
Measurement and Variation of Trace Metal Content  
of *Porphyra* Grown in Natural Environment.**

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(Figs 1 – 10; Table 1)

Studies of the trace metals in marine algae are rather few compared to those about the inorganic components in terrestrial plants or the organic components in marine algae. However, as the accumulation of radioactive substances and toxic metals in organisms has increasingly been examined, studies on the trace metals in marine algae have also increased at same pace.

In difference to terrestrial plants, the morphological structures of marine algae are relatively simple, only a few possess specialized organs, such as roots, stems, and leaves. The function of the tissues of roots, rhizoids or rhizoidal cells is simply to clamp onto other substances. Accordingly, nutrients are absorbed through the entire surface of the body including the rhizoids. Except the floating seaweeds which life on just by floating, most of the marine algae spend most of their lives just by clamping onto other substances (HIROSE<sup>1</sup>). Therefore, the effect of the surrounding sea water on them must be considered carefully. Even though the composition of sea water should be constant, trace metals are not always in ionic forms. They may be present as organic or inorganic complexes, or they may be distributed in suspending matters, detritus, sediments, or organisms (Tsubota<sup>2</sup>). If such various forms of trace metals subsist in equilibrium with the ionic forms, it should be considered that the algae growing in intertidal areas must be greatly affected by suspending matters and sediments as well as by the composition of the water. Therefore, we suppose that the content of trace metals in marine algae should be variable depending on their species, on the seasons, and on the horizontal or vertical regions.

As mentioned above, inorganic substances are absorbed through the entire surface of the thallus. It has been recognized by the assimilation experiments using [<sup>14</sup>C] labelled compounds that the absorption is uniform over the entire surface of the body when the algae are at the germ stage, then this absorption gradually becomes stronger at the margin area of the thallus as they grow. This points to some differences in metal absorption according to the growing stage and the parts of the thallus.

The parenchyma of the single layer cells of *Porphyra* is composed of five layers, the center of a cell part layer, both sides of middle (intercellular substance) layers and of the surface layer (cuticle layer), that can be regarded as having three components (MIRWA<sup>3</sup>). Frei and PRESTON<sup>4-6</sup>) showed by X-ray and electron microscopic analyses that the surface layer of *Porphyra* consists predominantly of crystalline mannan and only a little of crystalline xylan, that the middle layer consists of mannan, and the cell wall is made of xylan. If the absorption is defined as permeation through the cell wall into the cell and the adsorption as uptake of surface and middle layers, the analytical values obtained from leaves as a whole can not be regarded as representing the real absorption values. Thus, besides the total content, it is necessary to determine the metal content of the three component layers of fronds separately. We suppose that each layer has the proper characteristic pattern of absorption or adsorption.

Six elements, Fe, Zn, Mn, Cu, Pb, and Cd, were chosen particularly considering that they can be easily determined by an atomic absorption spectrophotometry and also that they are physiologically important elements of the algae. Among a number of decomposition methods ( $\text{HNO}_3\text{-HCl}$ ,  $\text{H}_2\text{SO}_4\text{-HNO}_3$ ,  $\text{HNO}_3\text{-HClO}_4\text{-HNO}_3$ , and  $\text{HNO}_3\text{-HClO}_4\text{-HCl}$  (TAKEUCHI<sup>10</sup>)) attempted for processing algal samples, the last one gave the best result and therefore we used throughout the present study.

## Materials and Methods

### (1) Measurement of Metals.

The atomic absorption spectrophotometer (HITACHI 208) was used for the analysis of metals, Fe at 2483, Zn at 2138, Mn at 2794, Cu at 3247, Pb at 2170, and Cd at 2288 Å. Optima results a fine adjustment of  $\pm 5$  Å was necessary for all metals. In order to analyze a sample by the atomic absorption spectrophotometry a calibration curve must be prepared first. The calibration curve is usually a straight line, but tends to fall off from a straight line in the higher concentrations. Since the slope of a calibration curve varies with the metals and with same other factors too such as the operating conditions of the machine, solutions of the same concentration may show different absorptions. Therefore, a new calibration curve must be prepared for each measurement.

### (2) Analyses of Algal Samples.

Algal samples were decomposed by the wet ashing process and then analyzed by the atomic absorption spectrophotometer. Before measuring the samples, it was necessary to check the level of metal recovery in the decomposition process. Thus, a metal solution of 1000 mg/l was diluted in distilled water to form solution of 0.25, 0.5, 1.0, and 2.0 mg/l, respectively. Fifty and 150 ml each of these solutions were treated with the reagents (20ml  $\text{HNO}_3$ , 5ml  $\text{HClO}_4$ , and 10ml  $\text{HCl}$ ) used for the decomposition. As the standard solutions, solutions of the same concentrations as those above were prepared by diluting the 1000 mg/l solution in 10%  $\text{HCl}$  instead of in distilled water. The blank tests were carried out using 50 and 150 ml distilled water in the same manner. Metal impurities in the reagents were also checked by the same treatment. The concentration

ranges of the calibration curves holding the straight lines and high recoveries were less than 1.0, 0.6, 1.0, 1.0, 1.0, and 0.6 mg/l for Fe, Zn, Mn, Cu, Pb, and Cd, respectively. To determine the metal content of algal samples, quantity and dilution of the samples should be adjusted so that the measurements can be made within these ranges.

The samples were decomposed by the following procedure. To 4g of dried alga in a 300 ml Erlenmyer flask 10ml HNO<sub>3</sub> were added, the sample was left to stand overnight and then carefully heated on a sand bath the following day, a vigorous reaction arose, after quiet down red-brown gas was generated, now 5 ml of 70% HClO<sub>4</sub> were added, and the content continued the same heating process until the solution turned completely colorless and the white smoke due to the excess of HClO<sub>4</sub> disappeared. The solution was then evaporated to 2-3 ml by further heating. After addition of 10ml of HCl the solution was boiled again, cooled off, and water was added to make the complete volume 100ml. This solution was used for the analyses of Fe, Zn, Mn, Cu, and Pb (ISHIBASHI<sup>7,8</sup>, MORII<sup>9</sup>). For the determination of Cd it was necessary to extract the sample solution by a solvent in order to eliminate the interferences of sodium ion. Thus 100ml of the sample solution were adjusted to pH 9.1 with ammonium hydroxide solution and poured into 500ml separatory funnel. To this solution were added 5ml of 1% diethyl dithiocarbamic acid (DDTC). Leaving it for 5 min. methyl isobutyl ketone (MIBK) was added, then the funnel was vigorously shaken for 5 min. After standing for another 15 min., the water layer was discarded, and the upper layer was stored as the sample solution for the Cd assay (TAKEUCHI<sup>10</sup>, YAMAMOTO<sup>11</sup>).

### (3) Contents of Trace Metals in *Porphyra*.

To investigate the presence of trace metals in *Porphyra* grown in natural environment, three sampling stations were set up from near to the coast to the off-shore sea on the laver cultural grounds located in the estuary of the Ashida river, Fukuyama (Fig. 1), and *Porphyra* was collected every two weeks during the cultural season from November, 1972 to April, 1973. Collected samples were put in nylon bags and immediately transported to the laboratory, rinsed successively with tap water, deionized water, and distilled water until the washings showed no trace of chloride and other ions. After removing the water droplets the wet material was dried at 105°C for about 6 hr. The dried samples were stored in a desiccator. Before analyzing the dry material was heated again at the same temperature for 4 hr., cooled off in a desiccator for 30 min., and then weighed. Three samples from each station were separately treated and analyzed, and the average of these values was taken as the metal content of the sample from that station.

Besides the trace metals, the content of chlorophyll *a* in fronds was also determined by extracting 10 mg of dry material from each station with 92% acetone and by measuring the acetone solution by means of a spectrophotometer. The content of chlorophyll *a* was calculated by the following equation.

$$\text{Chlorophyll } a \text{ (mg/g dry weight)} = 11.64 D_{663} - 2.16 D_{645} - 0.10 D_{630} \\ (\text{SCOR} - \text{UNESCO}^{12})$$

As the environmental factors of sampling stations, water temperature and specific gravity were measured, and chlorinity was derived from the specific gravity.

In order to compare the regional differences of metal content of *Porphyra*, samples were also collected from the cultural ground of Tajiri and Tashima, off-shore Fukuyama, and their metal contents were measured.

## Results

### (1) Metal Measurement by Atomic Absorption Spectrophotometry.

The sensitivity of the spectrophotometer was different by according to the metals. The recovery of each metal was over 95% when the concentration was 1.0mg/l. No trace of Fe, Mn, Cu, Pb, or Cd was detected in distilled water and in the reagents used for the decomposition, while Zn was detected in  $\text{HNO}_3$  (0.05  $\mu\text{g}$  in 20 ml),  $\text{HClO}_4$  (0.04  $\mu\text{g}$  in 5 ml), and in HCl (0.04  $\mu\text{g}$  in 10 ml).

### (2) Analyses of Algal Samples.

The recovery of metals in the decomposition of the alga was 95% for Fe, Mn, Zn, Cu, Pb and 100% for Cd, when measured by adding known metal solutions of low concentration.

In order to find the decomposition error the standard deviation was obtained by processing separately and measuring five to ten samples from a same source. Number of samples, average concentration, and standard deviation are as follows. Fe: 5 samples,  $486 \pm 66$ , Mn: 8 samples,  $116 \pm 39$  or  $698, \pm 44$ , Cd: 10 samples,  $58.5, \pm 5.5 \mu\text{g/g}$  dry tissues.

### (3) Contents of trace Metals in *Porphyra* Grown in Natural Environment.

The contents of trace metals in *Porphyra* grown in natural environment were as follows (see Fig. 2-7). Fe 100-1000, Zn 100-200, Mn 20-70, Cu 9-30, Pb 1-4, and Cd 0.1-0.5  $\mu\text{g/g}$  dry weight.

The seasonal and regional variations of trace metal content of the alga could not be clearly obtained as initially expected. However, Station 1 in the estuary, revealed a higher metal content than the other stations as shown by the average of all the collections. In the seasonal variation the Fe content was high at germ to growing stages, but it rapidly

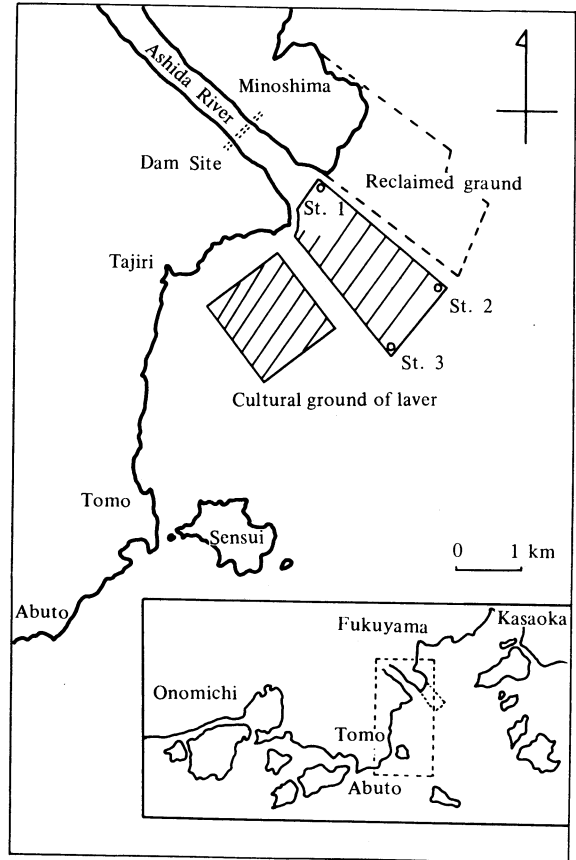


Fig. 1 Sampling stations

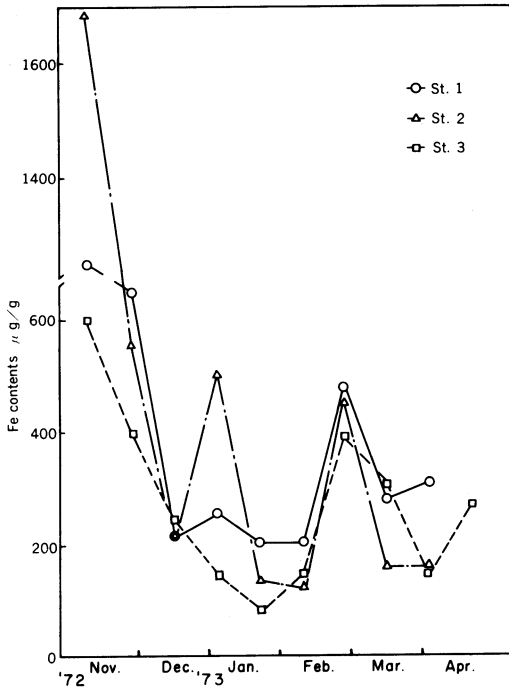


Fig. 2 Seasonal and regional variations of Fe content of *Porphyra*

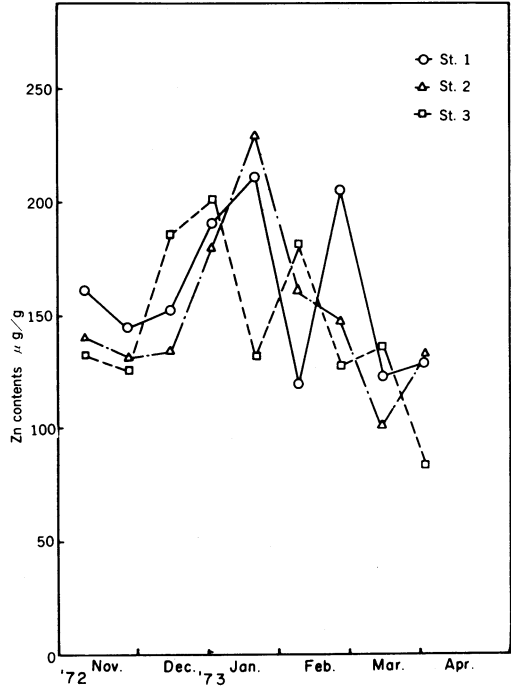


Fig. 3 Seasonal and regional variation of Zn content of *Porphyra*

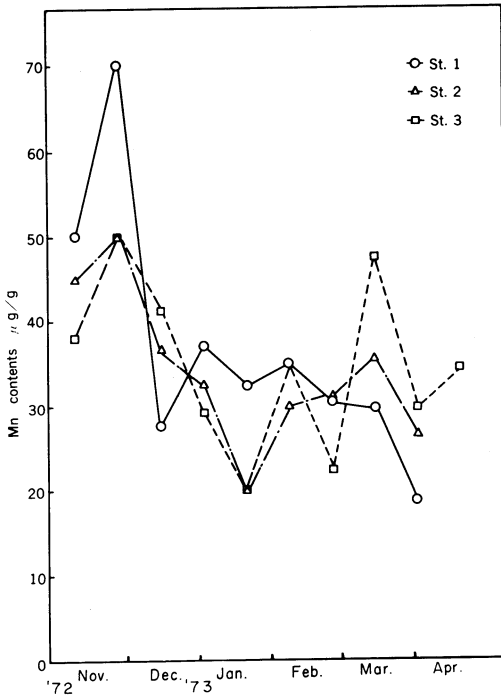


Fig. 4 Seasonal and regional variation of Mn content of *Porphyra*

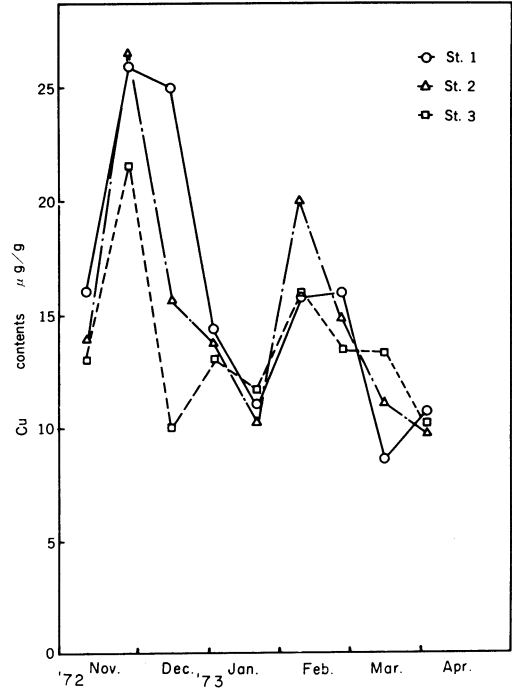


Fig. 5 Seasonal and regional variations of Cu content of *Porphyra*

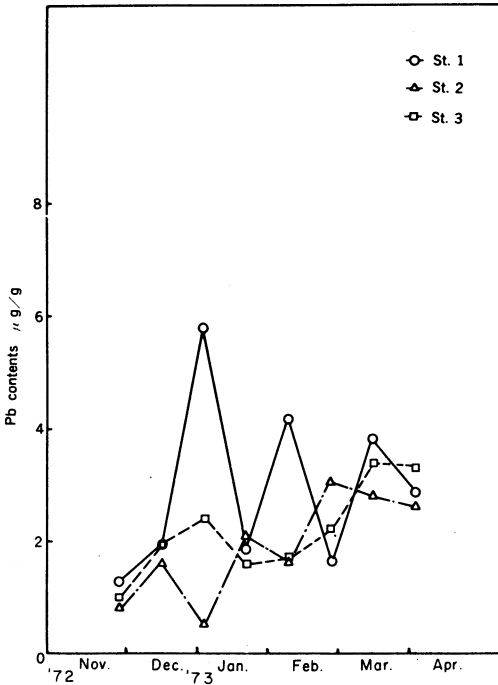


Fig. 6 Seasonal and regional variations of Pb content of *Porphyra*

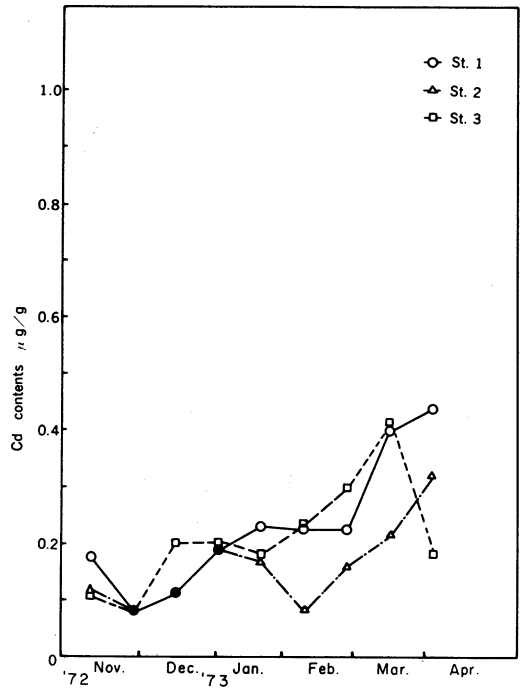


Fig. 7 Seasonal and regional variations of Cd content of *Porphyra*

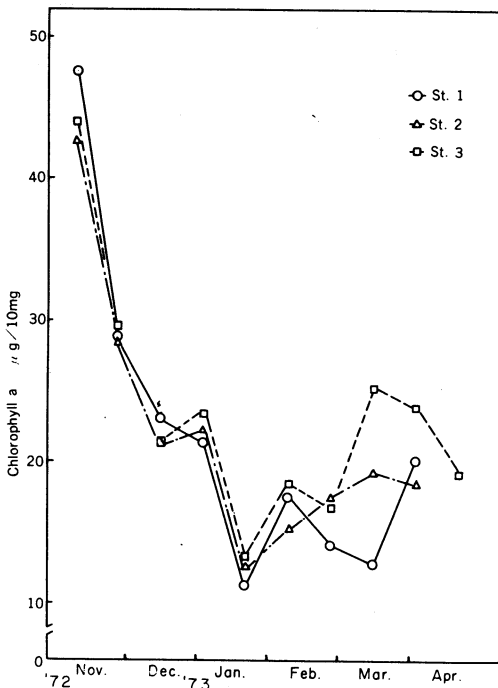


Fig. 10 Variations of chlorophyll a contents of *Porphyra*

decreased in the period of maturity. In general, the essential minerals, Fe, Mn, Zn, and Cu were higher at young stages but gradually decreased at the maturity of the fronds. On the other hand the contents of Pb and Cd were lower at the young stages, but they showed a tendency to gradual increase in the course of time. The content of nitrogen and chlorophyll *a* in *Porphyra* are the important factors affecting the quality of laver products. The seasonal variation of chlorophyll *a* content closely resembled to that of the Fe content (Fig. 2 and Fig. 10).

Although the lengths of fronds of the alga were not measured, rather considerable variations were observed. There were also great individual differences in the contents.

Water temperature did not show much difference at each station, yet it was

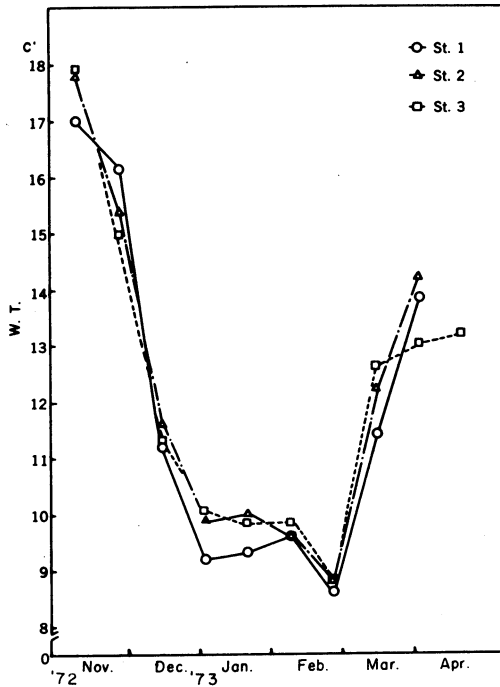


Fig. 8 Variations of water temperature of the sampling stations

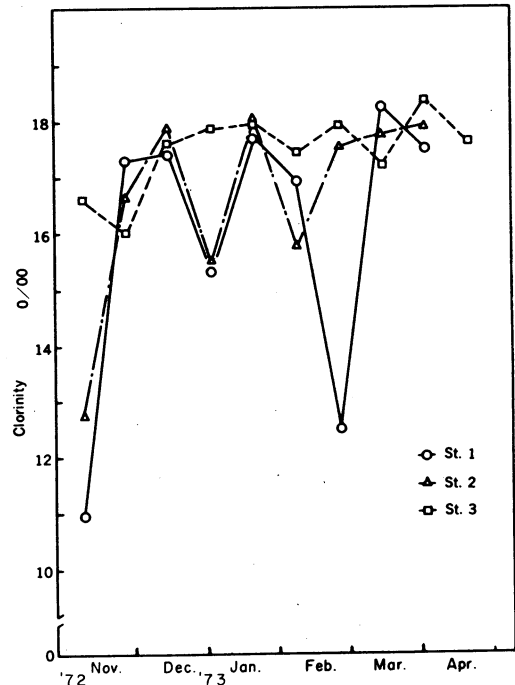


Fig. 9 Variations of chlorinity at the sampling stations

slightly lower and had greater variations near the shore than off-shore of the estuary. Salinity was also much lower and had greater variations near the shore than at off-shore (Figs. 8 and 9).

Table 1 shows the content of the trace metals and of chlorophyll *a* in *Porphyra* from regions other than the selected fixed stations. The Fe content is high in the laver of Tajiri and low in the samples of Tashima compared to those of the fixed stations. Zn is high at both Tajiri and Tashima, Mn is higher at Tajiri but without difference at Tashima, Pb is considerably higher at Tajiri but lower at Tashima, and Cu is slightly lower in the samples collected at Tajiri in Nov. 1972 compared to those of the fixed stations. Chlorophyll *a* showed no regional variations.

#### Discussion

The recovery of metals by the decomposition of the alga was over 95%. However, the metal content of the alga showed a 10–20% deviation. This deviation might be attributed to the decomposition process used and also to the differences in size and maturity of the alga. In general the seasonal and regional variations of trace metals in *Porphyra* grown in natural environments were not so different as initially expected. Only for the iron, the variation was rather remarkable even taking in account the high deviation. That is, the period from germ to growing stage showed a very high Fe content, while the period of maturity revealed a rapid decrease of the metal. It was also characteristic that the content of chlorophyll *a* indicated quite similar variations to that of iron. The rapid

Table 1. Contents of metals and chlorophyll a of *Porphyra* at various regions

Fe contents ( $\mu\text{g/g}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		650.0	550.0	400.0	600.0	
12. 19		216.0	216.0	248.0		125.0
'73. 3. 31		281.0	150.0	306.0	330.0	

Zn contents ( $\mu\text{g/g}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		143.0	133.0	126.0	300.0	
12. 19		153.0	135.0	185.0		250.0
'73. 3. 31		123.0	101.0	138.0	253.0	

Mn contents ( $\mu\text{g/g}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		70.0	50.0	50.0	70.0	
12. 19		27.5	36.9	41.7		27.6
'73. 3. 31		29.7	35.9	47.7	78.1	

Cu contents ( $\mu\text{g/g}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		26.0	26.7	21.7	14.0	
12. 19		25.0	15.0	10.0		25.0
'73. 3. 31		8.9	11.2	13.5	10.9	

Pb contents ( $\mu\text{g/g}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		1.30	0.80	1.00	0.90	
12. 19		2.00	1.65	2.00		0.36
'73. 3. 31		3.81	2.81	3.45	8.66	

Chlorophyll a contents ( $\mu\text{g/mg}$ )						
date	stations	St. 1	St.2	St. 3	Tajiri	Tashima
'72. 11. 24		2.88	2.86	2.96	3.08	
12. 19		2.31	2.14	2.17		2.07
'73. 3. 31		1.29	1.91	2.53	1.66	

Tajiri and Tashima are 5 to 10 kilometers away from the stations

decrease of Zn, Mn, and Cu at the maturity stage was also similar to that of the variation of iron. Those metals are essential elements for plants, and they have close relations with the action of a number of important enzymes which control the metabolism of plants. Most enzymes are a kind of conjugated proteins which involve non-protein and low molecular coenzymes. Coenzyme is essential for holoenzyme to perform its action. A part of coenzyme consists of such metals as Fe, Zn, Mn, or Cu. They also act as cofactors in the enzymic reaction. Therefore, decrease of these metals in *Porphyra* could be regarded as a decline of physiological activities.

On the other hand, on the contrary of those metals, the content of Pb and Cd showed a tendency to increase with the growth of the plant. The physiological significance of



this tendency may be explained as follows. Plant cells absorb substances in two ways. One by diffusion which occurs when the concentration of a substance inside the cells is lower than the surrounding medium. The other is the process that can be termed as active absorption which occurs in spite of the higher concentration inside the cells than in the medium. The latter process advances against concentration gradient by using the energy produced by respiration of plants themselves. However, these two processes can not be clearly distinguished one from the other. But certainly, plants absorb substances by these two processes. Therefore, Pb and Cd are gradually absorbed by algae during the period of their growth and considered to be accumulated without playing positive or physiologically meaningful roles in the cells.

Although the regional variations of the metal contents of *Porphyra* were obtained, it is difficult to judge whether the result is affected by the variations of metals in sea water, because the metal content of sea water was not measured in the present investigation. Only the regional variations were observed in the examination of the contents Zn and Pb in *Porphyra*.

### Summary

1. Trace metals in *Porphyra* were recovered for more than 95% by the treatment of the alga with  $\text{HNO}_3$ - $\text{HClO}_4$ - $\text{HCl}$ .
2. Ten to 20% of the standard deviations were obtained by the analyses of the alga.
3. The trace metals in *Porphyra* could be divided into two groups according to their tendencies of seasonal and regional variations, one is the group of Fe, Zn, Mn, and Cu and the other group is Pb and Cd.
4. The variation of chlorophyll *a* content was quite similar to that of Fe.
5. The content of metals was different in *Porphyra* collected from regions other than the fixed stations.

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## アマノリに含有する微量金属元素の測定方法と 自然状態アマノリの微量金属元素の含有状態

藤山虎也・前田正智

海藻中の微量金属に関する研究は陸上植物の無機成分や海藻中の有機成分にくらべて、はなはだ少ない。海藻の形態的構造は陸上植物と異なり比較的簡単で、根、茎、葉などの特別な器官の揃っているものは少ない。ただ他物に付着する組織だけを持ち、養分等の吸収については体の全表面で行うのが普通である。また、生活史の一時期を除けば海藻はほとんど付着生活である。それ故、海藻の生活環境である海水の影響を当然考えなくてはならない。潮間帯付近に生育する海藻にとっては海水の成分はもちろん、その底泥や懸濁物までも海藻に与える影響は大きいものと考えなければならない。したがって海藻中の微量金属の含有量は海藻の種類、季節、生育する場所（水平、垂直的）に大いに異なると予想される。

自然状態におけるアマノリ属の微量金属の含有量を調べるため、福山地先のノリ養殖場において1972年11月から1973年4月に至るまで半月ごとに岸寄りの河口付近から沖合にかけて三定点を設け、ノリを採取した。アマノリ試料は硝酸-過塩素酸-塩酸で分解し、Fe, Zn, Mn, Cu, Pb, Cdの6種の元素について原子吸光分光分析法により測定した。また同時に葉体のクロロフィルa量についても測定した。

原子吸光分光分析法による測定法で各元素とも95%以上の回収率が得られた。三定点におけるアマノリの微量金属含有量の変動はFe: 100~1000 $\mu$ g/g, Zn: 100~200, Mn: 20~70, Cu: 9~30, Pb: 1~4, Cd: 0.1~0.5であった。一般的に必須微量元素であるFe, Zn, Mn, Cuは葉体が若いときに含有濃度が高く、成熟期に減少し、Pb, Cdは反対に葉体が若いとき少なく徐々に増加する傾向を示した。クロロフィルa量の季節的変動はFe含有濃度の季節的変動によく似た傾向を示した。当初予想した葉体中の微量元素の季節的、地域的変動があまり明確にならなかった。