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Vitamin B_{12} Contents in Sea Water along the Coast of Fukuyama in 1970 and 1971.

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(Figs. 1-3; Tables 1-3)

Red water, the amber to red discoloration of sea water due to a dense bloom of plankton, has recently become a rather common occurrence in the inshore waters along the Pacific coast of Japan. In the Seto Inland Sea, in particular, owing to its enclosed topography and consequent accumlation of the nutrient salts disposed from the land, the red water is now of frequent occurrence chiefly during the summer (early June to late September), often causing extensive mortalities in the natural and cultured population of marine fishes and invertebrates. In addition, several phytoplankters whose names had never been mentioned in connection with red waters in this sea region have now become the leading species in some of the blooms; they include a species of ***Exuviaella*** (dinoflagellate)[1] and two forms of ***Eutreptiella*** (euglanoid).[1][2] We can now count about 30 species of phytoplankters among the list of the organisms that have caused red waters in the Seto Inland Sea, and some of them have been proved to be toxic to mice and blue gill sunfish.[3]

Although the mechanism of the outburst of a red water is not yet thoroughly understood, it is a well established fact that vitamin B_{12} (cyanocobalamin) or its analogues[4][5] are required as an essential micronutrient for growth by most of the species of phytoplankters constituting red waters, and that growth is at least strongly intensified by the presence of these substances in some other species.[6]~[8] On the other hand, it is also known that vitamin B_{12} and its analogues are not always equally active to an organism requiring them.[9]~[12]

For the purpose of evaluating the role that vitamin B_{12} might play in the outbursts of red waters, it should be taken as a pertinent approach to investigate the relationship between the vitamin B_{12} contents of the sea water and the fluctuation and succession of the phytoplankton populations which constitute the blooms.

In this present study the vitamin B_{12} content of sea water was measured at monthly intervals in the nearshore waters of Fukuyama, and its seasonal and geographical variations as well as its correlation to phytoplankton blooms were discussed.

MATERIALS AND METHODS

Sea water samples were collected generally at monthly intervals during 1970 and 1971 at the eight stations located in the northern part of Bingo Nada of the Seto Inland Sea (St. 1–9 in Fig. 1). These stations were 0.8 to 6 km distant from the mouth of the Ashida River, and water depths measured 2 to 6 m. The water samples were taken at a depth of 2 m at each station with the Kitahara water bottle at low tide. Sample waters were immediately brought to our laboratory, where they were filtered through the membrane filter (Sartorius, pore size 0.45 μ) to exclude the suspended matters. The filtrates were stored at $-25°C$ until use.

Vitamin B_{12} contents in the filtered sea water samples were determined by the microbiological assay method depending on ***Lactobacillus leichmanii*** ATCC 7830. The thawed sample was dialyzed, in a cellophane membrane, against distilled water to diminish the inorganic salts which might disturb the growth of the test bacteria. The dialysis was carried out two times each for 12 hours, and the distilled water

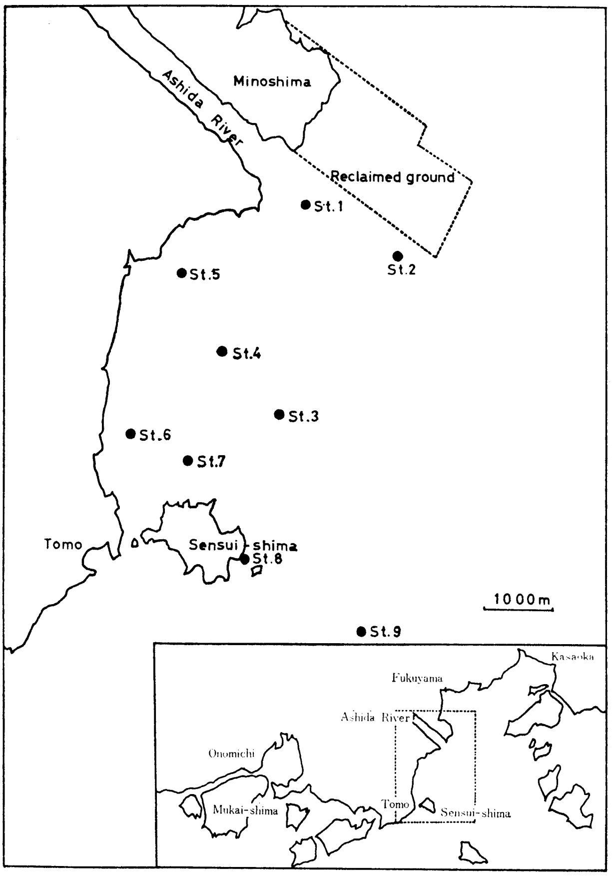

Fig. 1. Sampling stations.

was changed several times in each dialysis procedure. The aliquots, usually 5 mℓ, of the dialysates were subjected to the microbiological assay. The results of the assay for the triplicate subsamples coincided usually within 0.05 mμg vitamin B_{12} per litre.

In 1971 the chlorinity and the temperature of the sea water at 2.5 m depth were measured at St. 2 and 9 on the same days as the sea water samples were collected.

RESULTS

In the 60 samples collected on eight occasions during 1970, the concentration of vitamin B_{12} ranged between 0.42 and 6.43 mμg/ℓ (Table 1). In the 56 samples collected on seven occasions in 1971, it was within the range of 0.70—3.62 mμg/ℓ (Table 2). It is noteworthy that all the samples tested contained vitamin B_{12} in

Table 1. Vitamin B_{12} contents in sea water along the coast of Fukuyama in 1970. (mμg/ℓ)

Sampling date	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8
May 12	2.86	2.63	2.92	1.43	0.95	1.67	2.02	0.77
Jun. 9	1.11	2.90	2.54	3.08	3.26	6.43	2.00	1.46
Jul. 18	2.87	3.36	1.68	2.75	2.57	3.81	1.99	1.87
Aug. 6	2.63	2.14	4.27	1.96	1.03	2.66	2.74	1.91
Aug. 28	2.02	*	*	2.36	3.98	1.74	*	*
Sept. 8	2.34	2.42	2.67	2.55	2.36	2.15	2.13	2.45
Oct. 13	2.27	2.16	2.99	1.74	1.78	2.25	2.35	2.29
Dec. 15	5.92	1.64	1.32	0.62	0.56	1.63	0.87	0.42
Mean	2.75	2.46	2.63	2.06	3.06	2.79	2.01	1.59

* No measurement.

Table 2. Vitamin B_{12} contents in sea water along the coast of Fukuyama in 1971. (mμg/ℓ)

Sampling date	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8
Apr. 30	1.63	1.64	1.96	1.86	1.91	1.12	0.92	1.23
May 27	2.41	2.63	1.64	2.44	0.99	1.99	2.99	0.96
Jun. 29	3.62	2.77	2.71	2.72	2.77	2.69	2.74	2.88
Jul. 29	1.12	1.43	1.64	1.53	2.66	2.69	2.07	0.92
Aug. 27	0.70	1.56	1.46	2.53	2.42	2.00	2.13	0.88
Oct. 19	2.49	2.92	2.65	2.02	1.92	1.53	2.75	0.94
Nov. 16	1.42	1.65	1.76	1.83	2.83	2.00	1.46	1.09
Mean	1.91	2.09	1.97	2.13	2.21	2.00	2.15	1.27

concentrations well above the lower detection limit inherent in the present method of bioassay. In either year about 85% of the samples showed vitamin B_{12} contents between 1 and 4 mμg/ℓ, and about 10% of the samples, values less than 1 mμg/ℓ. Concentrations over 4 mμg/ℓ were shown only by the three samples collected in 1970. All these values are comparable with those hitherto reported from the coastal regions of Japan.[13)~15)]

When the vitamin B_{12} contents of the eight sea water samples collected on a same day were compared mutually, the sample from St. 8 usually showed lower values than most of the other samples. The six samples from St. 2 through St. 7 were usually somewhat similar in vitamin B_{12} contents, the ratio of the highest value to the lowest not exceeding 3. The water sample from St. 1 was apt to give an extraordinary low or high value. The low values at St. 8 were probably ascribable to the fact that this station, most distant from the river mouth (viz., 6 km), was subjected to the influence of the offshore water to greater degrees than the other stations. The horizontal chlorinity gradients in this sea region are reflected by the chlorinity difference between St. 2 and St. 9 as shown in Fig. 2. The rather great variations in the vitamin B_{12} contents at St. 1 were probably caused by the varying mixing ratio between the river drainage and the sea water, since this station was ver near to the river mouth (0.8 km). The annual mean of the vitamin B_{12} contents were within the range of 1.97—3.06 mμg/ℓ for each of St. 2 to St. 7, and relatively lower at St. 8 (1.27 and 1.59 mμg/ℓ).

In order to check the seasonal trend of the vitamin B_{12} concentration in the general area represented by St. 2 through St. 7, the concentrations at these six stations, as given in Tables 1 and 2, were averaged for each sampling day (Table 3). In both 1970 and 1971, these daily averages were kept at relatively high levels from

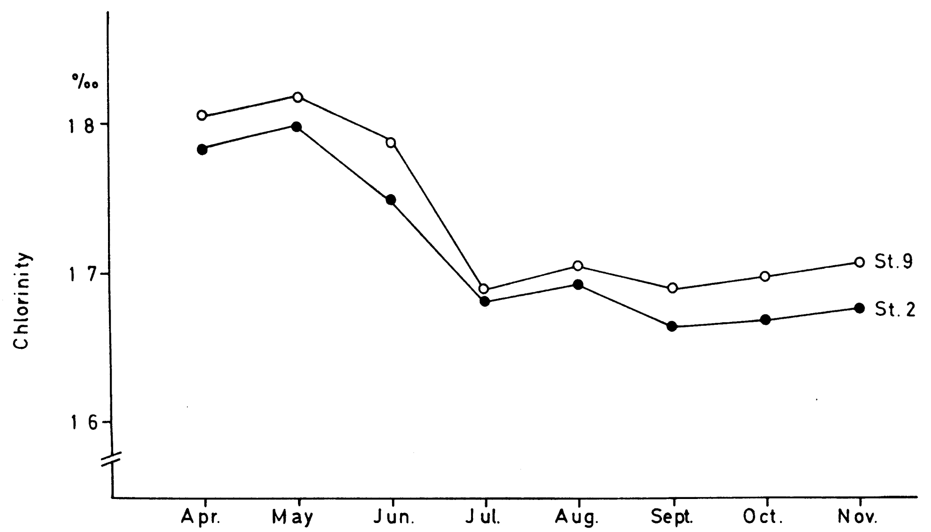

Fig. 2. Seasonal change in chlorinity of sea water in 1971.

Table 3. Seasonal trend of vitamin B_{12} content in sea water along the coast of Fukuyama. (mμg/ℓ)

1970		1971	
Sampling date	*Average for St. 2 to St. 7	Sampling date	Average for St. 2 to St. 7
May 12	1.94	Apr. 30	1.57
Jun. 9	2.76	May 27	2.11
Jul. 18	2.69	Jun. 29	2.73
Aug. 6	2.11	Jul. 29	2.00
Sept. 8	2.38	Aug. 27	2.02
Oct. 13	2.21	Oct. 19	2.30
Dec. 15	1.11	Nov. 16	1.92
Mean	2.17	Mean	2.09

* Values over 4 mμg/ℓ were excluded from the calculation.

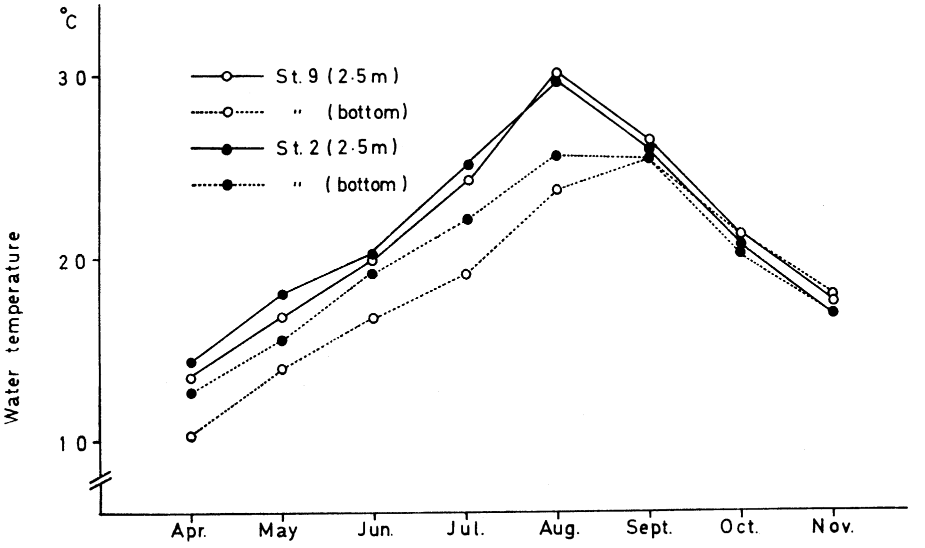

Fig. 3. Seasonal change in sea water temperature in 1971.

May to October, viz., in the months with water temperatures above about 18°C (Fig. 3). A higher maximum occurred in June or July and a lower maximum in September or October. The first maximum roughly coincided with the rainy period in the early summer, when the chlorinity of the local sea water dropped markedly owing to the diluting effect of the river drainage (Fig. 2). The annual mean of these daily averages happened to be nearly equal in 1970 and 1971 (*i. e.*, 2.17 and 2.09 mμg/ℓ).

Out of the 15 sample-collecting occasions during the two years, three coincided with red water blooms. On August 28, 1970, a dense bloom of a marine euglenoid (***Eutreptiella*** sp.) occupied St. 4, 5 and 6, and the sea water contained 2.36, 3.98 and 1.74 mμg/ℓ of vitamin B_{12} respectively. On June 29, 1971, the upper zone of the whole area represented by St. 2 throuth St. 7 was inhabited by a dense population of an unarmored dinoflagellate (***Heterosigma*** sp.), and about 2.7 mμg/ℓ of vitamin B_{12} were detected in the water samples from these stations. On August 27, 1971, St. 4, 5 and 6 were occupied by the red water bloom of another naked dinoflagellate (***Gymnodinium*** sp.) from the surface to the bottom, and the vitamin B_{12} contents of sea water were 2.53, 2.42 and 2.00 mμg/ℓ respectively. The vitamin B_{12} concentrations mentioned above fall within the range between the annual mean and the twice; in other words, it did not appear that the concentration of vitamin B_{12} in the sea water showed any conspicuous decrease in these red waters.

DISCUSSION

Vitamin B_{12} concentrations in oceanic and coastal sea waters have been reported by several authors.[16)~20)] Their results range from an undetectable level up to 150 mμg/ℓ, and are less than 10 mμg/ℓ in most cases. Since these authors used different test organisms for the bioassay, some of their results may reflect the combined effect of vitamin B_{12} and one or more of its analogues. The values obtained in the present study may be regarded to represent mainly vitamin B_{12} (cyanocobalamin) itself, because the employed test organism (***Lactobacillus leichmanii***) is specifically responsive to vitamin B_{12} and some of its analogues but not to other analogues such as Factors B and C.[21)] According to DROOP[7)], the concentrations of vitamin B_{12} analogues are far lower than that of vitamin B_{12} itself in ocean waters. If this is also the case with the sea waters of inshore regions, the figures in Tables 1 and 2 should approximate the concentration of vitamin B_{12}.

The contents of vitamin B_{12} in marine sediments were assayed by KURATA[14)], OHWADA and TAGA[22)] and others, and they concluded that vitamin B_{12} producing bacteria are rather commonly found in marine muds and are especially abundant in the surface layer of the bottom muds of coastal regions. Hence the suggestion arises that, in the inshore waters presently studied, the muddy bottom coupled to the shallow water depths is probably a proliferous source of vitamin B_{12} supply to the sea water throughout the year.

According to MENZEL and SPAETH[23)], in the Sargasso Sea where vitamin B_{12} concentration was very low, auxotrophic diatoms occurred in significant number only at the time of the vitamin B_{12} maximum, and the species that do not require this vitamin dominated the phytoplankton at other times. In contrast, in the inshore waters of Fukuyama the sea water contains 2 mμg/ℓ or more of vitamin B_{12} during the warm season of the year, which corresponds to the concentrations sufficient to grow chrysomonads to population densities over 10^5 cells/ℓ.[6)] Therefore, it seems probable that vitamin B_{12} should not be a limiting factor to the fluctuations and reccurrences of red waters in this region during the warm months of the year.

SUMMARY

In the nearshore region of the Seto Inland Sea off Fukuyama, where red water blooms of phytoplankton frequently occur, the vitamin B_{12} content of the sea water was determined at nearly monthly intervals except in winter during 1970 and 1971. Water samples were taken from a depth of 2 m. The vitamin B_{12} content of the sample water filtered through a membrane filter was determined by the microbiological assay depending on ***Lactobacillus leichmanii***. The results can be summarized as follows:

1) Vitamin B_{12} was detected in all the samples. Its concentration varied between 0.42 and 6.43 mμg/ℓ.

2) The vitamin B_{12} content was not conspicuously low in the sea water samples taken within red water blooms. Though its seasonal variation was not conspicuous, it was generally high in the months of high water temperatures (ca. 18°C or above); the maximum occurred in June or July when the chlorinity of sea water dropped due to the rainy season; a secondary maximum occurred in September or October.

3) At the station most distant from the river mouth (viz., 6 km) the sea water showed somewhat lower contents of vitamin B_{12} than at other stations. Besides the river drainage, the bottom mud was also considered as source of vitamin B_{12} supply.

4) In the present waters the fluctuation and succession of phytoplankton blooms took place during warm months. It does not seem probable, however, that these phenomena were controlled by any paucity of vitamin B_{12}, because the latter was present in these months usually in concentrations of 2 mμg/ℓ or above, which is sufficient to grow some of the vitamin B_{12} requiring plankters to a population density of about 10^5 cells/mℓ.

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1970年および1971年における福山周辺海域のビタミンB_{12}の分布

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赤潮が頻発する福山周辺海域に8定点を設け，1970—1971年の間，冬期を除きほぼ毎月海水中のビタミンB_{12}含量を測定した．採水は2m層から行ない，メンブランフィルターで沪過後，沪液について *Lactobacillus leichmanii* を用いてビタミンB_{12}を定量した．

結果を要約すると次の通りである．

1）この海域において，ビタミンB_{12}は海水中からたえず検出され，その量は0.42～6.43 mμg/ℓ であった．

2）この両年にも赤潮は頻発したが，発生時に海水中のビタミンB_{12}の含量に顕著な低下は認められなかった．季節変化は著しくはなかったが，高水温期（約18°C 以上）にはビタミンB_{12}含量は概して高く，梅雨期の塩分低下期である6—7月に最高を示し，9—10月にもやや増加の傾向を示した．

3）河口から最も遠い（約6㎞）定点では，他の定点よりも幾分含量が低かった．なお，ビタミンB_{12}の補給は，河水によるほか，海底泥にも由来するものと考えられた．

4）この海域においては，暖期には一般に 2 mμg/ℓ 以上のビタミンB_{12}が海水中に溶存した．これはビタミンB_{12}を要求するプランクトンが10^5 cells/mℓ 程度の集群を形成するに足る濃度である．従って，少なくともこの海域においては，ビタミンB_{12}が暖期における赤潮の消長現象を制約する因子とは考えにくい．