Distribution of Vitamin B_{12}, Thiamine, and Biotin in Hiuchi-Nada Sea

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Abstract: Distribution of vitamin B_{12}, thiamine, and biotin in the water and sediments of Hiuchi-Nada Sea were determined by microbiological assay methods. Also some environmental factors were measured and their relation to vitamin distribution were studied.

It was found that concentrations of dissolved vitamins in the water were considerably high: 0.68-15.28 ng/l for vitamin B_{12}, 0-730 ng/l for thiamine, and 0.2-34.7 ng/l for biotin. Content of particulate vitamin B_{12} was also high but that of thiamine and biotin were low compared with the contents in the water. In bottom sediments in Hiuchi-Nada Sea, each vitamin accumulated abundantly: 8.85-124 ng/g wet wt. for vitamin B_{12}, 0-379 ng/g wet wt. for thiamine, and 1.1-4.4 ng/g wet wt. for biotin.

The concentration of dissolved vitamin B_{12} required by many red tide flagellates did not reach to the level that maximizes their growth at any time during the year. Low content of dissolved vitamin B_{12} in the water could limit their growth in Hiuchi-Nada Sea.

Introduction

Many species of marine phytoplankton require B group vitamins for growth: most commonly vitamin B_{12}, thiamine, and biotin alone or in various combinations. The incidence of auxotrophy is known to be high in members of Euglenophyceae, Cryptophyceae, Dinophyceae, and Crysophyceae, including important red tide organisms.[1-5] Therefore, it is suggested that essential vitamins in seawater not only could exert a controlling influence on seasonal variation and/or species composition of phytoplankton communities but also could affect the initiation of red tides.[6-8]

Hiuchi-Nada Sea is polluted by the inflowing of domestic and industrial waste waters, and red tides have frequently occurred in the district during the warm seasons for several years.

The present paper describes the occurrence and distribution of vitamin B_{12}, thiamine, and biotin in the water and sediments of Hiuchi-Nada Sea.

Materials and Methods

Water region investigated Hiuchi-Nada Sea is located in central part of Seto Inland Sea in Japan. The sea has an area of 1,300 km^2, a mean depth of 17.8 m, and a vlume of 23.2 km^3. Its topography is enclosed as illustrated in Fig. 1; only the north part opens to Bingo-Nada Sea. The tide in the area is affected by the two tidal waves from the Pacific Ocean, through the Kii Channel and the Bungo Strait far away from the area. Therefore, the pattern of tidal currents in the sea is complicated and the current velocity is generally slow.[9]

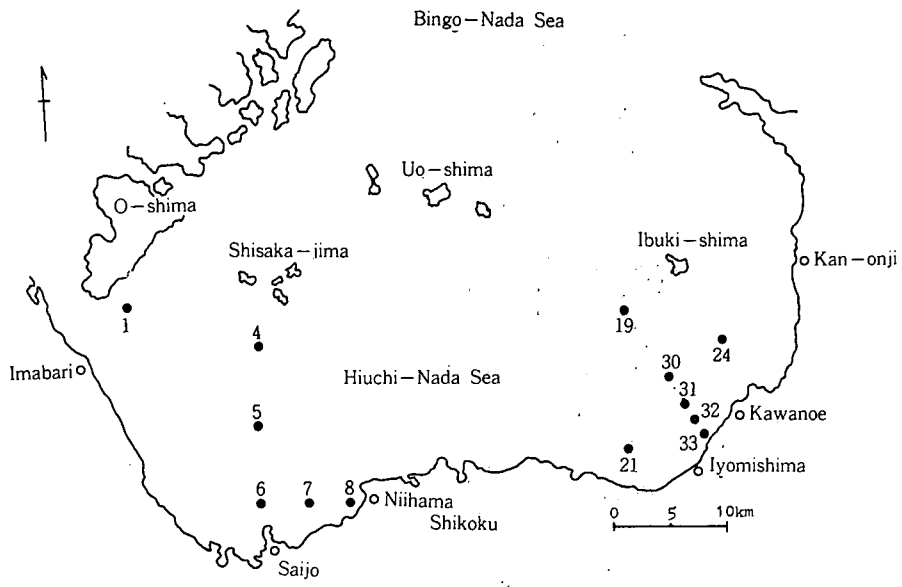

Fig. 1. Location of sampling stations in Hiuchi-Nada Sea.

A large amount of industrial and domestic drainage is being discharged into the sea: from the petrochemical plants and the textile industry into the western part, and from the paper and pulp industry into the eastern part.

Collection of samples Samples were collected at stations shown in Fig. 1 on August, 8,1973, December 23,1973, August 28,1975, and November 28,1975. The water samples for determination of inorganic nutrients, chemical oxygen demand (COD), and the dissolved B group vitamins were immediately filtered through a HA Millipore filter (pore size: 0.45 μm) and the filtrate was kept frozen in acid-cleaned bottles until the time of analysis. Particulate matter collected on the filter was used for the determination of the particulate B group vitamins. The sediment samples were also kept frozen until the time of analysis.

Vitamin assays The amounts of the B group vitamins in water and sediments were determined by microbiological assay methods. Vitamin B_{12} was assayed with *Euglena gracilis* strain z[10], thiamine with *Cryptococcus albidus* (Saito) Skinner[11], and biotin with *Achromobacter* sp. strain yH-51[12].

Dissolved vitamins in the water were determined for the filtrate, and particulate vitamins for the extract from materials left on the filter. Particulate materials for the vitamin B_{12} assay were extracted at 100℃ for 30 minutes in 0.15% potassium cyanide solution[13], those for the thiamine assay were hydrolyzed at pH 4.5 for 12 hours by Takasiastase after extraction at 100℃ for 30 minutes in 0.1 N hydrochloric acid solution[7], and those for the biotin assay were extracted at 120℃ for 30 minutes in 1 N sulfuric acid solution.[7]

The sediment samples for the vitamin B_{12} assay were extracted at 100℃ for 30 minutes

Table 1. *Concentrations of the dissolved vitamins and some environmental factors in the water of Hiuchi-Nada Sea (1973)*

Date	St.	Depth	Tr.	Sampling Depth	Temp.	Cl	DO		COD	Vitamin B_{12}	Thiamine	Biotin	Total aerobic heterotrophs
		m	m	m	°C	‰	mg/l	Satu.%	mg/l	ng/l	ng/l	ng/l	CFU/ml
Aug. 8 1973	4	32	5.0	0	28.4	17.61	11.37	168	1.46	5.00	216	10.4	1.2×10^4
				31	25.8	16.47	—	—	1.51	3.40	32	6.4	1.5×10^3
	6	11	1.9	0	28.9	17.61	13.42	200<	1.93	3.08	108	26.5	6.2×10^4
				10	28.6	19.06	6.63	100	1.87	8.52	196	15.5	7.6×10^4
	8	9	1.4	0	29.0	15.94	13.66	200<	1.62	5.20	424	22.3	2.2×10^5
				8	28.0	17.71	6.13	90	1.69	7.04	201	13.5	9.5×10^5
	19	19	10.6	0	28.9	17.68	9.38	140	1.71	3.16	76	15.5	4.7×10^3
				18	26.3	16.33	—	—	1.51	2.84	37	4.1	1.3×10^3
	31	24	8.6	0	28.4	17.40	9.49	140	2.61	5.32	54	16.9	1.8×10^5
				23	28.5	17.25	—	—	1.88	4.20	19	12.7	8.5×10^4
	33	14	2.9	0	28.5	17.40	8.46	125	1.80	4.84	108	18.6	2.3×10^5
				13	25.7	18.07	3.54	45	1.57	2.64	73	8.6	2.8×10^4
Dec. 23 1973	4	37	3.5	0	12.7	8.73	8.04	81.6	—	2.76	20	3.2	3.6×10^3
				36	13.2	18.42	8.05	92.4	—	4.36	7	0.2	1.1×10^3
	8	10	2.9	0	10.9	15.08	8.33	87.8	—	2.96	35	5.4	3.0×10^4
				9	10.9	18.40	8.29	91.0	—	4.00	27	4.3	5.0×10^4
	19	23	5.5	0	11.6	16.55	8.52	92.7	—	1.12	0	5.9	3.8×10^3
				22	11.8	17.75	8.25	91.5	—	3.24	11	5.8	2.6×10^3
	33	14	2.4	0	10.9	14.34	8.35	87.2	—	0.68	12	4.8	3.9×10^4
				13	10.8	14.71	8.25	86.4	—	2.24	193	2.7	3.4×10^4

in 0.15% potassium cyanide solution[13], those for the thiamine assay at 100℃ for 30 minutes in 0.2 N sulfuric acid solution[14], and those for the biotin assay at 120℃ for 30 minutes in 0.2 N sulfuric acid solution.[14]

All glassware used for vitamin assays was cleaned in chromic-sulfuric acid solution, rinsed with distilled water, and then baked at 200℃ for three hours.

Enumeration of aerobic heterotrophs The number of aerobic total heterotrophic bacteria was estimated by the most probable number (MPN) method or spread plate counting method in terms of colony forming units (CFU) using modified ZoBell's 2216E medium.[15] Media inoculated were incubated at 22℃ for two weeks, aud then MPN／m*l* or CFU／m*l* was determined.

Measurement of other chemical properties Chlorinity was measured by a salinometer model MC5／2 (E. S. LTD). Dissolved oxygen (DO) was determined by the azide modification of Winkler's method.[16] Chemical oxygen demand (COD) was estimated from the amount of potassium permanganate consumed by the alkaline reaction.[16] Ammonium nitrogen was determined by the indophenol method,[17] nitrite nitrogen by the GR method,[17] nitrate nitrogen by the cadmium-copper reduction method,[17] phosphate phosphorous by the heteropoly blue method,[17] and sulfide sulphur by the methylene blue reaction method.[18] Dissolved inorganic nitrogen (DIN) represents the sum of ammonium, nitrate, and nitrite nitrogen.

Results

Distribution of the B group vitamins in the water and the other chemical and physical properties of the water are shown in Table 1 (in 1973) and Table 2 and 3 (in 1975). Mean concentrations of each vitamin and its 95% confidence intervals are given in Table 4 (in 1973) and Table 5 (in 1975).

Water quality Transparency of the water was generally below 6.0 m except at the offshore stations. Especially at the inshore stations it was low because of suspended matter from industrial wastes.

Stratification of water formed in summer; dissolved oxygen was hypersaturated near the surface but was far from saturation near the bottom. DIN levels were generally low, ranging from 0.031 to 0.084 mg／*l* with a mean of 0.056 mg／*l*. The content of PO_4^{3-}-P ranged from 14.1 to 42.6 μg／*l* (mean: 25.1 μg／*l*). COD values ranged from 0.52 to 2.61 mg／*l* (mean: 1.35 mg／*l*). Total heterotrophic bacteria ranged from 10^3 to 10^5 MPN／m*l* or CFU／m*l*.

Vitamins in water Concentration of dissolved vitamin B_{12} ranged from 0.68 to 15.28 ng／*l*, but it was ordinarily below 8.52 ng／*l* except for unusually high concentration (15.28 ng／*l*) at St. 24 in December, 1975. The difference between dissolved vitamin B_1 content in summer and winter was not significant, and the mean value was 3.72 ng／*l* from all available data. Concentration of dissolved thiamine ranged from 0 to 730 ng／*l* (mean: 145 ng／*l*) and that of biotin ranged from 0.2 to 34.7 ng／*l* (mean: 10.3 ng／*l*).

Table 2. *Concentrations of some environmental factors in the water of Hiuchi-Nada Sea (1975)*

Date	St.	Depth	Transparency	Sampling depth	Temp.	Cl	DO		DIN	PO_4^{3-}-P	COD	Total aerobic heterotrophs
		m	m	m	°C	‰	mg/l	Satu.%	μg/l	μg/l	mg/l	MPN/ml
Aug. 28, 1975	1	34	5.2	0	27.4	17.40	7.48	108.3	78.7	40.6	1.14	5.2×10^3
				33	26.4	17.57	6.02	85.8	72.1	35.8	1.09	3.4×10^3
	4	33	6.1	0	27.4	17.04	7.59	109.4	67.6	22.4	0.57	6.6×10^3
				32	26.8	17.47	5.09	73.0	30.9	22.7	0.52	3.6×10^3
	6	12	2.5	0	28.1	17.07	8.64	126.2	30.8	36.8	1.55	1.2×10^5
				11	26.3	17.45	4.03	57.3	41.6	33.9	1.06	2.8×10^3
	7	16	4.5	0	28.1	16.81	8.77	127.7	31.1	32.6	2.09	8.0×10^6
				15	26.7	17.32	5.13	73.3	61.6	42.6	1.13	6.0×10^4
	21	19	6.5	0	28.6	17.03	10.56	155.3	33.7	22.4	0.52	1.0×10^5
				18	26.5	17.19	4.28	60.8	38.6	24.0	1.16	4.0×10^3
	24	24	5.0	0	28.8	17.01	10.06	148.7	41.0	32.0	0.85	2.0×10^5
				23	26.6	17.30	3.93	56.0	46.1	26.2	1.25	4.2×10^5
Nov. 28, 1975	1	40	—	0	17.8	17.93	7.14	88.5	69.9	14.7	1.15	2.4×10^4
				41	17.8	17.92	7.11	88.1	52.2	21.4	0.76	4.0×10^4
	4	36	5.5	0	16.8	17.89	8.18	99.5	57.3	21.8	1.48	7.0×10^3
				35	17.5	17.81	7.39	90.9	64.5	19.2	1.24	5.4×10^3
	6	13	3.9	0	15.9	17.87	8.32	99.6	67.8	16.0	1.30	8.0×10^4
				12	17.0	17.88	7.45	91.0	56.6	18.2	1.26	1.0×10^4
	7	18	3.7	0	16.6	17.94	7.20	87.4	83.3	19.5	1.24	1.4×10^4
				17	17.0	17.85	7.12	86.9	74.8	26.9	1.33	2.4×10^3
	21	20	8.5	0	16.6	17.61	7.91	95.6	44.7	18.6	1.32	3.0×10^3
				19	16.5	17.77	7.37	89.1	77.7	16.6	0.98	8.0×10^2
	24	23	6.0	0	16.4	17.78	7.74	93.4	60.8	24.0	1.66	1.8×10^5
				22	16.7	17.70	7.27	88.1	47.5	14.1	1.18	1.4×10^4

Table 3. *Concentrations of the dissolved and particulate vitamins in the water of Hiuchi-Nada Sea (1975)*

Date	Station	Depth	Vitamin B_{12}			Thiamine			Biotin		
			Dissolved	Particulate	P/D	Dissolved	Particulate	P/D	Dissolved	Particulate	P/D
		m	ng/l	ng/l	%	ng/l	ng/l	%	ng/l	ng/l	%
Aug. 28, 1975	1	0	2.72	3.76	138.2	293	25.0	8.5	19.7	0.17	0.9
		33	2.20	2.00	90.9	202	13.8	6.8	22.8	0.43	1.9
	4	0	2.76	2.54	92.0	219	10.5	4.8	19.9	0.37	1.9
		32	3.52	2.31	65.6	172	19.6	11.4	34.7	1.44	4.1
	6	0	5.64	6.37	112.9	416	16.7	4.0	12.9	1.93	15.0
		11	3.80	5.68	149.5	166	9.4	5.7	9.6	0	—
	7	0	2.92	3.62	124.0	420	11.8	2.8	6.4	0.53	8.3
		15	2.76	2.89	104.7	267	8.5	3.2	13.1	1.31	10.0
	21	0	3.16	4.72	149.4	434	9.9	2.3	12.3	1.13	9.2
		18	3.56	2.52	70.8	108	7.6	7.0	25.8	5.10	19.8
	24	0	2.27	3.18	116.9	730	11.3	1.5	10.2	0.65	6.4
		23	3.24	1.84	56.8	376	17.6	4.7	15.5	1.12	7.2
Nov. 28, 1975	1	0	3.03	0.61	20.2	66.8	5.4	8.1	4.1	0.11	2.7
		41	2.32	0.73	31.5	36.0	4.5	12.5	1.5	0.10	6.7
	4	0	1.66	2.43	146.4	73.2	8.0	10.9	2.6	0.14	5.4
		35	0.80	1.28	160.0	68.9	6.9	10.0	2.8	0.22	7.9
	6	0	1.73	6.93	400.6	82.9	13.5	16.3	2.8	0.25	8.9
		12	2.66	4.21	158.3	71.1	26.4	37.1	2.0	0	—
	7	0	2.23	3.78	169.5	38.2	7.1	18.6	2.4	0.13	5.4
		17	3.67	2.33	63.5	34.0	8.0	23.5	4.2	0.08	1.9
	21	0	3.66	2.69	73.5	51.0	8.5	16.7	1.8	0.09	5.0
		19	3.81	8.13	213.4	62.5	9.6	15.4	7.8	0.24	3.1
	24	0	15.28	4.75	31.1	68.9	8.4	12.2	5.7	0.16	2.8
		22	7.64	3.10	40.6	89.4	7.4	8.3	7.1	0.04	0.6

The seasonal and regional variation of thiamine content was similar to that of biotin: both vitamins were more abundant in summer than in winter, more abundant in the inshore than in the offshore stations, and more abundant it the surface water than in the bottom water.

Content of particulate vitamin B_{12} in the water ranged from 0.61 to 8.13 ng/l (mean: 3.43 ng/l). The difference between vitamin B_{12} content in summer and winter, and that among the stations were not significant. The ratio of particulate to dissolved vitamin B_{12} content, P/D ratio (%), was fairly high; it ranged from 20.2 to 400.6% and exceeded 100% in more than half of the samples (mean: 116%). Content of particulate thiamine was 4.5-26.4 ng/l (mean: 11.5 ng/l) and varied seasonally the same as dissolved thiamine: higher in summer than in winter. Its P/D ratio (%) was

Table 4. *The mean and its 95% confidence intervals of the concentration of dissolved vitamins in the water of Hiuchi-Nada Sea (1973)*

Vitamins	Date	Mean ng/l	Range * ng/l
Vitamin B_{12}	Aug. 30	4.60	3.46 — 5.74
	Dec. 23	2.67	1.60 — 3.74
Thiamine	Aug. 30	129	55.3 — 203
	Dec. 23	38.1	0 — 91.3
Biotin	Aug. 30	14.3	10.2 — 18.4
	Dec. 23	4.0	2.4 — 5.6

* Range represents the 95% confidence intervals of the mean.

Table 5. *The mean and its 95% confidence intervals of the concentration of dissolved and particulate vitamins in the water of Hiuchi-Nada Sea (1975)*

Vitamins	Dissolved or Particulate	Date	Mean ng/l	Range * ng/l
Vitamin B_{12}	Dissolved	Aug. 28	3.21	2.63 — 3.79
		Nov. 28	4.04	1.54 — 6.54
	Particulate	Aug. 28	3.45	2.53 — 4.37
		Nov. 28	3.41	1.93 — 4.89
Thiamine	Dissolved	Aug. 28	317	209 — 425
		Nov. 28	61.9	50.3 — 73.5
	Particulate	Aug. 28	13.5	10.2 — 16.8
		Nov. 28	9.48	5.81 — 13.2
Biotin	Dissolved	Aug. 28	16.9	11.8 — 22.0
		Nov. 28	3.73	2.4 — 5.1
	Particulate	Aug. 28	1.18	0.32 — 2.04
		Nov. 28	0.13	0.08 — 0.18

* Range represents the 95% confidence intervals of the mean.

Table 6. *Contents of the vitamins and some environmental factors in the sediments of Hiuchi-Nada Sea (1973)*

Date	Station	Depth	Temp.	Moisture	IL	Sulfide	Vitamin B_{12}	Thiamine	Biotin	Total aerobic hetorotrophs
		m	°C	%	%	mg/g *	ng/g *	ng/g *	ng/g *	CFU/g *
Aug. 30, 1973	4	32	25.8	60.1	9.2	0.02	17.3	11	2.9	7.6×10^5
	5	16	26.1	60.9	9.7	0.02	24.6	77	2.6	1.2×10^6
	6	11	26.6	62.2	9.0	0.02	53.0	0	3.2	1.9×10^7
	7	12	26.3	68.4	11.6	0.05	43.2	76	2.0	1.3×10^7
	8	9	26.1	65.4	10.0	0.06	28.9	12	1.6	3.0×10^6
	19	19	24.5	75.0	15.7	0.07	15.8	52	3.6	4.5×10^5
	30	23	24.4	77.0	14.7	0.04	18.0	14	3.0	1.5×10^6
	31	24	24.4	73.6	21.6	0.08	30.4	12	2.7	1.0×10^6
	32	18	25.2	57.7	8.9	0.07	47.2	0	2.2	1.4×10^7
	33	14	25.5	70.9	26.2	0.54	95.6	48	2.4	3.0×10^7
Dec. 23, 1973	4	37	13.2	48.1	7.8	0.03	—	0	1.3	2.3×10^5
	6	12	13.8	62.9	9.8	0.17	—	38	2.2	1.9×10^5
	8	10	11.6	64.4	9.4	0.11	—	115	4.4	5.4×10^6
	19	23	12.8	72.1	12.0	0.07	—	12	1.7	1.7×10^5
	31	14	12.0	69.6	11.6	0.08	—	48	3.1	9.0×10^4

* Values per wet weight are represented.

Table 7. *Contents of the vitamins and some environmental factors in the sediments of Hiuchi-Nada Sea (1975)*

Date	St.	Depth	Temp.	Moisture	IL	COD	Sulfide	Vitamin B_{12}	Thiamine	Biotin	Total aerobic heterotrophs
		m	°C	%	%	mg/g *	mg/g *	ng/g *	ng/g *	ng/g *	MPN/g *
Aug. 28, 1975	1	34	—	15.1	—	—	0.01	8.85	83	1.1	1.6×10^6
	4	33	26.2	48.4	8.2	3.85	0.06	53.2	216	3.3	8.6×10^5
	6	12	26.3	50.0	6.0	3.02	0.08	32.8	363	2.4	2.0×10^6
	7	16	26.2	64.9	9.1	3.04	0.12	61.4	379	2.8	5.0×10^5
	21	19	25.6	68.2	11.7	3.41	0.11	71.8	332	2.1	2.6×10^5
	24	24	25.3	73.3	13.2	3.77	0.11	87.9	216	1.2	8.0×10^6
Nov. 28, 1975	4	36	—	49.4	6.9	3.63	0.12	87.0	136	1.6	4.8×10^6
	6	13	16.6	63.1	8.6	2.99	0.17	110	150	1.3	9.6×10^6
	7	18	16.9	65.9	8.9	3.04	0.14	124	160	1.3	7.6×10^6
	21	20	17.3	69.1	9.9	2.19	0.12	117	90	1.2	7.8×10^6
	24	23	15.8	72.6	10.3	2.95	0.15	76.5	114	1.1	7.4×10^6

* Values per wet weight are represented.

small, 1.5-37.1% (mean: 11%), thus, particulate thiamine was about one tenth of the dissolved level. Particulate biotin contained 0-5.10 ng/l in the water and was higher in summer than in winter (mean: 0.66 ng/l). Biotin content in particulate matter was low, and its content in the water was less than one tenth of the dissolved biotin for almost all the samples.

Distribution of the B group vitamins in sediments and other chemical and physical properties of sediments are given in Table 6 and, 7, and the 95% confidence intervals of the vitamin contents are shown in Table 8.

Table 8. *The mean and its 95% confidence intervals of the vitamin contents in the sediments of Hiuchi-Nada Sea*

Vitamins	Date	Mean ng/g wet wt.	Range* ng/g wet wt.
Vitamin B_{12}	1973	37.4	20.1 — 54.7
	1975	75.5	51.8 — 99.2
Thiamine	1973	34.3	15.1 — 53.5
	1975	204	131 — 277
Biotin	1973	2.59	2.14 — 3.04
	1975	1.76	1.25 — 2.27

* Range represents the 95% confidence intervals of the mean.

Quality of sediments The value of IL ranged from 6.0 to 26.2% (mean: 11.2%), that of COD ranged from 2.19 to 3.85 mg/g wet wt. (mean: 3.19 mg/g wet wt.), and sulfide content ranged from 0.01 to 0.54 mg/g wet wt. (mean: 0.10 mg/g wet wt.). These values were generally larger in the eastern area than in the western area, moreover they were larger at the inshore stations than at the offshore stations. Aerobic heterotrophic bacteria were distributed from 10^5 to 10^7 MPN (or CFU) /g wet wt. in the sediments. At the inshore stations, heterotrophs were usually abundant: 10^6-10^7 MPN (CFU) /g wet wt. From these results the sediments in the inshore regions of Hiuchi-Nada Sea were found to be considerably polluted by organic waste waters.

Vitamins in sediments The content of vitamin B_{12} in sediments ranged from 8.85 to 124 ng/g wet wt. (mean: 3.19 ng/g wet wt). Its content was larger in summer than in winter, and also larger at the inshore stations than at the offshore stations. Thiamine content ranged from 0 to 379 ng/g wet wt. and the range of variation was the widest in the three vitamins. Seasonal and regional distribution of thiamine in sediments was similar to that of vitamin B_{12}. Biotin content in sediments ranged from 1.1 to 4.4 ng/g wet wt. (mean: 2.24 ng/g wet wt.), and its variation was the smallest of the three. Regional distribution of biotin was similar to the other vitamins but its seasonal variation was not significant.

Discussion

The present study indicates that the DIN levels in the water of Hiuchi-Nada Sea were close to the critical level that permits the occurrence of red tides in coastal waters: viz., 0.1 mg/l for DIN.[19] Furthermore, the levels of PO_4^{3-}-P and COD were found to be above the critical level (PO_4^{3-}-P: 15 μg/l and COD: 1 mg/l)[19] at almost all stations. Dissolved oxygen was hypersaturated near the surface water but was far from saturation in the bottom waters in summer. Total heterotrophic bacteria were also abundant in the water. These environmental factors characterize Hiuchi-Nada Sea to a eutrophic water according to Yoshida's definition.[20]

The present results reveal that concentrations of dissolved vitamin B_{12} in the water of Hiuchi-Nada Sea are somewhat lower than those of Tokyo Bay (23.2-73.2 ng/l),[21] Ise Bay (2.4-29.3 ng/l),[22] Kagoshima Bay (0-28.73 ng/l),[23] Uranouchi Inlet (0-56.1 ng/l),[24] Nomi Inlet (0-45.0 ng/l),[24] and Susaki Harbor (1.54-56.0 ng/l),[24] but that they are generally similar to those of Sagami Bay (0-14.3 ng/l),[7] Katagami Inlet (0.1-16.1 ng/l),[25] Okayama Coastal Region (0-9.8 ng/l),[26] Fukuyama Coastal Region (0.42-6.43 ng/l),[27] and Harima-Nada Sea (0-25 ng/l).[28] Dissolved thiamine concentrations in Hiuchi-Nada Sea are also comparable to those in Tokyo Bay,[21] Ise Bay,[22] Ago Bay,[29] and Hiuchi-Nada Sea (by Okaichi),[29] and dissolved biotin concentrations are similar to those of Sagami Bay.[7] Consequently, these vitamin contents in Hiuchi-Nada Sea were generally close to those in other coastal waters where red tides frequently occur.

Many red tide flagellates require vitamin B_{12} and occasionally thiamine for their growth. The critical level of each vitamin necessary to maximize their growth is known to be 10-20 ng/l for vitamin B_{12}[30-33] and about 200 ng/l for thiamine.[32] Concentrations of dissolved thiamine in Hiuchi-Nada Sea were found to be above this level in summer, 1975 but far below in winter, 1973 and 1975. On the other hand, concentrations of dissolved vitamin B_{12} were found not to reach this level any time during the year in 1973 and 1975. Consequently, the level of dissolved Vitamin B_{12}, not but that of dissolved thiamine, in the water could limit the development of a red tide by flagellates in Hiuchi-Nada Sea during summer.

Vitamin B_{12} was found to occur abundantly in particulate form in eutrophic seawater. This suggests that vitamin B_{12}-rich suspended organic solids, e.g., plankton, detritus, and organic pollutants, which were widely spreaded in such regions, could serve as an important source of dissolved vitamin B_{12}.

The present study indicates that the B group vitamins accumulated in bottom sediments more abundantly than in the water: about 10,000 fold for vitamin B_{12}, about 1,000 fold for thiamine, and about 1,000 fold for biotin. Thus, the sediments, in which each vitamin is abundantly contained, may be an important source of dissolved vitamins in the water.

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