Distribution of Vitamin B₁₂, Thiamine, and Biotin in the Water of Harima-Nada Sea

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Distribution of both dissolved and particulate vitamin B₁₂ and thiamine, and dissolved biotin in the water of Harima-Nada Sea were determined by microbiological assay methods between 1978 and 1981, and the regulatory influences on the distribution of these vitamins in the region were discussed.

The ranges in dissolved vitamin B₁₂, thiamine, and biotin were 1.38–25.0 ng/l, 3.40–2140 ng/l, and 1.51–33.2 ng/l, respectively. The levels of particulate vitamin B₁₂ and thiamine were 1.60–20.1 ng/l and 2.40–175 ng/l, respectively. These were generally greater in the northern part, where industrial and domestic wastes were loaded, than in the southern part. The contents of both dissolved and particulate vitamins in Harima-Nada Sea were found to be generally comparable to the other eutrophied coastal waters.

Both particulate vitamin B₁₂ and thiamine, and dissolved biotin were significantly greater in the summer than in the winter in the offshore stations. The level of particulate vitamin B₁₂ was far above that of dissolved vitamin B₁₂ in almost all the samples in the summer but not in the winter. No significant difference could be found between the summer levels of dissolved vitamin B₁₂ or thiamine and its winter levels.

It was suggested that the levels of both the dissolved and the particulate vitamins in eutrophied sea waters, such as Harima-Nada Sea, may be closely connected with the growth of phytoplankters and that they may be affected significantly by the discharge of various kind of waste waters.

It is known that many marine phytoplankters require B group vitamins for growth: most commonly vitamin B₁₂, thiamine, and biotin alone or in various combinations. Members of the Euglenophyceae, Cryptophyceae, Dinophyceae, and Chrysophyceae, including red tide organisms, have been found to show a clear predominance of auxotrophic species.^{1–5)} Therefore, it has been suggested that essential vitamins in seawater could not only have a regulatory influence on seasonal variation of phytoplankton communities but also affect the initiation of red tides.^{6–8)}

Harima-Nada Sea has been eutrophied for the last twenty years by inflowing of domestic and industrial waste waters. In this district red tides have occurred during the warm seasons for recent years, occasionally causing serious injury to fisheries resources.

The present paper describes distribution of the dissolved and particulate vitamins in the water of Harima-Nada Sea, and a regulatory influence on the distribution of these vitamins is discussed.

Materials and Methods

Water Region Investigated

Harima-Nada Sea is located in the central part of the Seto Inland Sea in Japan. The sea has an area of 3,426 km², a mean depth of 25.6 m, and a volume of 88.7 km³. Its topography is nearly enclosed as illustrated in Fig. 1. In the north littoral districts there are many factories and plants of steel industry, machinery, chemical industry, petrochemical industry, food industry, and others. A large amount of industrial, domestic, and agricultural waste waters have been discharged into the sea.

Collection of Samples

Water samples were collected at offshore stations between 1978 and 1979, as shown in Fig. 1, and at inshore stations between 1980 and 1981, as shown in Figs. 2–4. The samples for vitamin assays were filtered through an HA Milipore filter (pore

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Fig. 1. Location of offshore stations in Harima-Nada Sea.

Fig. 2. Location of inshore stations in the estuary of Aioi Bay.

Fig. 3. Location of inshore stations in the estuary of Himeji District.

Fig. 4. Location of inshore stations in the estuary of Harima District.

size: $0.45 \mu\text{m}$), then both filters and filtrates were kept frozen at -20°C until analyzed.

Vitamin Assays

Contents of the vitamins were determined by microbiological assay methods. Vitamin B_{12} was assayed with *Euglena gracilis* strain z (Hayashi

and Kamikubo),⁹⁾ thiamine with *Cryptococcus albidus* (Natarajan and Dugdale),¹⁰⁾ biotin with *Achromobacter* sp. strain yH-51 (Ohwada).¹¹⁾ Dissolved vitamins were determined for the filtrate, and particulate vitamins were determined for the extract from suspended matters on the filter. Particulate materials for vitamin B_{12} were ex-

Table 1. Concentration of dissolved vitamins and occurrence of aerobic heterotrophic bacteria in offshore stations of Harima-Nada Sea (June 22-23, 1978)

St.	Depth m	Sampling depth m	Vitamin B ₁₂ ng/l	Thiamine ng/l	Biotin ng/l	Aerobic heterotrophs MPN/ml
3	28	0	7.89	236	10.3	1.1×10^5
		10	7.36	124	6.52	4.9×10^3
		20	6.83	81.3	8.40	2.3×10^3
		26	8.00	120	7.80	4.9×10^2
5	39	0	9.13	208	10.7	1.3×10^5
		10	6.70	8.0	8.27	1.4×10^4
		20	6.96	17.0	5.04	6.9×10^3
		30	6.61	20.5	7.40	4.9×10^2
6	36	37	6.87		7.63	4.9×10^2
		0	9.71	56.8	8.44	2.2×10^5
		10	8.75	12.5	14.3	1.3×10^5
		20	6.40	46.6	8.92	1.3×10^5
7	21	24	5.97	46.6	9.36	1.7×10^3
		0	10.4	122	14.3	2.4×10^5
		10	5.87	45.5	8.92	3.3×10^4
		19	6.61	5.7	9.36	3.3×10^3
8	28	0	8.26	357	15.6	2.4×10^5
		10	6.70	200	9.64	4.9×10^4
		20	6.87	17.0	8.36	3.3×10^3
		26	7.65	4.5	8.95	2.3×10^3
12	27	0	9.07	100	8.11	1.1×10^4
		10	7.57	35.2	7.87	7.9×10^3
		20	7.47	25.0	7.83	7.9×10^3
		25	7.15	29.5	7.13	1.3×10^4
13	40	0	7.83	97.7	7.65	3.3×10^3
		10	7.56	8.0	6.87	7.9×10^3
		20	7.56		8.38	4.9×10^3
		30	6.61	9.1	6.99	3.3×10^3
15'	43	38	7.06	3.4	5.37	2.3×10^3
		0	6.51	131	9.08	1.7×10^3
		10	6.51	93.4	8.28	4.9×10^2
		20	5.33	81.2	7.88	2.3×10^3
17	44	30	5.76	62.3	7.60	3.3×10^3
		41	5.55	111	8.12	1.3×10^3
		0	7.47	200	7.24	4.9×10^4
		10	7.57	122	9.28	1.4×10^4
17	44	20	7.15	81.3	5.44	1.7×10^3
		30	6.19	93.6	7.88	7.9×10^2
		42	6.19	97.2	6.92	4.9×10^2

tracted at 100°C for 30 min in 0.15% potassium cyanide solution,¹²⁾ those for thiamine were hydrolyzed at pH 4.5 for 12 h by Takadiastase after extraction at 100°C for 30 min in 0.1 N hydrochloric acid solution.⁷⁾ All glassware used for vitamin assays was cleaned in chromic-sulfuric acid solution, rinsed with distilled water, and baked at 200°C for 3 h.

Enumeration of Aerobic Heterotrophs

The bacteriological treatments were made within one hour after sampling. The number of aerobic total heterotrophs was estimated by the most probable number (MPN) method using the broth of modified ZoBell's 2216E medium.¹³⁾ Media inoculated were incubated at 22°C for two weeks, and then the MPNs were determined.

Table 2. Concentrations of dissolved and particulate vitamins and occurrence of aerobic heterotrophs in offshore stations of Harima-Nada Sea (June 23–26, 1979)

St.	Depth m	Sampling depth m	Vitamin B ₁₂			Thiamine			Biotin Dissolved ng/l	Aerobic heterotrophs MPN/ml
			Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l	Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l		
1	19	0	3.74 (27.9)	9.67 (72.1)	13.4	271 (83.7)	52.8 (16.3)	324	12.6	2.4 × 10 ⁴
		10	6.90 (42.2)	9.45 (57.8)	16.4	113 (64.4)	62.4 (35.6)	175	11.4	1.5 × 10 ⁴
		18	5.42 (30.2)	12.5 (69.8)	17.9	175 (71.5)	69.6 (28.5)	245	6.55	9.3 × 10 ³
2	11	0	6.25 (32.1)	13.2 (67.9)	19.5	418 (71.5)	167 (28.5)	585	12.8	2.0 × 10 ⁴
		5	5.57 (26.1)	15.8 (73.9)	21.4	261 (66.6)	131 (33.4)	392	10.2	2.0 × 10 ⁴
		10	5.12 (28.4)	12.9 (71.6)	18.0	196 (87.5)	28.0 (12.5)	224	7.10	4.3 × 10 ⁴
3	26	0	3.86 (23.2)	12.8 (76.8)	16.7	251 (83.7)	48.8 (16.3)	300	11.0	9.3 × 10 ³
		10	5.36 (24.0)	17.0 (76.0)	22.4	170 (77.4)	49.6 (22.6)	220	11.0	2.4 × 10 ³
		25	8.35 (33.9)	16.3 (66.1)	24.7	135 (49.5)	138 (50.5)	273	6.85	2.4 × 10 ³
4	40	0	4.08 (20.1)	16.2 (79.9)	20.3	135 (50.9)	130 (49.1)	265	8.80	4.3 × 10 ²
		10	3.03 (15.8)	16.1 (84.2)	19.1	181 (65.9)	93.6 (34.1)	275	9.53	2.4 × 10 ³
		39	5.12 (21.4)	18.8 (78.6)	23.9	121 (83.4)	24.0 (16.6)	145	5.40	9.3 × 10 ²
5	38	0	11.8 (39.6)	18.0 (60.4)	29.8	215 (85.4)	36.8 (14.6)	252	6.50	1.5 × 10 ³
		10	15.8 (46.3)	18.3 (53.7)	34.1	155 (82.9)	32.0 (17.1)	187	6.80	9.3 × 10 ²
		37	7.76 (28.9)	19.1 (71.1)	26.9	75.5 (30.6)	171 (69.4)	247	8.03	3.9 × 10 ³
6	27	0	3.84 (16.0)	20.1 (84.0)	23.9	96.0 (75.5)	31.2 (24.5)	127	6.67	2.1 × 10 ³
		10	6.43 (30.0)	15.0 (70.0)	21.4	179 (80.3)	44.0 (19.7)	223	6.60	9.3 × 10 ²
		26	14.7 (75.7)	4.71 (24.3)	19.4	134 (79.6)	34.4 (20.4)	168	5.75	4.3 × 10 ³
7	20	0	5.26 (43.5)	6.84 (56.5)	12.1	200 (85.6)	33.6 (14.4)	234	7.25	3.9 × 10 ⁴
		10	5.51 (47.3)	6.15 (52.7)	11.7	112 (80.5)	27.2 (19.5)	139	5.45	4.3 × 10 ³
		19	5.38 (75.5)	4.31 (44.5)	9.69	125 (83.4)	24.8 (16.6)	150	5.50	4.3 × 10 ³
8	24	0	5.57 (38.8)	8.80 (61.2)	14.4	215 (79.8)	54.4 (20.2)	269	11.3	6.4 × 10 ⁴
		10	5.26 (41.8)	7.33 (58.2)	12.6	60.0 (73.5)	21.6 (26.5)	81.6	10.1	
		23	6.38 (50.2)	6.33 (49.2)	12.7	16.0 (28.2)	40.8 (71.8)	56.8	8.60	7.5 × 10 ³
9	10	0	6.25 (25.3)	18.5 (74.7)	24.8	151 (75.3)	49.6 (24.7)	201	12.6	4.6 × 10 ⁴
		5	7.32 (36.2)	12.9 (63.8)	20.2	211 (79.3)	55.2 (20.7)	266	13.0	2.4 × 10 ⁴
		9	12.3 (52.6)	11.1 (47.4)	23.4	171 (83.9)	32.8 (16.1)	204	13.3	1.5 × 10 ⁵
10	13	0	19.9 (53.9)	17.0 (46.1)	36.9	156 (72.0)	60.8 (28.0)	217	13.3	2.1 × 10 ⁴
		5	6.45 (35.1)	11.9 (64.9)	18.4	350 (85.4)	60.0 (14.6)	410	12.7	9.3 × 10 ⁴
		12	6.13 (40.9)	8.85 (59.1)	15.0	135 (74.8)	45.6 (25.2)	181	14.4	1.5 × 10 ⁴
11	21	0	7.00 (40.5)	10.3 (59.5)	17.3	109 (91.3)	10.4 (8.7)	119	10.2	2.4 × 10 ⁴
		10	6.45 (36.8)	11.1 (63.2)	17.6	133 (72.2)	51.2 (27.8)	184	11.4	2.4 × 10 ⁴
		20	6.13 (40.2)	9.10 (59.8)	15.2	156 (85.5)	26.4 (14.8)	182	13.6	4.3 × 10 ⁴
12	29	0	4.66 (36.8)	7.99 (63.2)	12.7		175		10.1	4.6 × 10 ⁴
		10	8.01 (61.0)	5.13 (39.0)	13.1	181 (82.8)	37.6 (17.2)	219	9.35	1.1 × 10 ⁵
		27	11.0 (71.0)	4.50 (29.0)	15.5	95.5 (86.9)	14.4 (13.1)	110	6.25	1.1 × 10 ⁵

* Per cent to the total concentrations.

Table 2. (Continued)

St.	Depth m	Sampling depth m	Vitamin B ₁₂			Thiamine			Biotin Dissolved	Aerobic heterotrophs
			Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l	Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l	ng/l	MPN/ml
13	38	0	6.58 (56.7)	5.02 (43.3)	11.6	225 (70.8)	92.8 (29.2)	318	9.30	9.3×10^3
		10	7.69 (60.1)	5.11 (39.9)	12.8	85.5 (64.4)	47.2 (35.6)	133	8.60	4.3×10^3
		37	18.4 (76.5)	5.65 (23.5)	24.1	120 (97.4)	3.20 (2.6)	123	7.83	2.1×10^4
14	29	0	4.13 (51.8)	3.84 (48.2)	7.97	144 (69.2)	64.0 (30.8)	208	10.5	2.4×10^3
		10	5.16 (47.9)	5.61 (52.1)	10.8	70.5 (44.3)	88.8 (55.7)	159	8.20	9.3×10^3
		27	8.40 (49.8)	8.48 (50.2)	16.9	15.5 (58.1)	11.2 (41.9)	26.7	6.67	2.4×10^3
15	40	0	16.1 (62.4)	9.70 (37.6)	25.8	146 (71.4)	58.4 (28.6)	204	10.7	9.3×10^3
		10	7.16 (45.0)	8.75 (55.0)	15.9	108 (97.8)	2.40 (2.2)	110	9.35	9.3×10^3
		39	8.36 (40.3)	12.4 (59.7)	20.8	46.5 (46.8)	52.8 (53.2)	99.3	8.60	1.1×10^3
16	34	0	7.00 (39.8)	10.6 (60.2)	17.6	61.0 (47.7)	67.0 (52.3)	128	10.2	2.1×10^4
		10	6.96 (38.1)	11.3 (61.9)	18.3	90.5 (77.0)	27.0 (23.0)	118	10.9	4.3×10^3
		33	8.12 (49.5)	8.30 (50.5)	16.4	11.5 (48.9)	12.0 (51.1)	23.5	11.8	9.3×10^3
17	44	0	6.38 (35.5)	11.6 (64.5)	18.0	102 (68.0)	48.1 (32.0)	150	10.6	9.3×10^3
		10	4.01 (28.2)	10.2 (71.8)	14.2	55.5 (70.8)	22.9 (29.2)	78.4	11.8	9.3×10^3
		41	6.83 (21.4)	9.12 (28.6)	16.0	56.5 (56.9)	42.8 (43.1)	99.3	9.76	4.6×10^4
18	36	0	2.36 (17.0)	11.5 (83.0)	13.9	61.2 (32.3)	128 (67.7)	189	10.6	2.4×10^3
		10	4.75 (32.9)	9.68 (67.1)	14.4	74.6 (48.7)	78.5 (51.3)	153	10.8	2.1×10^4
		34	11.5 (63.0)	6.76 (37.0)	18.3	32.5 (72.7)	12.2 (27.3)	44.7	10.6	9.3×10^3
19	34	0	3.24 (21.5)	11.8 (78.5)	15.0	71.5 (45.8)	84.7 (54.2)	156	11.1	7.5×10^3
		10	2.80 (24.2)	8.78 (75.8)	11.6	89.5 (62.5)	53.6 (37.5)	143	10.7	9.3×10^3
		33	7.80 (53.9)	6.66 (46.1)	14.5	41.0 (24.4)	127 (75.6)	168	10.3	2.4×10^3
20	34	0	4.51 (32.9)	9.20 (67.1)	13.7	114 (56.8)	86.7 (43.2)	201	11.0	4.3×10^3
		10	3.18 (23.6)	10.3 (76.4)	13.5	206 (79.4)	53.6 (20.6)	260	6.80	2.4×10^3
		33	4.69 (41.2)	6.70 (58.8)	11.4		127		6.73	2.4×10^3
21	26	0	1.38 (14.5)	8.11 (85.5)	9.49	296 (77.3)	86.7 (22.7)	383	11.1	9.3×10^3
		10	5.35 (39.4)	8.22 (60.6)	13.6	40.5 (39.1)	63.2 (60.9)	104	10.8	1.5×10^3
		24	4.23 (34.6)	8.16 (65.4)	12.5	81.5 (45.6)	97.4 (54.4)	179	11.1	9.3×10^3
22	33	0	3.88 (27.2)	10.4 (72.8)	14.3	34.0 (31.1)	75.5 (68.9)	110	11.4	1.2×10^4
		10	2.97 (25.0)	8.92 (75.0)	11.9	17.0 (16.1)	88.8 (83.9)	106	11.6	7.5×10^3
		32	3.90 (33.0)	7.91 (67.0)	11.8	39.5 (31.5)	85.7 (68.5)	125	8.50	2.1×10^4

* Per cent to the total concentrations.

Table 3. Concentrations of dissolved and particulate vitamins and occurrence of aerobic heterotrophs in offshore stations of Harima-Nada Sea (Nov. 13–16, 1979)

St.	Depth m	Sampling depth m	Vitamin B ₁₂			Thiamine			Biotin Dissolved ng/l	Aerobic heterotrophs MPN/ml
			Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l	Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l		
1	20	0	5.88 (48.3)	6.30 (51.7)	12.2	116 (70.0)	49.6 (30.0)	166	6.23	7.5×10^4
		10	7.38 (44.8)	9.09 (55.2)	16.5	56.0 (69.8)	24.2 (30.2)	80.2	7.91	7.5×10^2
		19	5.38 (46.9)	6.08 (53.1)	11.5	209 (82.3)	44.8 (17.7)	254	7.65	4.3×10^3
2	11	0	5.31 (44.0)	6.76 (56.0)	12.1	64.0 (55.7)	50.8 (44.3)	115	9.41	7.5×10^3
		5	9.90 (54.9)	8.14 (45.1)	18.0	53.5 (60.0)	35.6 (40.0)	89.1	9.88	4.3×10^3
		10	4.85 (46.0)	5.69 (54.0)	10.5	82.5 (67.2)	40.2 (32.8)	123	5.52	4.3×10^3
3	28	0	6.91 (54.7)	5.73 (45.3)	12.6	196 (80.7)	46.8 (19.3)	243	7.39	4.3×10^3
		10	6.07 (52.0)	5.60 (48.0)	11.7	226 (93.2)	16.6 (6.8)	243	5.50	7.5×10^3
		27	6.50 (46.1)	7.61 (53.9)	14.1	443 (95.4)	21.2 (4.6)	464	8.36	2.3×10^3
4	43	0	8.39 (58.2)	6.03 (41.8)	14.4	25.5 (46.3)	29.6 (53.7)	55.1	6.90	7.5×10^2
		10	5.32 (52.1)	4.90 (47.9)	10.2	191 (82.0)	42.0 (18.0)	233	7.78	6.4×10^2
		42	3.02 (63.8)	1.71 (36.2)	4.73	11.0 (27.2)	29.4 (72.8)	40.4	5.90	3.9×10^2
5	39	0	3.39 (64.0)	1.91 (36.0)	5.30	168 (76.2)	52.4 (23.8)	220	9.72	2.3×10^3
		10	2.97 (45.8)	3.52 (54.2)	6.49	400 (93.3)	28.6 (6.7)	429	8.40	9.3×10^3
		38	3.79 (35.1)	7.01 (64.9)	10.8	115 (76.9)	34.6 (23.1)	150	8.25	4.3×10^3
6	26	0	4.76 (58.8)	3.33 (41.2)	8.09	186 (86.1)	30.0 (13.9)	216	8.66	4.3×10^4
		10	2.95 (48.8)	3.10 (51.2)	6.05	163 (84.0)	31.0 (16.0)	194	7.53	1.5×10^4
		25	6.25 (69.1)	2.80 (30.9)	9.05	86.5 (74.1)	30.2 (25.9)	117	7.50	4.3×10^4
7	21	0	5.01 (60.4)	3.29 (39.6)	8.30	84.5 (64.7)	46.2 (35.3)	131	11.7	1.5×10^5
		10	4.63 (69.3)	2.05 (30.7)	6.68	182 (83.6)	35.8 (16.4)	218	10.1	9.3×10^4
		20	4.26 (64.4)	2.35 (35.6)	6.61	136 (88.7)	17.4 (11.3)	153	8.44	4.3×10^4
8	26	0	9.01 (73.4)	3.27 (26.6)	12.3	141 (87.9)	19.4 (12.1)	160	7.40	4.3×10^3
		10	7.14 (73.8)	2.53 (26.2)	9.67	105 (82.0)	23.1 (18.0)	128	10.0	1.5×10^3
		25	16.6 (80.1)	4.12 (19.9)	20.7	130 (77.8)	37.0 (22.2)	167	7.42	2.3×10^3
9	11	0	14.4 (65.5)	7.60 (34.5)	22.0	346 (88.5)	44.8 (11.5)	391	10.7	2.4×10^5
		5	4.12 (43.3)	5.40 (56.7)	9.52	273 (84.8)	48.8 (15.2)	322	10.9	2.3×10^4
		10	7.01 (50.0)	7.02 (50.0)	14.0	322 (88.0)	44.0 (12.0)	366	9.35	1.5×10^4
10	12	0	7.00 (49.4)	7.18 (50.6)	14.2	225 (79.2)	59.2 (20.8)	284	7.76	1.1×10^5
		5	9.20 (57.5)	6.81 (42.5)	16.0	185 (83.4)	36.8 (16.6)	222	7.67	4.6×10^3
		10	11.3 (61.7)	7.02 (38.3)	18.3	275 (86.4)	43.2 (13.6)	318	10.0	$1.1 \times 10^5 <$
11	25	0	8.18 (63.5)	4.70 (36.5)	12.9	90.5 (72.0)	35.2 (28.0)	126	6.90	9.3×10^4
		10	3.10			6.5 (12.7)	44.8 (87.3)	51.3	6.76	2.3×10^4
		24	8.44 (64.1)	4.72 (35.9)	13.2	201 (82.6)	42.4 (17.4)	243	8.42	2.3×10^4

* Per cent to the total concentrations.

Table 3. (Continued)

St.	Depth m	Sampling depth m	Vitamin B ₁₂			Thiamine			Biotin Dissolved ng/l	Aerobic heterotrophs MPN/ml
			Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l	Dissolved ng/l (%)*	Particulate ng/l (%)*	Total ng/l		
12	41	0	3.92 (38.0)	6.40 (62.0)	10.3	106 (77.3)	31.2 (22.7)	137	4.11	4.3×10^4
		10	6.51 (69.8)	2.81 (30.2)	9.32	64.5 (64.2)	36.0 (35.8)	101	6.01	2.3×10^4
		40	9.27 (74.3)	3.20 (25.7)	12.5	202 (81.1)	47.0 (18.9)	249	5.02	2.3×10^4
13	38	0	4.85 (64.0)	2.73 (36.0)	7.58	24.0 (63.8)	13.6 (36.2)	37.6	3.26	4.3×10^3
		10	5.01 (68.1)	2.35 (31.9)	7.36	156 (84.8)	28.0 (15.2)	184	1.51	9.3×10^3
		37	10.2 (82.3)	2.20 (17.7)	12.4	88.0 (72.8)	32.8 (27.2)	121	4.00	4.3×10^3
14	29	0	12.9 (84.9)	2.30 (15.1)	15.2	22.4 (63.6)	12.8 (36.4)	35.2	6.01	9.3×10^3
		10	16.1 (86.1)	2.59 (13.9)	18.7	18.3 (38.9)	28.8 (61.1)	47.1	6.88	1.5×10^4
		28	13.5 (76.2)	4.22 (23.8)	17.7	22.6 (48.5)	24.0 (51.5)	46.6	5.50	9.3×10^3
15	40	0	9.64 (80.7)	2.31 (19.3)	12.0	70.5 (74.0)	24.8 (26.0)	95.3	6.53	9.3×10^2
		10	12.5 (80.0)	3.12 (20.0)	15.6	22.0 (34.6)	41.6 (65.4)	63.6	5.41	2.3×10^2
		39	12.6 (81.9)	2.79 (18.1)	15.4	25.5 (56.0)	20.0 (44.0)	45.5	5.22	9.3×10^2
16	34	0	1.63 (40.4)	2.40 (59.6)	4.03	89.5 (79.4)	23.2 (20.6)	113	5.00	4.3×10^3
		10	5.62 (73.8)	2.00 (26.2)	7.62	29.0 (45.2)	35.2 (54.8)	64.2	5.50	2.1×10^3
		33	4.67 (56.1)	3.65 (43.9)	8.32	44.9 (60.3)	29.6 (39.7)	74.5	4.94	9.3×10^2
17	44	0	3.37 (53.0)	2.99 (47.0)	6.36	30.6 (43.8)	39.2 (56.2)	69.8	6.46	2.3×10^3
		10	4.99 (65.9)	2.58 (34.1)	7.57	32.0 (54.1)	27.2 (45.9)	59.2	6.11	9.3×10^3
		42	4.62 (62.3)	2.80 (37.7)	7.42	32.0 (47.6)	35.2 (52.4)	67.2	6.15	4.3×10^2
18	36	0	3.59 (68.0)	1.69 (32.0)	5.28	69.5 (68.5)	32.0 (31.5)	102	7.32	7.5×10^4
		0	3.63 (64.5)	2.00 (35.5)	5.63	43.5 (59.5)	29.6 (40.5)	73.1	6.20	3.9×10^3
		35	5.14 (56.2)	4.00 (43.8)	9.14	18.5 (39.1)	28.8 (60.9)	47.3	4.59	4.3×10^3
19	35	0	4.62 (63.4)	2.67 (36.6)	7.29	38.9 (58.9)	27.2 (41.1)	66.1	7.91	7.5×10^2
		10	5.32 (69.6)	2.32 (30.4)	7.64	24.9 (43.2)	32.8 (56.8)	57.7	6.75	1.5×10^3
		34	6.52 (67.6)	3.12 (32.4)	9.64	27.1 (51.4)	25.6 (48.6)	52.7	5.74	2.3×10^3
20	30	0	5.76 (63.6)	3.30 (36.4)	9.06	101 (79.3)	26.4 (20.7)	127	6.96	2.3×10^3
		10	5.50 (65.2)	2.93 (34.8)	8.43	145 (83.0)	29.6 (17.0)	175	7.65	4.3×10^3
		29	5.01 (75.8)	1.60 (24.2)	6.61	50.0 (60.4)	32.8 (39.6)	82.8	5.87	2.3×10^3
21	26	0	4.99 (50.9)	4.81 (49.1)	9.80	185 (86.2)	29.6 (13.8)	215	8.40	9.3×10^3
		10	6.68 (70.4)	2.81 (29.6)	9.49	181 (82.5)	38.4 (17.5)	219	6.96	7.5×10^3
		25	6.24 (58.1)	4.50 (41.9)	10.7	27.1 (47.1)	30.4 (52.9)	57.5	7.05	1.5×10^3
22	33	0		5.51		18.5 (32.1)	39.2 (67.9)	57.7	8.28	2.3×10^3
		10		4.55		19.5 (57.5)	14.4 (42.5)	33.9	8.31	2.4×10^4
		32		4.79		40.1 (64.2)	22.4 (35.8)	62.5	6.44	2.3×10^3

* Per cent to the total concentrations.

Results

The vitamin contents and the number of aerobic heterotrophic bacteria in Harima-Nada Sea are shown in Tables 1-3 for offshore stations and Table 4 for inshore stations. By the statistic analysis based on these data, the seasonal, regional, and vertical variations of both dissolved and particulate vitamins were studied and the results are shown in Tables 5-7.

Total Aerobic Heterotrophic Bacteria

The total number of aerobic heterotrophs oc-

curing in the water from offshore stations ranged from 10^2 to 10^8 cells/ml with most samples ranging from 10^3 to 10^4 cells/ml. No apparent differences could be found between counts in summer and those in winter. Aerobic heterotrophs, however, were more abundant in the northern part than in the southern part and frequently least in the central part of the sea.

Vitamin B₁₂ Contents

Dissolved vitamin B₁₂ concentrations in the water were fairly high and ranged from 1.38 to 19.9 ng/l with a mean of 6.87 ng/l in offshore stations and from 4.82 to 25.0 ng/l with a mean of

Table 4. Concentrations of dissolved vitamins in inshore stations of Harima-Nada Sea

Date	St.	Sampling depth m	Vitamin B ₁₂ ng/l	Thiamine ng/l	Biotin ng/l
June 30, 1980	105	0	23.0	2140	18.0
	105	7	6.52	42.7	9.50
	106	0	5.89	154	7.30
	107	0	5.67	80.0	11.7
	107	10	5.99	49.9	10.0
	109	0	6.58	172	11.0
	109	11	7.47	77.7	9.50
	110	0	8.02	48.1	11.6
	110	8	6.68	163	8.30
	112	0	5.06	143	11.2
	112	13.5	6.17	88.9	9.50
	113	0	7.94	148	10.0
	113	14	10.9	52.7	13.5
	114	0	8.53	106	10.3
	114	17.5	8.96	52.0	9.70
	115	0	4.82	84.2	10.7
	115	17	6.46	48.7	8.80
May 20, 1981	201	0	12.6	42.0	5.90
	201	5.5	9.20	73.0	8.90
	202	0	7.80	55.0	9.50
	203	0	14.8	36.0	8.30
	203	7	11.1	46.0	9.60
	204	0	10.6	110	8.70
	205	0	11.5	42.0	7.10
	206	0	10.3	31.0	8.30
	207	0	25.0	45.0	11.1
	208	0	11.9	125	10.0
	208	14	7.80	126	10.4
	209	0	14.8	35.0	8.30
	210	0	12.6	154	33.2
	211	0	14.6	115	14.5
	212	0	15.2	30.0	3.90
	213	0	14.4	48.0	9.40
	213	6	8.30	90.0	7.90
	214	0	10.0	50.0	8.60
	214	3	10.1	36.0	5.80
	214	10	10.3	68.0	5.70
	215	0	10.2	50.0	8.90

Table 5. Statistic analysis for seasonal variation (summer/winter) of vitamin contents in offshore stations of Harima-Nada Sea

Vitamins	Samples	Number of Samples	Vitamin contents		Difference of Means
			Ranges ng/l	Means ng/l	
Vitamin B ₁₂					
Dissolved	Summer (78/79)	105	1.38–19.9	6.93	No* ¹
	Winter (79)	62	1.63–16.6	6.78	
Particulate	Summer (79)	66	3.84–20.1	10.5	P<0.001* ²
	Winter (79)	66	1.60–9.09	4.10	
Total	Summer (79)	66	7.97–36.9	17.3	P<0.001* ²
	Winter (79)	62	4.03–22.0	10.9	
Thiamine					
Dissolved	Summer (78/79)	101	3.40–418	114	No. * ¹
	Winter (79)	66	6.50–443	115	
Particulate	Summer (79)	66	2.40–175	61.4	P<0.001* ²
	Winter (79)	66	12.8–59.2	33.1	
Total	Summer (79)	64	23.5–585	190	P<0.05* ³
	Winter (79)	66	33.9–464	148	
Biotin					
Dissolved	Summer (78/79)	105	5.04–15.6	9.26	P<0.001* ²
	Winter (79)	66	1.51–11.7	7.12	

*¹ Not significant.*² Significant at the 0.1% level.*³ Significant at the 5% level.

10.2 ng/l in inshore stations, for the combined data from all surveys. Particulate vitamin B₁₂ contents ranged from 1.60 to 20.1 ng/l in offshore stations and the mean was 7.32 ng/l. Total vitamin B₁₂ contents, the sum of the dissolved and particulate vitamin content in the water, were also generally high and they ranged from 4.03 to 36.9 ng/l with a mean of 14.2 ng/l in offshore stations.

Each content of dissolved and particulate vitamin B₁₂ in the offshore stations was generally higher in the northern part than in the southern part, and only dissolved one was occasionally highest in the central part. As shown in Table 5, there was no significant difference between observed mean dissolved vitamin B₁₂ levels in summer and those in winter, but the mean contents of both particulate and total vitamin B₁₂ observed in offshore stations were significantly larger in summer than in winter ($P<0.001$). The statistic analysis on the regional variation in the warm seasons, as shown in Table 6, demonstrated clearly that dissolved vitamin B₁₂ concentrations were considerably higher in inshore stations than in offshore stations (significant $P<0.001$). But no significant differences of vertical profiles could be established for dissolved vitamin B₁₂ contents observed in both summer and winter (Table 7).

Particulate vitamin B₁₂ contents were far above that of dissolved ones in almost all of the summer

samples, but not in the winter samples; the ratios of particulate content to the total for vitamin B₁₂ ranged from 23.5 to 85.5% with a mean of 60.5% in summer and from 13.9 to 64.9% with a mean of 38.4% in winter.

Thiamine Contents

Dissolved thiamine concentrations ranged from 3.40 to 443 ng/l in offshore stations and from 30.0 to 2140 ng/l in inshore stations for all of the combined data; the mean was 114 and 133 ng/l, respectively. Particulate contents of thiamine in offshore stations ranged from 2.40 to 175 ng/l with a mean of 47.3 ng/l. Total thiamine contents in offshore stations were 23.5–585 ng/l (mean: 168 ng/l).

Regional variation of thiamine contents observed in offshore stations was found to be similar to that of vitamin B₁₂; both dissolved and particulate thiamine, in general, were more abundant in the northern part than in the southern part and scantiest in the central part. From the variance analysis of seasonal distribution observed in offshore stations (Table 5), there were significant seasonal differences in both particulate ($P<0.001$) and total thiamine contents ($P<0.05$); both these contents were higher in summer than in winter. But no significant difference could be found between dissolved thiamine levels observed in

Table 6. Statistic analysis for the variance of dissolved vitamins in offshore and inshore stations of Harima-Nada Sea in warm seasons*¹

Vitamins	Samples	Number of Samples	Vitamin Contents		Difference of Means
			Ranges ng/l	Means ng/l	
Vitamin B ₁₂	Offshore (78/79)	70	1.38–19.9	6.65	P<0.001* ²
	Inshore (80/81)	38	4.82–25.0	10.2	
Thiamine	Offshore (78/79)	68	8–418	131	No* ³
	Inshore (80/81)	38	30–2140	133	
Biotin	Offshore (78/79)	70	5.04–15.6	9.69	No* ³
	Inshore (80/81)	38	3.9–33.2	10.1	

*¹ Samples of above 20 meters depth.*² Significant at the 0.1% level.*³ Not significant.**Table 7.** Variance analysis for vertical difference in dissolved vitamin concentrations observed in offshore stations of Harima-Nada Sea

Vitamin	Seasons	Number of Samples	Means			Difference of Variance
			Surface ng/l	10 m ng/l	Bottom ng/l	
Vitamin B ₁₂	Summer	31	6.90	6.35	7.65	No* ¹
	Winter	20	6.27	6.58	7.34	No* ¹
Thiamine	Summer	29	168	111	80.1	P<0.005* ²
	Winter	22	109	117	118	No* ¹
Biotin	Summer	31	10.4	9.58	8.51	P<0.001* ³
	Winter	22	7.41	7.26	6.70	No* ¹

*¹ Not significant.*² Significant at the 0.5% level.*³ Significant at the 0.1% level.

summer and those in winter, and also between dissolved ones found in offshore stations and those in inshore waters in the warm seasons (Table 6). Dissolved thiamine concentrations, however, significantly decreased with increasing depth of water in summer but not in winter (Table 7). The difference in vertical distribution of particulate thiamine contents observed in both seasons were not clear.

The ratios of particulate content to the total for thiamine observed in both summer and winter were much smaller than that for vitamin B₁₂; the ratios of thiamine ranged from 2.2 to 83.9% with a mean of 33.4% in summer and from 4.6 to 87.3% with a mean of 32.0% in winter.

Biotin Contents

Dissolved biotin concentrations ranged from 1.51 to 15.6 ng/l (mean: 8.43 ng/l) in offshore stations and from 3.90 to 33.2 ng/l (mean: 10.1 ng/l) in inshore stations for all combined data. In offshore stations, as was similar to the other vitamins, the dissolved biotin concentration was generally higher in the northern part than in the

southern part and frequently least in the central part. As shown in Table 5, dissolved biotin in offshore stations were generally more abundant in summer than in winter (significant $P<0.001$). But there was no significant difference between dissolved biotin contents found in offshore station and those in inshore stations in the warm seasons (Table 6). The difference in vertical profiles for the mean dissolved biotin contents was statistically significant; the dissolved biotin generally decreased with increasing depth of water (Table 7).

Discussion

Concentrations of dissolved vitamin B₁₂, thiamine, and biotin in sea water are reviewed by some authors,^{5,14,15} and it is known that these are greater in coastal regions than in open seas. The concentrations of dissolved vitamin B₁₂, thiamine, and biotin observed in coastal waters of Japan are shown in Table 8 including the most recently published data.

The concentrations of dissolved vitamin B₁₂ in Harima-Nada Sea are rather high among such

Table 8. Concentrations of dissolved vitamin B₁₂, thiamine, and biotin observed in the coastal waters in Japan

Regions	Vitamin B ₁₂ ng/l	Thiamine ng/l	Biotin ng/l	Authors
Tokyo Bay	23.2–73.2	71–912		Ohwada (1973) ¹⁶⁾
Sagami Bay	0–14.3	0–142	0–17.3	Ohwada <i>et al.</i> (1972) ⁷⁾
Suruga Bay		0–71	0–3.4	Ohwada (1971) ¹⁷⁾
Ise Bay	2.4–29.3	0–1500		Noda (1982) ¹⁸⁾
Ago Bay		244–404		Okaichi (1972) ¹⁹⁾
Maizuru Bay	0–3.8			Kurata (1969) ²⁰⁾
Seto Inland Sea	0–72			Tsuda (1975) ²¹⁾
Harima-Nada Sea	1.38–19.9	3.4–443	1.51–15.6	This study
	0–25			Furuki <i>et al.</i> (1985) ²²⁾
Harima-Nada Sea (Inshore)	4.82–25.0	30–2140	3.90–33.2	This study
Hiuchi-Nada Sea	0.68–15.3	0–730	0.2–34.7	Nishijima <i>et al.</i> (1986) ²³⁾
		80–584		Okaichi (1972) ¹⁹⁾
Bingo-Nada Sea	0.20–9.32	44–516	0.5–60.0	Nishijima (1985) ²⁴⁾
Katagami Inlet	0.1–16.1			Fujisawa <i>et al.</i> (1973) ²⁵⁾
Coast of Okayama Pref.	0–9.8	0–0.302		Fujisawa <i>et al.</i> (1975) ²⁶⁾
Coast of Fukuyama Pref.	0.42–6.43			Inoue <i>et al.</i> (1973) ²⁷⁾
Uranouchi Inlet	0–9.36	0–1062	1.6–35.7	Nishijima <i>et al.</i> (1978) ²⁸⁾
Nomi Inlet	0–45.0	0–504	0–38.0	Nishijima <i>et al.</i> (1978, 1979) ^{28, 29)}
Susaki Harbor	1.54–56.0	40–490	2.04–75.0	Nishijima <i>et al.</i> (1979) ²⁹⁾
Omura Bay		16–165		Okaichi (1971) ³⁰⁾
Sacki Bay		100–150		Okaichi (1972) ¹⁹⁾
Kagoshima Bay	0–15			Kashiwada <i>et al.</i> (1957) ³¹⁾
	0–28.73			Inoue (1979) ³²⁾
Kagoshima Bay (Laver-farm)	1.5–24.4			Kashiwada <i>et al.</i> (1964) ³³⁾

coastal waters of Japan; these are similar to the concentrations found in several coastal regions, except for Maizuru Bay and Tokyo Bay, listed in Table 8. Dissolved thiamine concentrations in the sea are also comparable to those observed in some regions of Japan, but considerably greater than those measured in Suruga Bay and Omura Bay. Dissolved biotin concentrations in the sea were also similar to those found in many coastal waters of Japan. Consequently, the range in dissolved concentrations of the three vitamins in Harima-Nada Sea are generally comparable to those observed in such coastal waters including the area which has been frequently invaded by red tides.

Present results demonstrated that both particulate contents of vitamin B₁₂ and thiamine were also fairly high in the water of Harima-Nada Sea. The range in particulate vitamin B₁₂ contents recorded in this region is similar to that found in the coast of Georgia (2.7–130 ng/l)³⁴⁾ and Bahia Fosforescente (2.5–146.7 ng/l)³⁵⁾ but considerably greater than the range found in oceanic and

estuarine waters near Sapelo Island (0.8–14.0 ng/l).³⁶⁾ The content of particulate thiamine found in this study is also higher than that in Aburatubo Inlet (1.1–14.4 ng/l).⁷⁾

In the present study, both dissolved and particulate vitamin B₁₂, thiamine, and biotin were demonstrated to be generally greater in the northern part, which is loaded with industrial and domestic waste waters, than in the southern part of Harima-Nada Sea. These regional profiles of these vitamins are similar to the following factors indicating eutrophication; the bacterial distribution in this study and transparency, phytoplankton abundance, and contents of chlorophyll *a*, SS, PON, and POC measured in the same water samples as this study in 1978 and 1979 by Okaichi and Endo.* In addition dissolved vitamin B₁₂ is apparently greater in inshore stations than in offshore stations, and the higher concentrations were generally observed at the mouth of bays, in which waste waters inflow, and in estuaries of polluted rivers, *e.g.*, Ichi River Senba River, Ama River, and others. These suggest that the river

* "Kaiyo Kankyo Tokusei to Akashio Hassei nikansuru Kisokenkyu" Data Book (Harima-Nada Sea 1978–1980), 1981, pp. 1–49.

water as well as various waste waters is a major source of these vitamins.

Dissolved thiamine and biotin contents correlated positively with chlorophyll *a* content (significant $P < 0.05$ and $P < 0.01$, respectively). Even though dissolved vitamin B₁₂ didn't correlate significantly with chlorophyll *a*, it was sometimes greatest in the central part where phytoplankton communities were usually poor. Since both particulate vitamin B₁₂ and thiamine contents were found to correlate with chlorophyll *a* contents in this region ($P < 0.01$ and $P < 0.05$, respectively), particulate matter in the water may mainly consist of vitamin-rich phytoplankters and/or the detritus derived from them. Furthermore, particulate vitamin B₁₂ was greater than the dissolved vitamin B₁₂ in most of the summer samples, but not in the winter samples. It suggests that phytoplankters not only excrete thiamine and/or biotin into the water but also take up dissolved vitamin B₁₂ during their growth. As a result, particulate vitamin contents may increase with the development of phytoplankton communities during the warm seasons.

Consequently, in eutrophied sea waters such as Harima-Nada Sea, the levels of these three vitamins, both in dissolved and particulate form, may be closely connected with the growth of phytoplankters as well as being affected by the discharge of waste and polluted river waters.

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