Distribution of Thiamine, Biotin, and Vitamin B₁₂ in Lake Kojima-I Distribution in the Lake Water

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Concentrations of dissolved thiamine, biotin, and vitamin B₁₂ in the water of a eutrophic lake, Lake Kojima, have been determined by microbiological assay methods at intervals of 2 to 4 months from August, 1971 to April, 1972. Also the relations between the distribution of the B group vitamins and other physical, chemical, and biological environmental factors were studied. Thiamine was assayed with *Lactobacillus fermenti* ATCC 9338, biotin with *L. plantarum* ATCC 8014, and vitamin B₁₂ with *L. leichmannii* ATCC 7830. It was found that Lake Kojima is an extremely eutrophic lake and that concentrations of the B group vitamins were fairly high: thiamine 20–2300 ng/l, biotin 2.3–101.6 ng/l, and vitamin B₁₂ 2.1–36.0 ng/l. Thiamine and vitamin B₁₂ were most abundant in summer and markedly decreased from autumn and winter to spring, while the biotin concentration was highest in winter and lowest in spring. Concentrations of thiamine and vitamin B₁₂, not biotin, correlated negatively with abundances of both phytoplankton and aerobic heterotrophic bacteria. It was suggested that the seasonal fluctuation of the B group vitamins concentrations may be connected with the growth of phytoplankton species.

Many species of marine and freshwater phytoplankton, so far as have been studied, require B group vitamins for the growth; most commonly vitamin B₁₂, thiamine, and biotin, singly or in various combinations.¹⁻⁴⁾ Since it has been suggested that the B group vitamins may regulate the growth and succession of phytoplankton species and the initiation of water blooms or red tides,³⁻⁸⁾ important ecological roles of the B group vitamins on the biotic production and eutrophication in natural waters have attracted special interest.

Lake Kojima is a eutrophic man-made freshwater reservoir and heavy water blooms usually occur in the water all the year round. This paper describes the distribution and seasonal variations of thiamine, biotin, and vitamin B₁₂ (cyanocobalamin) in the water of Lake Kojima, and also the relations between the distribution of the B group vitamins and the other physical, chemical and biological environmental factors.

Materials and Methods

Lake Kojima is located in the south part of Okayama Prefecture. The lake is a man-made freshwater reservoir built by damming across Kojima Bay in 1956, and it is about 8.2×10^6 m² in area and 1.77×10^7 m³ in volume. The mean depth of the lake is 1.6 m, with the maximum depth of 9.0 m.⁹⁾ There are two inflowing rivers, the Sasagase

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Table 1. Concentrations of the B group vitamins and the

Date	Sta.	Depth m	Temp. °C	pH	DO		Chlorinity ‰	NH ₄ ⁺ -N mg/l
					O ₂ mg/l	Satu. %		
Aug. 4, 1971	3	0	29.2	8.4	6.76	84.4	0.52	0.27
		1	29.0	8.4	7.64	95.0	0.53	0.27
		3	27.7	6.8	1.53	19.5	5.10	0.51
		5	25.8	7.2	0.00	0.0	9.95	1.96
	5	0	28.5	7.0	5.43	66.8	0.14	0.25
		1	28.5	7.1	6.13	75.4	0.13	0.31
	8	0	28.8	8.2	6.89	85.6	0.25	0.26
		1	29.0	6.0	6.66	82.8	0.27	0.31
Oct. 29, 1971	3	0	16.5	9.2	10.8	108	0.77	0.18
		1	16.0	9.2	11.6	115	0.77	0.23
		3	—	8.0	0.00	0.0	7.58	0.74
		5	—	8.0	0.00	0.0	8.58	0.40
	5	0	—	9.2	12.5	—	0.68	0.25
		1	—	9.2	12.6	—	0.69	0.23
	8	0	—	9.2	12.2	—	0.65	0.22
		1	—	9.2	11.6	—	0.66	0.25
	10	0	—	9.0	14.7	—	0.34	0.35
		1	—	8.8	13.6	—	0.51	0.80
	11	0	—	8.1	10.3	—	0.33	1.86
		1	—	8.8	12.3	—	0.49	0.73
Dec. 19, 1971	3	0	7.5	9.2	15.3	127	0.83	1.03
		1	7.5	9.2	16.2	135	0.84	1.06
		3	9.3	8.8	8.52	75.3	2.70	0.39
		5	10.5	8.2	5.87	56.8	8.56	0.54
	5	0	8.2	8.6	14.6	124	0.92	1.94
		1	7.8	9.1	16.0	135	1.46	1.84
	8	0	7.8	9.2	16.4	138	1.36	0.68
		1	8.0	9.2	15.1	128	1.34	0.53
	10	0	8.0	8.2	13.3	112	1.19	2.73
		1	8.4	9.2	14.8	127	1.58	0.80
	11	0	7.8	8.2	13.7	116	0.92	1.83
		1	8.0	9.1	15.3	129	1.12	0.48
Apr. 25, 1972	3	0	17.5	9.2	12.6	129	0.50	0.01
		1	17.5	9.2	12.8	131	0.46	0.00
		3	15.5	8.2	2.84	28.6	2.84	0.53
		5	14.2	8.0	2.78	28.8	8.00	0.50
	5	0	17.5	9.4	14.0	143	0.50	0.01
		1	17.7	9.2	14.1	145	0.46	0.01
	8	0	17.8	9.4	13.7	141	0.58	0.00
		1	17.8	9.4	13.8	142	0.32	0.01
	10	0	18.0	8.4	11.8	122	0.12	0.81
		1	18.0	8.4	11.8	121	0.12	0.84
	11	0	18.5	8.8	13.1	136	0.12	0.23
		1	18.5	8.6	13.1	136	0.11	0.30

environmental factors in the water of Lake Kojima

NO ₂ ⁻ -N	NO ₃ ⁻ -N	Total inorg. N	PO ₄ ³⁻ -P	SiO ₂ -Si	Thiamine	Biotin	Vitamin B ₁₂	Chlo. <i>a</i>	Aerobic heterotrophs
mg/l	mg/l	mg/l	mg/l	mg/l	ng/l	ng/l	ng/l	μg/l	cells/ml
0.006	0.039	0.32	0.161	0.289	1640	9.2	33.0	52.8	9.0 × 10 ³
0.006	0.037	0.32	0.167	0.298	1370	3.2	26.5	64.5	9.3 × 10 ³
0.011	0.045	0.57	0.155	0.208	2300	2.2	22.0	22.0	9.0 × 10 ²
0.006	0.016	1.99	0.226	0.157	—	7.2	6.5	7.2	2.1 × 10 ³
0.004	0.008	0.26	0.514	0.270	1230	4.4	31.0	124	4.0 × 10 ³
0.005	0.017	0.33	0.127	0.272	2070	8.4	27.5	123	2.3 × 10 ⁴
0.005	0.005	0.27	0.164	0.301	1190	6.4	36.0	99.0	4.0 × 10 ³
0.006	0.000	0.31	0.164	0.295	2030	9.6	34.5	53.9	1.1 × 10 ⁴
0.005	0.038	0.22	0.049	0.223	490	6.0	19.2	117	1.3 × 10 ⁴
0.005	0.038	0.28	0.128	0.225	490	9.2	17.2	123	1.7 × 10 ⁴
0.007	0.036	0.78	0.230	0.142	1140	14.4	11.2	23.8	1.7 × 10 ⁴
0.005	0.053	0.46	0.167	0.134	740	9.2	13.6	24.9	4.6 × 10 ⁴
0.005	0.005	0.26	0.350	0.213	290	3.3	17.6	121	2.4 × 10 ⁴
0.005	0.022	0.26	0.110	0.201	240	14.4	16.4	120	7.9 × 10 ³
0.005	0.024	0.25	0.099	0.212	40	31.2	14.8	132	1.7 × 10 ⁴
0.005	0.028	0.28	0.131	0.221	330	16.4	18.4	116	7.0 × 10 ³
0.064	0.138	0.55	0.096	0.183	200	14.0	8.8	165	1.7 × 10 ⁴
0.027	0.069	0.90	0.119	0.210	80	51.6	15.2	157	1.7 × 10 ⁴
0.065	0.210	2.14	0.176	0.213	450	16.4	19.2	74.4	7.7 × 10 ⁴
0.029	0.258	1.01	0.150	0.199	290	9.2	18.8	119	7.0 × 10 ⁴
0.038	0.198	1.27	0.034	0.044	570	25.8	16.8	111	1.1 × 10 ⁴
0.040	0.184	1.28	0.043	0.044	490	19.2	14.8	110	1.6 × 10 ³
0.025	0.164	0.58	0.055	0.020	980	72.0	17.2	103	2.2 × 10 ⁴
0.027	0.101	0.67	0.077	0.048	860	13.6	16.8	26.6	3.3 × 10 ⁴
0.050	0.254	2.24	0.226	0.116	290	37.2	24.8	50.5	1.6 × 10 ³
0.052	0.300	2.19	0.081	0.068	120	72.8	16.4	96.9	1.7 × 10 ³
0.068	0.155	0.90	0.043	0.046	370	34.4	16.0	99.1	1.1 × 10 ³
0.045	0.170	0.74	0.038	0.076	400	101.6	20.0	109	7.9 × 10 ⁴
0.050	0.237	3.01	0.289	0.144	120	92.4	28.0	56.7	1.4 × 10 ³
0.046	0.178	1.03	0.041	0.064	240	22.8	14.8	127	3.5 × 10 ³
0.054	0.248	2.13	0.199	0.154	530	88.4	27.6	54.5	1.6 × 10 ³
0.043	0.112	0.63	0.028	0.052	650	34.4	19.6	117	2.8 × 10 ³
0.041	0.223	0.27	0.033	0.128	130	4.0	6.7	130	4.9 × 10 ³
0.040	0.252	0.29	0.030	0.128	180	5.0	4.2	102	2.2 × 10 ³
0.019	0.167	0.72	0.099	0.062	210	—	3.4	29.4	7.9 × 10 ⁴
0.011	0.116	0.62	0.093	0.034	190	—	3.8	13.2	3.3 × 10 ⁴
0.024	0.159	0.20	0.022	0.130	90	2.0	2.1	145	3.3 × 10 ³
0.028	0.293	0.33	0.021	0.074	20	4.4	4.1	92.8	1.1 × 10 ³
0.039	0.209	0.25	0.036	0.142	210	6.0	5.8	125	7.9 × 10 ⁴
0.027	0.163	0.20	0.035	0.102	60	4.4	6.0	126	1.3 × 10 ³
0.095	0.541	1.45	0.295	0.124	100	7.2	5.8	136	4.9 × 10 ³
0.097	0.590	1.53	0.295	0.130	100	8.0	9.2	139	2.3 × 10 ⁴
0.039	0.455	0.73	0.088	0.147	20	10.4	5.4	127	1.7 × 10 ³
0.054	0.485	0.84	0.099	0.167	90	10.0	6.8	123	3.3 × 10 ³

and the Kurashiki rivers, through which a large amount of industrial wastes, night soil disposal waste, domestic drainage and farming effluents are brought into the lake.

Sampling was carried out at intervals of 2 to 4 months from August, 1971 to April, 1972. Water samples were collected from 5 stations shown in Fig. 1, with plastic Kita-

Fig. 1. The stations set up in Lake Kojima.
() Depth in m; ... 1.0 m depth lines.

hara-type water bottles. The water samples for determination of inorganic nutrient salts and the B group vitamins were immediately filtered through HA Millipore filters (pore size: $0.45\ \mu\text{m}$) and were kept frozen in acid-cleaned polyethylene bottles until the time of analysis.

Dissolved oxygen was determined by the azide modification of Winkler's method,¹⁰⁾ chlorine by the mercury thiocyanate method,¹¹⁾ ammonium nitrogen by the direct nesslerization method,¹⁰⁾ nitrite nitrogen by the Griess-Romijn method,¹⁰⁾ nitrate nitrogen by the cadmium-copper reduction method,¹²⁾ phosphate phosphorous by the heteropoly blue method,¹³⁾ and silicate silicon by the molybdosilicate method.¹⁰⁾ The chlorophyll *a* content of the particulate matter was determined by the spectrophotometric method.¹⁴⁾

The enumeration of aerobic heterotrophic bacteria was carried out by the most probable number (MPN) method¹⁰⁾ using Henrici's medium¹⁵⁾ for freshwater samples or ZoBell's 2216E medium¹⁶⁾ for seawater samples. Inoculated media were incubated at 20°C for two weeks, and the growth was estimated visually.

Amounts of the B group vitamins in the water were determined by microbiological assay methods.¹⁷⁾ Thiamine was assayed with *Lactobacillus fermenti* ATCC 9338 (IFO 3071*), biotin with *Lactobacillus plantarum* ATCC 8014 (IFO 3070*), and vitamin B₁₂ with *Lactobacillus leichmannii* ATCC 7830 (IFO 3376*).

Results and Discussion

The quality of the water of Lake Kojima is shown in Table 1. Presumably as a result of vigorous photosynthesis by phytoplankton, relatively high pH values, and the oxygen supersaturation were generally found in the surface samples (zero and one meter samples). Chlorine contents varied within the range of 0.11–1.58‰ in the surface sam-

* Culture number of the Institute for Fermentation, Osaka (IFO)

ples, 8.00–9.95‰ in deeper samples. These variations were owing to influx and leakage of seawater through the lock-gate and dam, and to the inflow of salty water eliminated from the reclaimed farmland.

The concentration of nutrient salts in the lake water was found to be at a high level all the year round: far above the general criterion in a eutrophic lake, viz., nitrogen 0.15 mg/l and phosphorous 0.02 mg/l.¹⁸⁾ Presumably as a result of the inflow of various waste waters from the rivers and the consumption by phytoplankton in the lake, the concentration of the nutrient salts was considerably higher at the influxes of the rivers (Stations 10 and 11) than those in the lake (Stations 3, 5, and 8).

Chlorophyll *a* contents of more than 50 µg/l were generally measured in the surface samples, and heavy water blooms were observed throughout all the seasons.

The number of aerobic heterotrophic bacteria was comparatively large: about 10^3 – 10^6 cells/ml, and the bacterial population tended to be large in the cold months and smaller in the warm months. This tendency may be chiefly attributed to the stirring-up of the bottom sediments by water movement resulting from strong seasonal winds during the cold months.

Thus it is apparent that Lake Kojima is an extremely eutrophic lake and that both the biotic production and the decomposition of organic matter are vigorously proceeding in the water.

The concentrations of the B group vitamins in the water were fairly high: thiamine 20–2300 ng/l, biotin 2.3–101.6 ng/l, and vitamin B₁₂ 2.1–36.0 ng/l.

Diurnal fluctuations in the concentrations of the B group vitamins, dissolved oxygen, chlorophyll *a*, and bacterial population in the water are shown in Fig. 2. Dissolved oxygen decreased during the night, but recovered in the daytime. On the other hand, there were no regular diurnal fluctuations in the concentrations of the B group vitamins, chlorophyll *a*, and the population of heterotrophs.

The concentration of vitamins in a lake has been found to be related to the fertility of the lake; the vitamin concentration in eutrophic lake waters is higher than in oligotrophic lake waters. KASHIWADA and KAKIMOTO¹⁹⁾ surveyed the water of ten Japanese lakes and found the low vitamin B₁₂ concentration (0–1.3 ng/l) in oligotrophic lakes and higher levels (0.9–11.9 ng/l) in eutrophic lakes. Similarly DAISLEY²⁰⁾ found from 0 to 6 ng/l of vitamin B₁₂ in low-productivity lakes, from 2.5 to 20 ng/l in nutrient-rich lakes of nine English lakes. CARLUCCI and BOWES²¹⁾ reported that the concentration of vitamin B₁₂, thiamine, and biotin in a eutrophic Cleare Lake (California, USA) was 4.5 ng/l, 400 ng/l, and 6.4 ng/l respectively.

From the present investigation it is apparent that concentrations of the B group vitamins as well as nutrient salts were very high in the water of Lake Kojima, and it is in good agreement with these reports.

Fig. 2. Diurnal fluctuations of the concentrations of the B group vitamins and some environmental factors in the water of Lake Kojima (Station: 5, Depth: 1.0 m, Aug. 26–27, 1971).

Results of the variance analysis of vitamin concentrations in the surface samples at Stations 3, 5, and 8 are given in Table 2. There were significant differences among seasonal

Table 2. Analysis of variance of logarithmic transformed values of the B group vitamin concentrations in the water of Lake Kojima

Vitamins	Factors	s.s.	d.f.	m.s.	F ₀
Thiamine	Season	471.2	3	157.1	18.3**
	Location	41.4	2	20.7	2.4
	Depth	0.3	1	0.3	<1
	Residual	146.2	17	8.6	
	Total	659.1	23		
Biotin	Season	336.7	3	112.2	22.4**
	Location	35.6	2	17.8	3.6
	Depth	9.4	1	9.4	1.9
	Residual	84.3	17	5.0	
	Total	466.0	23		
Vitamin B ₁₂	Season	228.9	3	76.3	65.2**
	Location	2.6	2	1.3	1.1
	Depth	0.1	1	0.1	<1
	Residual	19.9	17	1.2	
	Total	251.5	23		

** Significant at the 1% level.

variations of the B group vitamin concentrations, but no significant differences among both locational and vertical distributions. Seasonal variations of the B group vitamin concentrations in the surface samples are given in Table 3. As a whole, thiamine and vitamin B₁₂ in the lake water were most abundant in summer and markedly decreased from autumn and winter to spring. On the other hand, the concentration of biotin was highest in winter and lowest in spring.

Correlation coefficients between concentrations of the B group vitamins (ng/l) and

Table 3. Seasonal variation in the concentration of the B group vitamins in the surface samples of Lake Kojima

Date	Thiamine ng/l		Biotin ng/l		Vitamin B ₁₂ ng/l	
	Mean	Range*	Mean	Range*	Mean	Range*
Aug. 4, 1971	1550	1200-2000	6.37	3.99-10.2	31.2	27.4-35.5
Oct. 29, 1971	246	92.2-655	10.5	4.56-24.2	17.2	15.6-18.9
Dec. 19, 1971	336	187-601	40.9	21.1-79.1	17.8	14.6-21.8
Apr. 25, 1972	90.0	36.2-223	4.11	2.75-6.13	4.51	2.89-7.04

* Range represents the 95% confidence interval.

abundances of both chlorophyll *a* ($\mu\text{g/l}$) and heterotrophic bacteria (log cells/ml) in the euphotic zone were calculated for the combined data from Stations 3, 5, and 8 over the whole period of study. There was a significant, negative correlation between phytoplankton and thiamine ($r = -0.47$, $P < 0.05$); a negative, but nonsignificant correlation between phytoplankton and biotin; a significant, negative correlation between phytoplankton and vitamin B₁₂ ($r = -0.55$, $P < 0.01$). Negative, significant correlations were found between the abundance of bacteria and concentrations of both thiamine ($r = -0.51$, $P < 0.05$) and vitamin B₁₂ ($r = -0.58$, $P < 0.01$), but there was no significant correlation between the abundance of bacteria and the biotin content.

OHWADA *et al.*^{7,8)} demonstrated the ecological relationships between the seasonal fluctuations of the dominant phytoplankton species and the B group vitamins concentrations in Lake Tsukui and Lake Sagami; the dissolved vitamin B₁₂ concentration decreased remarkably in the surface water of the lakes in most of the time when diatoms, such as *Cyclotella* sp., *Nitzschia acicularis*, *N. paleacea*, and *Synedra acus*, were dominant, while thiamine and biotin concentrations increased very much in the surface water during the same period. They suggested that dissolved vitamin B₁₂ in the surface water would be taken up by the dominant diatoms and some other phytoplankton during their growing seasons, while thiamine and biotin would be produced and released to lake water by them. ADACHI²²⁾ observed the succession of phytoplankton species in Lake Kojima from 1969 to 1970; the dominant species were Cyanophyceae from early summer to autumn, Bacillariophyceae and unknown species during winter, and Chlorophyceae during spring. From the present investigation, thus, the seasonal fluctuations of the B group vitamins concentrations in this lake water may be connected with the growth of the dominant species of phytoplankton.

The B group vitamins found in any one sample must represent a momentary state of the balance between vitamin-producing and vitamin-removing factors. The producers, so far as is known, are bacteria, phytoplankton, and perhaps seaweeds; the direct consumers are other bacteria, phytoplankton, protozoa, and perhaps invertebrates.¹⁻⁴⁾ Although there was no evidence that the concentration of the B group vitamins limited the

overall growth of phytoplankton in this lake during the period of study, still individual species of phytoplankton may have been affected.

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