Distribution of Vitamin B₁₂, Thiamine and Biotin in Marine Sediments*

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Abstract: Vitamin B₁₂, thiamine and biotin in the sediments collected from different 41 stations at the Eastern China Sea, the Pacific Ocean and the bays and inlet along the Pacific coast of Japan have been determined by microbiological assay methods using *Lactobacillus leichmannii*, *Lactobacillus fermenti* and *Saccharomyces cerevisiae* respectively. Vitamin B₁₂ contents in the surface sediments were rather higher at such a shallow area as the Aburatsubo Inlet (1.14–7.14 m μ g/g) than those at other areas, the Japan Trench, the Mid-Pacific Ocean, the Eastern China Sea, the Suruga Bay and the Sagami Bay (0–2.10 m μ g/g). Thiamine contents were similar levels in the sediments at all areas investigated (average of 0.31 μ g/g), whereas, biotin was more abundant in the sediments at the Mid-Pacific Ocean (average of 5.0 m μ g/g) than the other areas (average of 2.0 m μ g/g). Vertical distribution of vitamin concentrations in the cores revealed, in general, the gentle decreasing trends with depth, except for a few irregular fluctuations. In comparing the relationship between vitamin contents and some of the environmental factors in the sediments, significant correlations were observed between vitamin B₁₂ or thiamine contents and values of loss on ignition. On the other hand, there seemed to be no clear relation between biotin contents and values of loss on ignition.

1. Introduction

Vitamin B₁₂, thiamine and biotin have been shown to be required indispensably by certain marine unicellular algae, which play the important role as primary producers in marine ecosystem. After their reviewing the accumulated informations, DROOP (1962) and PROVASOLI (1963) reported that some 60 % of strains of algae so far studied proved to be auxotrophs, 80 % of them required vitamin B₁₂, 53 % thiamine, and 10 % biotin. In recent years, considerable interest has centered also around the possible role of these vitamins in the outbreak of algal bloom and in the succession of algal population, because available concentration of vitamins in the aquatic environment might often regulate the algal production. Subsequently to the aforementioned scope, the vitamin cycles in the sea should be comprehended extensively for the evaluation of the importance of these vitamins in marine ecology.

Informations on the distribution of vitamin B₁₂, thiamine and biotin in sea water have been accumulated, and were reviewed by PROVASOLI (1963).

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As for the distributions of vitamins in marine sediments, although BURKHOLDER and BURKHOLDER (1956) and STARR (1956) measured very high concentrations of vitamin B₁₂ in the marsh muds compared with others found in sea water, the extensive data are so scarce up to now.

This paper describes the results of assays for vitamin B₁₂, thiamine and biotin in the sediments collected from different 41 stations at the Eastern China Sea, the Pacific Ocean and its adjacent bays around Japan. Environmental factors in relation to the vitamin contents are also discussed.

2. Materials and Methods

Collection of sediment samples

The locations of sampling stations where the samples were collected, dates of sampling, depths of water and types of sediments are shown in Table 1. The charts of the sampling stations at the Aburatsubo Inlet, the Japan Trench and the Eastern China Sea, the Mid-Pacific Ocean, the Suruga Bay and the Sagami Bay are shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4 respectively. The sediment samples from the Japan Trench (St. 1, St. 2, St. 3 and St. 4) were collected during the cruise of the Leg IV of Zetes (Depac

Table 1. Locations and types of sediments used in the present study.

Station number	Location		Date	Depth of water (m)	Type of sediment
	Latitude	Longitude			
1	33°37'N	141°58'E	May 31, 1966	9,247	clay
2	30°42'N	142°25'E	June 3, 1966	9,628	clay
3	32°05'N	142°06'E	June 3, 1966	9,489	clay
4	33°59'N	140°26'E	June 4, 1966	1,660	silt
5	39°49'N	145°44'E	Aug. 10, 1967	5,205	clay
6	38°28'N	145°49'E	Aug. 11, 1967	5,250	clay
7	38°49'N	143°00'E	Aug. 13, 1967	1,600	silt
8	38°39'N	144°08'E	Aug. 14, 1967	7,370	clay
9	00°00'N	150°05'E	Dec. 27, 1967	5,235	clay
10	00°40'S	160°37'E	Jan. 5, 1968	2,940	foram. ooze
11	00°49'N	164°00'E	Jan. 6, 1968	4,330	foram. ooze
12	02°18'N	167°00'E	Jan. 7, 1968	4,260	foram. ooze
13	03°28'N	170°09'E	Jan. 8, 1968	4,450	clay
14	05°10'N	173°08'E	Jan. 9, 1968	4,850	clay
15	07°42'N	177°54'E	Jan. 11, 1968	5,400	clay
16	18°04'N	175°13'W	Jan. 17, 1968	1,450	foram. ooze
17	12°17'N	174°16'W	Jan. 19, 1968	5,000	clay
18	31°23'N	128°10'E	May 19, 1968	150	coarse sand
19	31°36'N	128°10'E	May 19, 1968	141	coarse sand
20	31°42'N	127°19'E	May 20, 1968	123	fine sand
21	31°43'N	126°48'E	May 20, 1968	103	fine sand
22	31°47'N	125°52'E	May 20, 1968	70	silt
23	31°47'N	125°09'E	May 20, 1968	45	silt
24	31°50'N	124°32'E	May 20, 1968	40	fine sand
25	31°51'N	124°01'E	May 20, 1968	42	coarse sand
26	30°56'N	124°58'E	May 21, 1968	58	fine sand
27	30°33'N	125°18'E	May 21, 1968	62	fine sand
28	30°08'N	125°43'E	May 21, 1968	76	fine sand
29	29°52'N	126°01'E	May 21, 1968	88	fine sand
30	29°25'N	126°28'E	May 22, 1968	97	fine sand
31	28°40'N	126°49'E	May 22, 1968	192	fine sand
32	30°26'N	124°31'E	May 23, 1968	64	fine sand
33	29°52'N	124°29'E	May 23, 1968	66	fine sand
34	29°10'N	124°32'E	May 23, 1968	80	fine sand
35	28°28'N	124°51'E	May 23, 1968	105	fine sand
36	28°00'N	125°00'E	May 24, 1968	103	coarse sand
37	27°24'N	125°00'E	May 24, 1968	103	coarse sand
38	26°54'N	125°00'E	May 24, 1968	121	coarse sand
39	34°56'N	138°36'E	Oct. 10, 1968	835	fine sand
40	35°00'N	138°59'E	Oct. 11, 1968	1,425	fine sand
41	35°07'N	139°25'E	Oct. 13, 1968	1,120	fine sand

X) Expedition of the Scripps Institution of Oceanography, University of California, by R/V *Argo* (REID, 1966; SEKI and ZOBELL, 1967). The other samples at the Japan Trench, the sediments at the Mid-Pacific Ocean area and at the Eastern China Sea area were taken during the cruise of KH-67-2, KH-68-2 respectively by

R/V *Hakuho-maru* of the Ocean Research Institute, University of Tokyo. The samples at the Suruga Bay and the Sagami Bay were collected during the cruise of KT-68-20 by R/V *Tansei-maru* of the same Institute. The other samples at the Aburatsubo Inlet were taken by the boat of the Misaki Marine Biological Station, Univer-

Fig. 1. Sampling stations in the Aburatsubo Inlet.

Fig. 2. Map of sampling stations at the Japan Trench and the East China Sea.

Fig. 3. Map of sampling stations at the Mid-Pacific Ocean.

Fig. 4. Sampling stations at the Suruga Bay and the Sagami Bay.

sity of Tokyo.

Four kinds of samplers were adopted to take the sediments; *i.e.*, the piston corer, the Phleger-type gravity corer and the Smith-McIntyre grab were used to collect the sediments at the open sea, the Ekman-Birge sampler was particularly used at the shallow region such as the Aburatsubo Inlet.

As soon as the sediments were hauled aboard the ship, the samples were transferred aseptically to sterile Petri dishes, then a part was treated for the enumeration of heterotrophic bacteria and for the pH and Eh measurement. The rest of the samples were stored at -20°C in deep freezer until the other experiments were performed.

Estimation of the bacterial number

Three different methods were used for the enumeration of heterotrophic bacteria in the sediment; *i.e.*, surface colony counts method, membrane filter counts methods and most probable number (MPN) method.

For the method of surface colony counts, 0.1 ml from each dilution was pipetted on to the nutrient agar plate in Petri dish and spread out evenly with a sterile spreader.

For the method of membrane filter counts, bacteria in the sediments of each dilution were collected aseptically on the sterilized Millipore® filters (Type: HA-47 mm) by filtration. The inoculated filters were placed on the nutrient agar plate in Petri dishes.

For the method of MPN counts, the standard method was used (American Public Health Association, 1965).

These inoculated plates were incubated at 20°C for two weeks before bacterial colonies on the plates were counted.

Following medium was used for the surface colony counts method and the membrane filter method.

PPES II Polypeptone (Daigo eiyo kagaku):	2g
Proteose-peptone No. 3 (Difco):	1g
Bacto-soytone (Difco):	1g
Bacto-yeast extract (Difco):	1g
Ferric phosphate soluble (Merck):	0.1g
Agar (Wako):	15g
Marine mud extract:	100ml
Aged sea water:	900ml
pH adjusted to 7.6 to 7.8.	

For the MPN counts method, the following medium was used;

Bacto-casitone (Difco):	5 g
Beef extract:	3 g
KH ₂ PO ₄ :	0.1 g
Fe-EDTA:	6 mg
KNO ₃ :	0.5 g
Sea water:	1000 ml
pH adjusted to 7.6 to 7.8.	

pH and Eh measurement

The pH and redox potential of the samples were measured with pH meter of Model DM-1A (Toa Electronics Ltd.) and redox meter of Model RM-1 (Toa Electronics Ltd.) respectively.

Estimation of loss on ignition

In order to evaluate approximately the amount of organic matter in a sediment, loss on ignition was determined by ashing the sediment samples in a electric furnace at about 700°C (HANNA, 1914).

Vitamin assays

Amounts of vitamin B₁₂, thiamine and biotin in the sediments were determined by microbiological assay method. The assay organisms and the extraction procedures used are as follows;

Vitamin B₁₂ Samples were extracted for 10 minutes at 120°C in phosphate-citrate buffer (pH 4.5) containing 0.01 % of KCN. Aliquots of each extract were assayed using *Lactobacillus leichmannii* ATCC 7830 (IFO 3376*) as an assay organism (The Association of Vitamin Chemists, 1966).

Thiamine Samples were extracted for 30 minutes at 100°C in 0.2 N sulfuric acid solution. Assay organism used was *Lactobacillus fermenti* ATCC 9338 (IFO 3071) by the method of CHELDELIN *et al.* (1946).

Biotin Samples were extracted for 30 minutes at 120°C in 0.2 N sulfuric acid solution. *Saccharomyces cerevisiae* ATCC 7754 (IAM 4626**) was used as an assay organism (HERTZ, 1943).

Cultures of *Lact. leichmannii* and *Lact. fermenti* were provided from the Institute for Fermentation, Osaka, and culture of *Sacch. cerevisiae* from the Institute of Applied Micro-

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

** Culture number of the Institute of Applied Microbiology (IAM), University of Tokyo.

Table 2. Vitamin contents and other characteristics of the sediments collected at the Aburatsubo Inlet (July 23, 1968).

Station	Water depth (m)	Depth in core (cm)	pH	Eh (mV)	Loss on ignition (%)	Heterotrophs* (cells/g wet mud)	Vitamin contents**		
							Vitamin B ₁₂ (mμg/g)	thiamine (μg/g)	biotin (mμg/g)
St. A	5.8	0-2	8.8	394	20.6	1.2×10^7	7.14	0.50	1.3
		2-5	8.2	404	21.2	2.0×10^6	9.30	0.80	2.3
		5-10	8.3	64	14.7	1.0×10^6	6.80	0.68	1.8
		10-15	8.6	24	24.3	1.0×10^7	5.28	0.65	0.5
		15-20	8.3	-76	18.0		2.00	0.52	1.2
		20-25	9.0	244	15.8	4.0×10^6	1.83	0.45	0
St. B	2.0	0	7.9	297	9.5	4.0×10^6	1.99	0.37	0.8
St. C	5.4	0	7.6	139	19.0	4.0×10^7	2.14	0.53	0.9
St. D	1.0	0	7.6	334	8.0	6.0×10^6	1.14	0.26	2.6
St. E	6.0	0-2	8.9	324	18.6	1.0×10^7	4.94	0.16	1.4
		2-4	8.8	314	17.7	2.0×10^7	3.06	0.23	1.3
		4-8	8.8	244	13.9	1.0×10^7	3.58		1.7
		8-15	8.6	224	18.2	1.0×10^7	4.41	0.09	1.1
		15-20	8.6	174	15.7		5.83	tr***	1.1
		20-25	9.0	288	11.0		3.83	tr	0.6
		25-30	9.0	214	9.7		2.07	tr	0.5

* These numbers were estimated by the surface colony counts method.

** Values per dry weight are represented.

*** tr represents undetectable amounts.

Table 3. Vitamin contents and other characteristics of the deep sediments collected at the Japan Trench and its adjacent region.

Station number	Depth in core (cm)	pH	Eh (mV)	Loss on ignition (%)	Heterotrophs* (cells/g wet mud)	Vitamin contents**		
						vitamin B ₁₂ (mμg/g)	thiamine (μg/g)	biotin (mμg/g)
1	0	7.6	363	6.0		0.18	0.11	2.1
2	0	7.0	408	6.3	1.0×10^4	0.20	0.12	1.8
3	0	7.0	408	5.6	1.5×10^4	0.29	0.16	1.4
4	0	7.8	338	7.3	1.0×10^5	0.14	0.10	2.4
5	0-5	8.2	328	13.9	1.2×10^2	1.20	0.39	1.5
	15-20	8.4	359	4.5	3.0×10^2	0.30	0.11	0.9
6	0-5	7.6	322	5.4	6.0×10^2	2.10	0.06	1.5
	15-20	8.6	375	4.3	5.0×10	0.46	0.06	1.3
7	0	8.5	361	5.2	1.0×10^5	2.80	0.09	2.4
8	0-5	8.3	351	4.9	1.7×10^3	0.76	0.15	1.7
	15-20	8.5	362	5.5		0.29	0.05	1.6

* These numbers were estimated by the membrane filter counts method.

** Values per dry weight are represented.

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3. Results

Vitamin contents and other characteristics of the sediments collected at the different 41 stations are presented in Tables 2, 3, 4, 5 and 6.

Nature of the sediment samples

The sediments from St. A, St. C and St. D

of the Aburatsubo Inlet were similar in appearance, consisting of muds, having a slight odour of hydrogen sulfide. The values of loss on ignition and the population of heterotrophic bacteria in the samples were also similar levels and did not have clear trend from surface to 30 cm depth of core. The sediments from St. B, composed of granule, and the sample from St.

Table 4. Vitamin contents and other characteristics of the sediments collected at the Mid-Pacific Ocean.

Station number	Depth in core (cm)	pH	Eh (mV)	Loss on ignition (%)	Heterotrophs* (cells/g wet mud)	Vitamin contents**		
						vitamin B ₁₂ (mμg/g)	thiamine (μg/g)	biotin (mμg/g)
9	0-5	7.8	321	9.4	3×10 ⁶	1.32	0.79	1.7
	10-15	8.0	332	9.1	8×10 ⁵	1.57	1.07	1.5
	30-35	7.1	379	10.0		0.79	0.79	0.7
	45-50	7.6	310	9.2		2.99	0.27	0
10	0	8.6	365	9.1	below 10 ³	0.64	0.14	6.2
11	0	8.6	366	26.4	9×10 ⁶	1.03	0.13	4.8
12	0	8.3	346	19.7	2×10 ⁴	1.06	0.10	8.1
13	0-5	8.5	364	22.5	7×10 ³	0.93		7.3
	30-35	8.7	373	12.1	below 10 ³	0.63		3.1
	60-65	8.7	372	24.7	below 10 ³	1.01		tr***
14	0-5			26.6	5×10 ²	1.47		
	20-25			14.5	8×10 ³	tr		
	75-80			13.8	6×10 ²	tr		
15	0-5			8.6	3×10 ³		tr	5.1
	20-25			10.0	5		1.15	4.0
	85-90			16.3	below 10		0.74	4.2
16	0			5.5	below 10		0.23	2.4
17	0-5			13.4	2×10 ⁴		0.91	4.9
	20-25			10.2	3×10 ²			3.9
	45-50			11.7	1×10 ³			7.6

* These numbers were estimated by the surface colony counts method.

** Values per dry weight are represented. *** tr represents undetectable amounts.

Table 5. Vitamin contents and other characteristics of the sediments collected at the Eastern China Sea.

Station number	pH	Eh (mV)	Loss on ignition (%)	Heterotrophs* (cells/g wet mud)	Vitamin contents**		
					vitamin B ₁₂ (mμg/g)	thiamine (μg/g)	biotin (mμg/g)
18	8.2	347	3.6		0.06	0.26	0.9
19	8.0	337	9.5	3.5×10 ⁵	0.45	0.39	1.8
20	8.5	335	8.2		0.98	0.15	1.0
21	8.4	330	12.0		1.37	0.31	3.7
22	7.8	319	9.9		1.93	1.34	3.4
23	8.2	317	4.2		0.97	0.24	0.6
24	8.5	337	2.9	1.6×10 ⁶	0.95	0.15	2.0
	8.1	325	4.4		0.40		0.4
	8.6	346	4.0		1.18	tr***	0.2
27	8.3	325	5.8		0.94	0.14	0.9
28	8.6	346	6.4	2.2×10 ⁵	1.10	0.20	1.7
29	8.2	340	7.2		0.75	0.64	2.2
30	7.9	326	7.3		0.59	0.75	1.4
31	8.4	335	12.8		0.82	0.21	2.0
32	8.4	329	5.9		1.22	0.33	2.1
33	8.7	346	6.0		1.17	tr	0.4
34	8.5	340	6.3	1.7×10 ⁵	0.50	tr	1.1
	7.5	303	7.0		0.44	0.80	2.0
	8.0	352	6.4		0.31	0.63	2.4
37	8.4	332	4.8	1.3×10 ⁵	0.19	0.10	0.5
	8.3	326	1.5		0.30	tr	1.0

* These numbers were estimated by the most probable number method.

** Values per dry weight are represented. *** tr represents undetectable amounts.

Table 6. Vitamin contents and other characteristics of the sediments collected at the Suruga Bay and the Sagami Bay.

Station number	Depth in core (cm)	Loss on ignition (%)	Heterotrophs* (cells/g wet mud)	Vitamin contents**		
				vitamin B ₁₂ (mμg/)	thiamine (μg/g)	biotin (mμg/g)
39	0	4.3	9.2×10 ⁴	0.57	0.12	2.5
40	0	5.3	5.4×10 ⁵	0.40	0.06	0.6
41	0-3	15.8	7.9×10 ⁴	tr***	0.70	5.7
	6-9	15.2		1.14	0.44	4.9
	12-15	10.9		0.39	0.35	5.9
	21-24	11.6	3.3×10 ³	0.27	0.21	1.1
	33-36	10.2		0.34	0.14	4.5
	45	10.4		0.34	0.15	2.9

* These numbers were estimated by the most probable number method.

** Values per dry weight are represented.

*** tr represents undetectable amounts.

D, composed of sand had lower values of loss on ignition.

The pH values of the deep sea sediments of the Japan Trench lay between 7.0 and 8.7, and the Eh between 322 and 408 mV. These values are close to others found for red clay and other deep sea sediments (BAAS BECKING, KAPLAN and MOORE, 1960). The population of heterotrophic bacteria in the deep sea sediments enumerated by the membrane filter counts method was range from 10² to 10⁴ per gram (wet weight).

The sediment samples of the Mid-Pacific Ocean were composed of red clay in the area deeper than about 4,500 meters, on the other hand, the shallower sediments were consisted of foraminifera ooze. All the samples showed quite oxidizing conditions.

The surface sediments collected at the Eastern China Sea, consisting of fine sand or silt, showed lower values of loss on ignition than the sediments at the other parts used in this study. Population of heterotrophic bacteria estimated with MPN counts method was range from 10⁵ to 10⁶ per gram (wet weight).

Distribution of vitamins in the surface sediments

Surface samples from the Aburatsubo Inlet have been found to range from 1.14 to 7.14 mμg of vitamin B₁₂ per gram of dry weight, 0.16-0.53 μg of thiamine and 0.8-2.6 mμg of biotin. Except for the biotin content of St. D, vitamins were found more abundantly in the muddy sediments (St. A, St. C and St. E) than in the sandy or granular sediments. This result agrees

Table 7. Analysis of variance of logarithmic transformed values of three vitamin contents in the sediments from five different areas.

Vitamin B ₁₂				
Factor	s.s.	d.f.	m.s.	F ₀
Area	4193.2	4	1048.3	4.40**
Residual	8569.3	36	238.0	
Total	12762.5	40		

** Significant at 1% level.

Thiamine				
Factor	s.s.	d.f.	m.s.	F ₀
Area	148.6	4	37.2	0.44
Residual	3059.4	36	85.0	
Total	3208.0	40		

Not significant at 5% level.

Biotin				
Factor	s.s.	d.f.	m.s.	F ₀
Area	20402	4	5101	5.88**
Residual	32977	38	868	
Total	53379	42		

** Significant at 1% level.

well with the observation by STARR (1956).

Both vitamin B₁₂, thiamine and biotin were found in significant amounts in the deep sea sediments from the Japan Trench. In average concentrations were observed 0.80 mμg of vitamin B₁₂ per gram, 0.17 μg of thiamine and 1.7 mμg of biotin in the sediments at this region. These values were not so lower than the values found in the samples from the other shallower areas.

Fig. 5. The mean with the 95 % confidence interval of the estimated values of three vitamins from five different areas.

Fig. 6. Profile of distribution of vitamins and some environmental factors in the core collected at St. A in the Aburatsubo Inlet (July 23, 1968).

In order to confirm if there exist significant differences in the concentrations of each vitamin in the surface sediments among the five different areas, the data were examined statistically by the analysis of variance. The results obtained are given in Table 7. According to the result, the significant differences were defined in the vitamin B₁₂ contents and biotin contents among the five

different areas at 1 % level, on the other hand, thiamine contents were found not to be significant at 5 % level.

The estimated mean values of vitamin contents with the 95 % confidence intervals in the sediments at the five areas are shown in Fig. 5. It could be considered from this figure that the concentrations of vitamin B₁₂ in the sediments

Fig. 7. Profile of distribution of vitamins, population of heterotrophic bacteria and value of loss on ignition in the core collected at St. E. in the Aburatsubo Inlet (July 23, 1968).

Fig. 8. Profile of distribution of vitamins and value of loss on ignition in the core collected at St. 41 in the Sagami Bay.

at the Aburatsubo Inlet were relatively higher than the concentrations at the other areas, whereas, there were not so much differences in the thiamine contents among the five areas. The sediments at the Mid-Pacific Ocean seemed to be richer in biotin than the sediments at the other areas.

Vertical distribution of the vitamins in the sediments

Vertical distribution of each vitamin in the sediments seems to have the general tendency that there exists the general decreasing slope even if it sometimes increases along the core depths. The examples of profile of distribution

Fig. 9. Relation between vitamin B₁₂ contents and value of loss on ignition in the sediments examined by the Sign Test.

of vitamins and some environmental factors in the cores are given in Fig. 6, Fig. 7 and Fig. 8.

Relation between the vitamin contents and the environmental factors

Relation between the contents of each vitamin and each environmental factor was examined statistically by the Sign Test. It will be obvious from Fig. 9 that there exists significant correlation between vitamin B₁₂ contents and values of loss on ignition in the sediments at 5% level.

A significant correlation was found also between thiamine contents and values of loss on ignition at 1% level, as shown in Fig. 10, but no clear relationship between biotin contents and values of loss on ignition was found. There could not be found any correlation between the contents of each vitamin and population of heterotrophic bacteria, pH values or Eh values.

4. Discussion

The assay organism, *Lactobacillus leichmannii*, responds to several of the vitamin B₁₂-factors

Fig. 10. Relation between thiamine contents and value of loss on ignition in the sediments examined by the Sign Test.

and deoxiribosides except for factor B, factor D and factor E (COATES and FORD, 1955). BURKHOLDER and BURKHOLDER (1956) assayed marsh muds collected along the coast of Georgia for vitamin B₁₂ by *Escherichia coli* mutant 313, and reported 0.670–0.757 µg of vitamin B₁₂ per gram of mud (dry weight). By the same assay organism, STARR (1956) found average of 0.13 µg of vitamin B₁₂ per gram in the marsh muds and average of 0.07 µg per gram in the sandy sediments. *E. coli* is known to be able to make use of most of the known naturally occurring forms of vitamin B₁₂. Although the vitamin B₁₂ concentrations of shallow muds in the Aburatsubo Inlet assayed by the present authors were remarkably different from those of the marsh muds, which were obtained by the former authors, these discrepancies may be somewhat attributable to the difference of the assay organisms used in each case. HASHIMOTO (1954) observed 8–38 mµg of vitamin B₁₂ per gram in bottom muds

of the fresh water ponds by using *Euglena gracilis* as an assay organism. *Euglena gracilis* responds to several of the vitamin B₁₂-factors, in much the same fashion as *Lact. leichmannii* (COATES and FORD, 1955). These values were similar levels to the values found in the sediments from the Aburatsubo Inlet.

Incubation time of thiamine assay by *Lact. fermenti* was adopted 24 hours in the present study. Higher values of thiamine were obtained by the assay organism, *Lact. fermenti*, than those assayed by *Lact. viridescens*, which is known to respond only to intact molecules of thiamine, in the analysis of the same samples. This difference may be taken to indicate that *Lact. fermenti* responds also to pyrimidine and thiazole moieties in addition to thiamine at the assay condition adopted. HUTCHINSON (1943) measured 2.2-3.1 μg of thiamine per gram of mud (dry weight) collected at Linsley Pond, by using *Phycomyces blackesleeanus*, which responds to both thiazole and pyrimidine moieties. On the other hand, ROULET (1948) found 19.3 $\text{m}\mu\text{g}$ per gram of thiamine in the soil by the same assay organism.

Besides biotin, *Saccharomyces cerevisiae* makes use of desthiobiotin, biocytin and other spectrum of biotin-vitamins (ROBINSON, 1966). Values of biotin contents of the sediments, therefore, is considered to represent the values of total biotin. ROULET (1948) found 0.62 $\text{m}\mu\text{g}$ of biotin per gram of soil at depth of 10 cm, decreasing to 0.23 $\text{m}\mu\text{g}$ per gram at 50 cm. He also observed seasonal variation of the biotin contents in the surface soil.

Almost all the core samples used in the present study for assay of vitamins showed oxidizing condition. Although the concentrations of vitamin B₁₂, thiamine and biotin fluctuated irregularly, there seemed to be a gentle decreasing tendency with depth in the core. According to DEGENS (1965), the concentrations of amino acids in the reducing sediments of Santa Barbara Basin, California, do not systematically decrease or increase with depth, and the values fluctuate around the 500 $\text{m}\mu\text{g}$ level per gram of sediment. While, amino acids in oxidizing sediments show a trend to diminish with depth, probably approaching "steady state" conditions at a depth of 2.5

meters. If the sediment cores having reducing condition were examined, the different patterns of the vertical distributions of vitamin B₁₂, thiamine or biotin might be observed similarly as those of amino acids.

Vitamins found in sediments are considered to be mainly originated with the activities of microbes. LOCHHEAD (1957), in his study on the synthesis of B-vitamins by the predominant bacterial flora, found that out of 107 isolates from the soil, 35.5 % of them were thiamine producers, 19.6 % biotin producers, 39.2 % riboflavin producers, 29.9 % vitamin B₁₂ producers and 50.5 % producers of one or more factors. As for marine bacteria, it has been reported that physiologically significant amounts of biotin and thiamine were produced by about half of the 1,054 isolates, while nicotinic acid and vitamin B₁₂ were synthesized by somewhat fewer isolates (BURKHOLDER, 1963). Preliminary result of the present authors has also shown that out of 68 strains isolated from the sediments at the Mid-Pacific Ocean, 20 cultures synthesized significant amounts of biotin, 18 isolates of vitamin B₁₂ and 12 isolates of thiamine. Accordingly, it might be assumed from the above-mentioned results that the bacterial population and their activity producing vitamins would influence on the vitamin content of a sediment *in situ* as one of the environmental factors.

The activities of bacteria are influenced by many environmental factors, such as temperature, pH value, Eh value, organic matter etc. (ZOBELL, 1946). In the present study, pH value, Eh value, organic matter approximated by value of loss on ignition and population of heterotrophic bacteria in the sediments were examined as to the relations with vitamin contents. Positive correlations between values of loss on ignition and vitamin B₁₂ or thiamine contents were found statistically. On the other hand, no clear relationship was found between values of loss on ignition of sediments and their biotin contents. Relations between vitamin contents and population of heterotrophic bacteria, pH values or Eh values did not show any correlations in the present study. It would be possible, however, from the previously mentioned findings (LOCHHEAD, 1957; BURKHOLDER, 1963) that the content of each

vitamin was related to the bacterial population producing each vitamin.

As to the concentrations of vitamins dissolved in sea water, it has been reported that vitamin B₁₂ in the North Pacific Ocean was from undetectable amounts to 3.7 $\mu\text{g/l}$ (CARLUCCI and SILBERNAGEL, 1966), thiamine in the Pacific Ocean 0 to 147 $\mu\text{g/l}$ (NATARAJAN and DUGDALE, 1966) and biotin in the Gulf of Mexico 0 to 4.6 $\mu\text{g/l}$ (LITCHFIELD and HOOD, 1965). It has been also reported that inland and coastal waters contain higher levels of vitamins. VISHNIAC and RILEY (1961) observed 3.9–16 $\mu\text{g/l}$ of vitamin B₁₂ in Long Island Sound, NATARAJAN and DUGDALE (1966) found an average of 184 $\mu\text{g/l}$ of thiamine and CARLUCCI and BELSER (1963) observed 10 $\mu\text{g/l}$ of biotin in sea water off the pier at the Scripps Institution of Oceanography. Each vitamin content in the sediments, according to the present study, were about 1,000 times higher than that in sea water, which was reported by other authors. This finding seems to be very significant in view of the vitamin cycles in the sea, as it was suggested previously by PROVASOLI (1960) that the origin of vitamin B₁₂ could be located in the muds.

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海底土におけるビタミン B₁₂, チアミン, ビオチンの分布

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要旨 東支那海大陸棚水域, 北西および中部太平洋水域, 駿河湾, 相模湾, 油壺湾などの観測点から41個の異なった海底土試料を採取し, それら試料中のビタミン B₁₂, チアミンおよびビオチン含量を測定した. これらビタミンの測定は微生物定量法によって行ない, 定量菌株としては, *Lactobacillus leichmannii* (ビタミン B₁₂), *Lactobacillus fermenti* (チアミン), *Saccharomyces cerevisiae* (ビオチン) が用いられた. 表層底土のビタミン B₁₂ の含量は, 内湾的な油壺湾に多くみられ (1.14~7.14 mμg/g), 日本海溝, 中部太平洋水域, 東支那海水域および駿河湾・相模湾水域などの海底上には少なかつ

た (0~2.10 mμg/g). チアミン含量は, これらの水域の間には顕著な差がみられなかった (平均 0.31 μg/g). ビオチン含量は, 中部太平洋水域の底土に多く (平均 5.0 mμg/g), 他の4水域では, 顕著な差が認められなかった (平均 2.0 mμg/g). 柱状試料における鉛直的变化をみると, ビタミン含量は一般的な傾向として上層から下層に減少することが認められた. 底土中のビタミン含量と環境要因との関係を調べた結果, ビタミン B₁₂ およびチアミン含量と底土の灼熱減量との間には, 正の相関関係が認められたが, ビオチンについては, この関係が認められなかった.