Study on the Scarcity of Red Tide in the Eutrophicated Waters of ARIAKE Bay—I.

Dissolution of Phosphate Ion from Bottom Mud by Hydrogen Sulfide

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In order to know the causes of the scarcity of red tide in the eutrophicated waters of ARIAKE bay, the characteristics of the bottom mud of this bay were investigated together with those of HAKATA bay, where benthos have been seriously damaged every summer by red tide. In both bays, the total N and total P in the mud were not so different, but phosphate-P removable from this mud by adding 1% sodium sulfide solution were remarkably different and the contents of this type of phosphate-P were estimated to range within 236 ± 227 mg/kg dry mud in ARIAKE bay and 80 ± 50 mg/kg dry mud in HAKATA bay, respectively. This phosphate was presumed to be ferric phosphate which is changed by soluble sulfides into ferrous sulfide liberating phosphate ion. The average content of sulfide-S in the mud in HAKATA bay was about 3.5 times more than that in ARIAKE bay. Thus, it was judged that hydrogen sulfide releases phosphate ion from the mud into the water in HAKATA bay causing red tide and can be destructed in ARIAKE bay because of the rapid tidal current which disperses this mud into the water with sufficient oxygen. Therefore, ferric phosphate, which is gradually utilized by phytoplankton with a high availability, is accumulated in the mud and never dissolved abruptly by hydrogen sulfide in ARIAKE bay. This was regarded to be the most important cause of the scarcity of red tide and, concurrently, of the high fishery productivity in ARIAKE bay. The liberation of phosphate ion from the mud by sodium sulfide was interrupted by adding calcium hydroxide.

In these several years, the cultures of yellow tails in Seto Sound have been damaged seriously in every summer by red tide due to such a dinoflagellate as *Chattonella* sp.^{1,2)} The cause of the emergence of these red tides is now regarded to be attributable to the eutrophication of waters. However, such outstanding red tides have never been observed in ARIAKE bay, although the situation of ARIAKE bay is changing into more eutrophicated condition owing to the fertilization of water with such as ammonium sulfate for culturing *Porphyra tenera* in large scale during winter season. We considered that the cause of the scarcity of red tide in this bay can associate either with the repression of phosphate supply from bottom mud into water or with the discharge of industrial wastes which contain toxic substances to kill such as dinoflagellates in this bay.

In ARIAKE bay except the coastal waters around SHIMABARA peninsula, there are well developed intertidal zones and such a range of tide as large as 5.0 m resulting in a rapid tidal current,

thereby, the mass of plankton can be scattered and the bottom mud can be enforced to be suspended into water saturated with oxygen. Consequently, hydrogen sulfide formed in the bottom mud can be oxidized, otherwise, such phosphate as ferric phosphate in the bottom mud can be converted into ferrous sulfide accompanying the dissolution of phosphate ion into water. However, the deficiency of hydrogen sulfide thus attained allows this type of phosphate to retain in the bottom mud. The repression of phosphate supply from mud thus attained can interrupt the blooming of phytoplankton before developing to a large scale of red tide.

It was reported that benzanthrone, which was found as the causal substance of algal tumor of *Porphyra tenera*¹⁾, is contained, in bottom mud in the vicinity of Ohmuta industrial city and the extremely low concentrations of this compound such as 0.2 to 0.4 ppb induce the mitotic delay, 1 ppb the mortality of a certain type of dinoflagellate, *Gyrodinium* sp.²⁾ Such compounds in the

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industrial wastes of Ohmuta have been discharged for many decades into ARIAKE bay and could be distributed widely in the bottom mud causing the destruction of dinoflagellates.

As mentioned above, we were able to refer two causes concerning about the scarcity of red tide in the eutrophicated waters of ARIAKE bay. In this study, we are intending to compare the phosphate supplies from bottom mud of both ARIAKE bay and the eastern half of HAKATA bay where red tide occurs so often in summer that no benthos can survive, and to confirm whether the oxidation of hydrogen sulfide in the bottom mud can deeply associate with the scarcity of red tide in ARIAKE bay or not.

Methods and Materials

For the propagation of phytoplankton so densely that it can be called "red tide", the supplies of inorganic nitrogen (N) and inorganic phosphorus (P) are necessary³⁾. Therefore, during the period of red tide, N and P in the bottom mud must transfer into water. Since the dissolution of these N and P from mud depends on the bacterial decomposition of organic substances in the mud, the content of carbon in the bottom mud can also change simultaneously. Since there are found benthic diatoms together with deposited phytoplankton on the bottom mud of shallow waters, such organic substances as chlorophylls and lipids can be extracted from the bottom mud with acetone. The bacterial decomposition of phytoplank-

ton in the mud can induce anaerobic condition and hydrogen sulfide can be formed. Injurious hydrogen sulfide⁴⁾ reacts with ferric hydroxide forming ferrous sulfide and sulfur. Phosphate ion liberated from phytoplankton by mineralization also reacts with ferric hydroxide forming ferric phosphate. If the differences on the contents of these compounds in the bottom mud of both eastern half of HAKATA bay and ARIAKE bay can be observed, the key for knowing the mechanism of the emergence of red tide may be found. Accordingly, we collected the bottom mud of these bays during or just after the summer of 1979 and determined the contents of chlorophyll, acetone extract, total carbon, total nitrogen, total phosphorus, total sulfide-S and total iron.

Sampling of Bottom Mud

The sampling points of both eastern half of HAKATA bay and ARIAKE bay are indicated in Figs. 1 and 2.

Since most of these sampling points are located in the areas covered with soft-bottom deposit, a small modified-EKMAN dredge was employed to collect these soft mud laying from bottom surface to the depth of 8 cm. At first the sampling was performed in ARIAKE bay at these sampling points shown in Fig. 2: at 6 points from F₁ to F₂₁ on July 4th, at 6 points from S₁ to S₆ on July 12th, at 7 points from K₁ to K₇ on August 3rd and at 5 points from N₁ to N₅ on August 4th, 1979. The sampling in the HAKATA bay was carried out on September 7th, 1979 at 15 points indicated in Fig.

Fig. 1. Sampling points of bottom mud in the Eastern half of HAKATA bay on Sept. 7th, 1979.

This bay has been eutrophicated by discharge of treated and untreated municipal sewages from towns with population more than one million and benthos have been seriously damaged in every summer by red tide mostly of phytoplankton other than dinoflagellates.

Fig. 2. Sampling points of bottom mud in ARIAKE bay during the summer of 1979.

This bay, especially northern part, has been eutrophicated by fertilizers such as ammonium sulfate for cultivating *Porphyra tenera* during winter season and discharge of industrial and municipal wastes, but never damaged by red tide, rather, nicely productive in fisheries.

1. The mud collected was put in a duplicated vinyl bag each about 2 kg, and frozen immediately in a dry ice box, then kept in a refrigerator at -19°C until their analyses began.

Preparation of Dry Mud

After defrosting of frozen mud, four different samples of mud each 1 kg or so were transferred into four steel tubes and subjected to centrifugation for 1 hour under 830 to $900\times g$. After this centrifugation, the separated water of these mud was removed. Since the mud of upper layer in each tube contained much water, this part was dehydrated using a BUCHNER's funnel under reduced pressure. Foreign materials such as shells or gravels in these mud were removed by squashing them with fingers, then these mud were spread on plastic screens and dried in an air conditioned room for 24 hours. After being dried, these mud were powdered using a mixer of Toshiba JC 4000 type and the powdered mud were passed through a sieve with 40 meshes. The solid percentages of these

powdered mud ranged from 95.6 to 98.3% in sandy mud and from 79.5 to 94.5% in soft mud. These were kept in polyvinyl plastic bottles until used for analysis.

Analyses of Total Chlorophylls and Acetone Extracts in Mud

Either 35 or 40 g powdered sample of mud was extracted with acetone using a SOXHLET apparatus and the extraction was continued until any pigment does not seep out the paper extraction thimble. After the evaporation of acetone and the removal of slight amount of moisture from extracts in the extraction flask using a vacuum desiccator, the acetone extracts were obtained by subtracting the weight of the extraction flask from the total weight.

Then, the acetone extracts were dissolved with 35 or 40 ml acetone so that the extracts from 1 g mud were contained in 1 ml acetone. After the dilution of this solution more than three to ten times with acetone, the solution was transferred into a 1 cm absorption cell. According to "tri-

chromatic method" presented by JEFFREY and HUMPHREY,⁵⁾ the absorbances at the wave length of 630, 647, 664 and 730 nm were measured using a Hitachi Model 101 spectrophotometer, the contents of chlorophyll a, b and $c_1 + c_2$ were calculated in making use of three equations presented by them and converted to the contents in 1 kg dry mud. The sum of these contents was regarded to be total chlorophyll in mud.

Analyses of Total Carbon (C) and Total Nitrogen (N) in Mud

After the removal of moisture from these samples of powdered mud in an oven at 110°C, the total carbon and nitrogen in these mud were analyzed using a Yanagimoto high speed C. H. N. Corder MT type 2 at the Service Center of the Elementary Analysis of Organic Compounds in Kyushu Univ.

Analysis of Total Phosphorus (P) in Mud

A 400 mg powdered mud sample was taken into a 50 ml Kjeldahl flask and 5 ml (1:1) sulfuric acid was added to it together with 3 drops of 30% hydrogen peroxide. The flask was heated for the complete digestion. The content in the flask was diluted to 100 ml with distilled water. To 8 ml distilled water in a test tube were added 2 ml of this digested solution and 1 ml of STRICKLAND's color developing solution.⁶⁾ After 10 min, the absorbance of the colored solution was measured in a 2 cm absorption cell at 885 nm. Standard solutions with phosphate-P from 0.1 to 1.0 ppm were colored and the absorbances were measured in the same way. The content of total phosphorus of the sample mud were computed out using these absorbances of both digested and standard solutions. The high concentration of ferric iron of the digested solution of mud seems to interfere the color development in the analysis of phosphate-P, but the STRICKLAND' color developing solution makes the concentration of ascorbic acid to be more than 10 times as large as that of ferric iron in the colored solution. Thus, the interference due to the high concentration of ferric iron is completely destroyed.

Analysis of Phosphate-P Eluted from Mud by 1% Na₂S Solution

A 400 mg powdered mud sample was taken into a 50 ml Kjeldahl flask and 20 ml 1% Na₂S solution was also added to the same flask. The flask was then kept for 1 hour in boiling water and cooled. The content in the flask was filtered using a filter paper under reduced pressure. The filtrate

was diluted to 100 ml with distilled water after acidic air-oxidation. A 2 ml portion of this diluted filtrate was added to 8 ml distilled water in a test tube and 1 ml STRICKLAND's color developing solution was also added to the same test tube. Similarly, the absorbance at the wave length of 885 nm was measured after 10 min and the content of phosphate-P eluted from mud was calculated.

Analysis of Total Iron in Mud

An appropriate amount of the sample of powdered mud ranging from 2 to 8 g was taken in a 50 ml beaker. After the ascertainment of the weight of mud, the mud was heated with 25 ml conc. HCl in the beaker for 45 min on a hot plate adjusted at 200°C. About 20 ml solution remained in the beaker was diluted with distilled water to 80 ml or so and filtered through a filter paper fixed on a Millipore filter holder under reduced pressure. The mud remained on the filter paper was washed with distilled water. All the filtrates were combined and filled up to 100 ml with distilled water. A 100 μ l portion of this filtrate was taken and diluted to 50 ml with distilled water. The concentration of the total iron of this solution was measured by *o*-phenanthroline method⁷⁾ and the content of total iron in the sample mud was calculated in accordance with the equation described in the text.

Analysis of Sulfide-S in Mud

The mud frozen at -19°C was defrosted and mixed in the duplicated vinyl bag. About 5 g portion of this mud was taken into a 50 ml beaker and weighed quickly and accurately by a Mettler Type P-1000 analytical balance. This mud was subjected to steam distillation using Tomiyama's apparatus⁸⁾: the mud was charged in the apparatus with 30 ml distilled water and a flask containing 5 ml 1 M zinc acetate solution was prepared and so set that the outlet of a Liebig condenser was inserted into the solution of this flask. Hydrogen sulfide was distilled soon after the introduction of 5 ml (1:1) sulfuric acid and steamed into the apparatus. Distilled hydrogen sulfide was caught by the zinc acetate solution in the flask with condensed water. The distillation was continued until the condensate amounted to 50 ml. Together with 0.4 ml (1:1) sulfuric acid, 5.0 or 10.0 ml N/100 iodine solution was added to this distillate and the remained iodine was titrated with N/100 sodium thiosulfate solution using a 0.5% starch solution as an indicator. The error due to these procedures was made clear to range within $\pm 11\%$

of the mean by repeating the titration using the same amount of the same sample. In order to know the solid percentages of these sample mud, another 5 g of each mud was taken and dried up for 2 hours in an oven at 130°C, then weighed after being cooled. The solid percentage of sample mud was calculated from these weight of both dried and wet mud.

Test of Eluting Phosphate Ion from Bottom Mud with 0.1 N NaOH Solution

Since the 1% Na₂S aqueous solution indicated pH 12.35 at 25°C, the capability of this solution of eluting phosphate ion from mud might be ascribable to its alkaline pH. Thus, 20 ml 0.1 N NaOH solution was added to 400 mg dry mud in a 50 ml Kjeldahl flask and this flask was autoclaved at 120°C for 1 hour. After being cooled, the mixture was filtered through Toyo filter paper of 5 C using a Millipore filter holder under reduced pressure. After this filtrate was neutralized using phenolphthalein it was subjected to the same procedures for analyzing phosphate-P.

The Inhibition Test Due to Calcium Hydroxide against the Action of Sodium Sulfide Solution which Liberates Phosphate Ions from Mud

A 400 mg dried mud sample of ARIAKE bay which contains much phosphate removable by sodium sulfide was taken into a 50 ml Kjeldahl flask and 100 mg calcium hydroxide in 5 ml suspension was added to the same flask. The flask was then autoclaved at 120°C for 30 min and cooled. Into the flask, 20 ml 1% Na₂S solution was added and the flask was kept in boiling water for one hour. The content in the flask was filtered through Toyo filter paper of 5 C under reduced pressure. The filtrate thus obtained was adjusted to pH 2.8 to 3.5 by adding 100 μ l (1:1) sulfuric acid. The oxidation of hydrogen sulfide by atmospheric oxygen soon started and the consequent sulfur gave some turbidity to the solution. For the completion of this oxidation and elimination of free hydrogen sulfide, the solution was heated for a while on a hot plate kept boiling. After the solution was stood at room temperature overnight, it was filtered in the same way and neutralized by adding 1 N NaOH solution using phenolphthalein. After the total volume of this solution was adjusted to 100 ml with distilled water, its concentration of phosphate-P was measured by the method mentioned above and the amount of phosphate-P eluted from 1 kg dried sample mud was calculated.

Chemical Behavior of Ferric Phosphate to Sodium Sulfide

Phosphates present in bottom mud should be hard soluble in water. There can be seen such hard soluble phosphates as AlPO₄, FePO₄, Ca₃(PO₄)₂, Ca₁₀(PO₄)₆(OH)₂ and Ca₁₀(PO₄)₆F₂ in the bottom mud³⁾. Phosphate capable of releasing phosphate ion by adding sodium sulfide solution must form such a sulfide that is more insoluble in water than the original phosphate, because the substitution of phosphate group with sulfide group can be, thereby, attained. Among these phosphates, only ferric phosphate can correspond to this type of phosphate. Thus, we intended to confirm whether ferric phosphate behaves just as the phosphate which is present in mud and can release phosphate ion by adding 1% Na₂S solution or not. Since the authentic sample of ferric phosphate was not available, the purity of a reagent grade ferric phosphate with the formula of FePO₄·4H₂O was checked by analyzing phosphate-P in a certain weight of this compound and confirmed to be 89.9%. A crystal of Na₂S·9H₂O with the weight of 4.7129 g with the purity of Min. 90% was dissolved into 750 ml distilled water. The ferric phosphate with the weight of 2.9344 g was so added to this sodium sulfide solution as to satisfy the condition for the reaction advancing as follows:

If this reaction can be realized, the recovery of phosphate ion must be 100%. Therefore, after 3 and 6 hours from the start of this reaction, each 40 ml of this solution was taken from the reaction system and centrifuged at 11,000 rpm for 30 min. Then, each 100 μ l portion of this solution was taken and the liberated phosphate ion was analyzed by STRICKLAND's method.⁶⁾

Furthermore, we tried to add 1.9369 g FePO₄·4H₂O with the same purity of 6.2322 g Na₂S·9H₂O with the same grade dissolved in 750 ml distilled water. In this case, the amount of sodium sulfide was added twice by molecule as much as the first reaction needed. After 3 and 9 hours of the beginning of this reaction, the recovery of phosphate ion was checked, because we always added sufficient amount of sodium sulfide for eluting phosphate ion from the bottom mud of HAKATA and ARIAKE bays and we should know the recovery of these phosphate ion.

Moreover, we attempted to add 4.5874 g FePO₄·9H₂O with the same purity to 3.7081 g Na₂S·9H₂O with the same grade dissolved in 750 ml

distilled water. In this time, the amount of sodium sulfide was added half by molecule as much as the first reaction needed. After 6 hours of the start of this reaction, 40 ml of this solution was taken and centrifuged in the same way. From its upper layer, 100 μ l of this solution was taken and subjected to the analysis of phosphate-P, because if the reaction obeys the equation, the liberated phosphate ion of this reaction must corresponds to a half of the total phosphate in this reaction system.

In order to examine the similarity of the phosphate, which is removable from bottom mud by adding sodium sulfide solution, with this ferric phosphate, calcium hydroxide was added previously to ferric phosphate suspensions in these three cases. The amounts of calcium hydroxide added here were limited to 10 times by molar concentration as large as those of ferric phosphate, although calcium hydroxide was usually employed in the range of 90 to 350 times by molar concentration for the inhibition test due to calcium hydroxide against phosphate elution from mud by adding sodium sulfide solution. After three days from the addition of calcium hydroxide, the amounts of phosphate-P liberated from ferric phosphate were examined by adding sodium sulfide. The

details of these conditions of the reaction will be illustrated later in Table 9.

Results

Characteristics of Bottom Mud in HAKATA Bay and ARIAKE Bay

The contents of chlorophyll, total carbon, total nitrogen, total phosphorus, acetone extract, sulfide-S and total iron of bottom mud collected immediately after the summer of 1979 at fifteen sampling points in the eastern half of HAKATA bay and during the same summer at twenty-four sampling points in ARIAKE bay are listed in Tables 1 and 2. Their mean values, standard deviations and the correlation coefficients with the total chlorophyll are given near and at the bottom of individual columns.

Comparison of the Characteristics of Bottom Mud in HAKATA Bay and ARIAKE Bay

In Table 3, the means of total chlorophyll, C, N, P, acetone extract, sulfide-S and total iron of bottom mud in these two eutrophicated bays are listed with their standard deviations.

Total chlorophyll in the bottom mud of ARIAKE bay is 18.9 ± 9.2 mg/kg dry mud and that in

Table 1. Total chlorophyll, total carbon (C), total nitrogen (N), total phosphorus (P), acetone extract, sulfide-S and total iron (Fe) contents of the bottom mud of the eastern half of HAKATA bay on Sept. 7th, 1979

Sampling point	Total chlorophyll (mg/kg dry mud)	C (g/kg dry mud)	N (g/kg dry mud)	P (g/kg dry mud)	Acetone extract (g/kg dry mud)	Sulfide-S (g/kg dry mud)	Total Fe (g/kg dry mud)
4	0.75	10.271	0.157	0.266	0.238	0.013	5.3
10	4.13	10.720	0.352	0.289	0.431	0.030	17.1
12	20.30	22.115	1.372	0.518	1.673	0.273	29.4
9	40.98	28.247	2.241	0.639	4.264	0.493	33.9
14	30.11	37.320	3.183	0.453	3.577	0.696	31.2
28	32.90	30.043	1.412	0.496	2.146	0.368	41.5
20	28.88	28.030	1.807	0.545	4.910	0.245	36.6
21	33.67	26.737	1.675	0.491	2.485	0.335	35.1
23	49.93	27.290	2.141	0.328	3.567	1.748	35.4
26	10.15	12.404	0.854	0.384	0.761	0.163	40.8
25	48.06	23.910	1.984	0.494	3.890	1.357	33.1
24	65.00	26.021	2.180	0.554	8.053	1.435	31.7
19	33.18	47.366	2.129	0.509	2.572	0.487	24.6
18	74.09	37.692	3.219	0.632	4.726	1.055	27.1
17	41.55	33.462	2.286	0.612	3.271	0.977	34.5
Mean	34.25	26.775	1.799	0.481	3.104	0.645	30.5
St. Dev.	± 20.57	± 10.285	± 0.878	± 0.118	± 2.023	± 0.545	± 9.3
Cor. Coef. with total chlorophyll		+0.783	+0.679	+0.562	+0.781	+0.701	+0.355

Table 2. Total chlorophyll, total carbon (C), total nitrogen (N), total phosphorus (P), acetone extract, sulfide-S and total iron (Fe) contents of the bottom mud of ARIAKE bay during the summer from July 4th to August 3rd of 1979

Sampling point	Total chlorophyll (mg/kg dry mud)	C (g/kg dry mud)	N (g/kg dry mud)	P (g/kg dry mud)	Acetone extract (g/kg dry mud)	Sulfide-S (g/gk dry mud)	Total Fe (g/kg dry mud)
F ₁	4.72	5.538	0.692	0.475	0.536	0.095	29.1
F ₈	15.77	23.188	2.196	0.641	1.201	0.218	29.8
F ₁₁	28.06	25.627	2.337	1.057	2.285	0.100	48.4
F ₁₆	26.66	27.314	2.306	0.698	2.060	0.229	35.6
F ₁₇	8.74	23.859	0.667	0.086	0.607	0.068	54.9
F ₂₁	10.12	23.351	0.667	0.116	0.712	0.137	28.9
S ₁	31.71	25.089	2.445	0.843	2.114	0.442	64.3
S ₂	18.24	25.312	2.347	0.810	0.493	0.493	49.4
S ₃	40.41	27.825	2.462	0.838	2.576	0.595	34.5
S ₄	24.66	28.121	2.024	0.704	2.580	0.171	35.6
S ₅	7.40	29.012	0.643	0.402	1.867	0.164	17.1
S ₆	14.29	15.761	1.186	0.932	2.822	0.185	32.9
K ₁	7.91	9.804	0.295	0.086	1.019	0.088	34.4
K ₂	20.45	15.095	1.148	0.184	2.676	—	48.8
K ₃	20.95	19.890	1.597	0.900	1.399	0.223	66.6
K ₄	15.86	10.200	0.667	0.134	1.111	0.106	49.8
K ₅	14.45	6.988	0.947	0.125	2.614	0.062	57.6
K ₆	19.47	17.927	1.403	0.163	1.309	0.088	54.8
K ₇	14.94	9.318	0.774	0.102	0.680	0.074	33.3
N ₁	28.05	17.998	1.787	0.164	1.611	0.273	51.5
N ₂	31.77	19.502	2.289	0.181	1.689	0.141	60.4
N ₃	28.44	21.138	1.821	0.175	1.201	0.141	34.9
N ₄	5.66	10.415	0.520	0.092	0.450	0.055	15.4
N ₅	15.46	39.561	0.950	0.148	0.803	0.107	16.2
Mean	18.92	19.910	1.424	0.419	1.517	0.185	41.0
St. Dev.	±9.24	±8.348	±0.739	±0.342	±0.791	±0.143	±15.0
Cor. Coef. with total chlorophyll		+0.454	+0.831	+0.173	+0.568	+0.610	+0.480

Table 3. Comparison of the characters of bottom mud in the eastern half of HAKATA bay and ARIAKE bay. Figures in parentheses show the correlation coefficients between total chlorophyll and substances indicated uppermost.

Waters	Total chlorophyll (mg/kg dry mud)	C (g/kg dry mud)	N (g/kg dry mud)	P (g/kg dry mud)	Acetone extract (g/kg dry mud)	Sulfide-S (g/kg dry mud)	Total Fe (g/kg dry mud)
ARIAKE bay	18.9 ± 9.2	19.9 ± 8.4 (+0.454)	1.42 ± 0.74 (+0.831)	0.42 ± 0.34 (+0.173)	1.52 ± 0.79 (+0.568)	0.185 ± 0.143 (+0.610)	41.0 ± 15.0 (+0.480)
Eastern half of HAKATA bay	34.3 ± 20.6	26.8 ± 10.3 (+0.783)	1.80 ± 0.88 (+0.679)	0.48 ± 0.12 (+0.562)	3.10 ± 2.02 (+0.781)	0.645 ± 0.545 (+0.701)	30.5 ± 9.3 (+0.355)
Av. ratio between both waters	1: 1.81	1: 1.35	1: 1.27	1: 1.14	1: 2.03	1: 3.49	1: 0.74

HAKATA bay is 34.3 ± 20.6 mg/kg dry mud. The ratio between the contents of total chlorophyll of both ARIAKE bay and HAKATA bay is 1/1.8. The contents of C, N and P in the mud

of ARIAKE bay are 19.9 ± 8.4 , 1.42 ± 0.74 and 0.42 ± 0.34 g/kg dry mud, respectively. Those of HAKATA bay are shown to be 26.8 ± 10.3 , 1.80 ± 0.88 and 0.48 ± 0.12 g/kg dry mud, respectively.

The ratios of these contents of C, N and P in the mud of ARIAKE bay to those of HAKATA bay are 1/1.35, 1/1.27 and 1/1.14, respectively. Clearly, these contents of C, N and P in both waters are not so different when their differences are compared with the difference found on the total chlorophyll in these two waters. However, these acetone extract of ARIAKE bay is 1.52 ± 0.79 g/kg dry mud and that of HAKATA bay is 3.10 ± 2.02 g/kg dry mud. Thus, the average contents of acetone extract in HAKATA bay is approximately twice as large as that of ARIAKE bay. The sulfide-S in the mud of ARIAKE bay is 0.185 ± 0.143 g/kg dry mud, although that of HAKATA bay amounts to 0.645 ± 0.545 g/kg dry mud. The ratio of the average content of sulfide-S in the mud of ARIAKE bay to that of HAKATA bay is 1/3.49. The total Fe in the mud of ARIAKE bay is 41.0 ± 15.0 g/kg dry mud and that of HAKATA bay is 30.5 ± 9.3 g/kg dry mud, respectively. The average content of total Fe in the mud of HAKATA bay is three fourth as small as that of ARIAKE bay.

"Na₂S Eluted Phosphate-P" in the Mud of HAKATA and ARIAKE Bays

In Tables 4 and 5, the contents of phosphate-P eluted from the mud of both HAKATA and

Table 4. Phosphate-P eluted from bottom mud of HAKATA bay by adding 1% Na₂S solution. The mud was collected on Sept. 7th, 1979 and dried in an air conditioned room.

Sampling point	PO ₄ -P eluted with Na ₂ S sol. (mg/kg dry mud)	Total PO ₄ -P (mg/kg dry mud)	Eluted PO ₄ -P / Total P
4	8	266	0.030
10	1	289	0.003
12	43	518	0.083
17	67	612	0.109
18	88	632	0.139
19	39	509	0.077
24	95	554	0.171
25	178	494	0.360
26	95	384	0.247
23	159	328	0.485
21	45	491	0.092
20	75	545	0.138
28	88	496	0.177
14	124	453	0.274
9	100	639	0.156
Mean	80	481	0.169
St. Dev.	± 50	± 118	± 0.127

Table 5. Phosphate-P eluted from bottom mud of ARIAKE bay by adding 1% Na₂S solution. The mud was collected within one month from July 4th to August 3rd, 1979 and dried in an air conditioned room.

Sampling point	PO ₄ -P eluted with Na ₂ S sol. (mg/kg dry mud)	Total PO ₄ -P (mg/kg dry mud)	Eluted PO ₄ -P / Total P
F ₁	142	475	0.299
F ₈	164	641	0.256
F ₁₁	903	1,057	0.854
F ₁₆	378	698	0.542
F ₁₇	78	86	0.907
F ₂₁	81	116	0.698
S ₁	614	843	0.728
S ₂	333	810	0.411
S ₃	496	838	0.592
S ₄	415	704	0.589
S ₅	114	402	0.284
S ₈	591	932	0.634
K ₁	49	86	0.570
K ₂	130	184	0.707
K ₃	362	900	0.402
K ₄	94	134	0.701
K ₅	44	125	0.356
K ₆	47	163	0.288
K ₇	97	102	0.951
N ₁	143	164	0.872
N ₂	131	181	0.724
N ₃	107	175	0.611
N ₄	53	92	0.576
N ₅	86	148	0.581
Mean	236	419	0.589
St. Dev.	± 227	± 342	± 0.205

ARIAKE bays by adding 1% Na₂S aqueous solution are listed together with those of total phosphate-P. The ratios of the contents of "Na₂S eluted phosphate-P" to the contents of total phosphorus are also indicated in these same tables.

The contents of Na₂S eluted phosphate-P can be expressed statistically as 80 ± 50 mg/kg dry mud in HAKATA bay and 236 ± 227 mg/kg dry mud in ARIAKE bay, respectively. Evidently, the average content of Na₂S eluted phosphate-P in ARIAKE bay is almost three times as large as that of HAKATA bay. The ratios of the Na₂S eluted phosphate-P to the total phosphorus in HAKATA bay can be expressed as 0.169 ± 0.127 and those in ARIAKE bay as 0.589 ± 0.205 , respectively. The ratio of the average content of Na₂S eluted phosphate-P of ARIAKE bay to that of HAKATA bay is 3.49.

Table 6. Phosphate-P eluted from the bottom mud of HAKATA and ARIAKE bays by adding 0.1 N NaOH solution, successively autoclaving at 120°C for half an hour

Sampling point	NaOH eluted PO ₄ -P (mg/kg dry mud)	Eluted PO ₄ -P Total P	Sampling point	NaOH eluted PO ₄ -P (mg/kg dry mud)	Eluted PO ₄ -P Total P
4	4	0.015	F ₁	0	0
10	35	0.121	F ₃	0	0
12	51	0.098	F ₁₁	436	0.412
17	77	0.125	F ₁₆	67	0.096
18	87	0.138	F ₁₇	0	0
19	54	0.106	F ₂₁	21	0.181
24	91	0.164	S ₁	194	0.230
25	89	0.180	S ₂	156	0.193
26	7	0.018	S ₃	157	0.187
23	156	0.476	S ₄	117	0.166
21	56	0.114	S ₅	35	0.087
20	28	0.051	S ₈	235	0.252
28	46	0.093	K ₁	2	0.023
14	81	0.179	K ₂	4	0.021
9	33	0.052	K ₈	26	0.029
			K ₄	72	0.537
			K ₅	12	0.096
			K ₆	186	1.000
			K ₇	0	0
			N ₁	189	1.000
			N ₂	94	0.519
			N ₁	167	0.954
			N ₄	2	0.022
			N ₅	44	0.297
Mean	60	0.129	Mean	92	0.262
St. Dev.	±38	±0.106	St. Dev.	±132	±0.313

"0.1 N NaOH Eluted Phosphate-P" of Bottom Mud of HAKATA and ARIAKE Bays

In Table 6, the contents of "0.1 N NaOH eluted phosphate-P" of these bottom mud of HAKATA and ARIAKE bays are listed. The contents of this type of phosphate-P in HAKATA bay vary from 4 to 156 mg/kg dry mud and can be expressed as 60 ± 38 mg/kg dry mud. Similarly, the contents of the same type of phosphate-P in ARIAKE bay are observed in the range from 0 to 436 mg/kg dry mud and expressed as 92 ± 132 mg/kg dry mud. The ratios of this NaOH eluted phosphate-P to total phosphate-P can be given as 0.129 ± 0.106 in HAKATA bay and 0.262 ± 0.313 in ARIAKE bay. Since the ratios of the av. content of 1% Na₂S eluted phosphate-P to that of total phosphate-P in ARIAKE bay is 0.589 ± 0.205 , clearly the capability of 0.1 N NaOH solution for eluting phosphate-P from bottom mud is by far weaker than that of 1% Na₂S solution in ARIAKE bay. It is also obvious that the mud of HAKATA bay which did not release so much amounts of phosphate-P by adding 1% Na₂S solution also do not release so much

amounts of phosphate-P by adding 0.1 N NaOH solution.

Inhibiting Action of Calcium Hydroxide against the Elution of Phosphate Ion from Bottom Mud Due to Sodium Sulfide Solution

In Table 7, the amounts of phosphate-P eluted by 1% Na₂S solution from the bottom mud of ARIAKE bay which were previously autoclaved at 120°C for half an hour with calcium hydroxide suspension are indicated.

Table 7. Phosphate-P eluted from bottom mud of ARIAKE bay by adding 1% Na₂S solution after autoclaving at 120°C for half an hour with calcium hydroxide suspension. In this case, 5 ml 2% Ca(OH)₂ suspension was added to each 400 mg dry mud.

Sampling point	Dried mud taken (mg)	Na ₂ S eluted phosphate-P (mg/kg dry mud)
12 points of F ₁ to S ₈	402.2 ± 0.7	0~4
12 points of K ₁ to N ₅	402.2 ± 0.8	0~1

The values of detected phosphate-P are extremely low. Therefore, the autoclave of these mud with calcium hydroxide suspension appears to convert the phosphate to the other type of phosphate which does not allow to release phosphate ion even when sodium sulfide is added.

To each 800 mg dried bottom mud of a sampling point in ARIAKE bay was added each 10 ml of 2, 1, 0.5, 0.25 and 0.125% calcium hydroxide suspensions, but substantially no liberation of phosphate was observed as shown in Table 8. If the reaction of calcium hydroxide with ferric phosphate proceeds obeying next equation,

Table 8. The effect of previous adding of calcium hydroxide suspension on eluting phosphate-P from a certain bottom mud of ARIAKE bay by adding 1% Na₂S solution

Ca(OH) ₂ added to mud (mg)	Mud taken (mg)	pH after adding Ca(OH) ₂ suspension	Eluted PO ₄ -P (mg/kg dry mud)
Not eluted by Na ₂ S solution but Ca(OH) ₂ suspension alone			
200	801.4	12.70	0
100	800.2	12.65	0
50	801.4	12.55	0
25	800.2	12.45	0
12.5	800.8	11.90	1
0	800.2	7.35	2
Autoclaved at 120°C for half an hour			
100	401.2		0
50	401.9		0
25	401.0		1
12.5	402.2		1
6.25	401.5		50
0	400.5		276
Stood at room temperature for 3 days			
100	401.1		5
50	401.0		2
25	401.2		4
12.5	401.8		14
6.25	401.4		58
0	400.7		287

the consequent Ca₃(PO₄)₂ can give the concentration of phosphate-P as high as 4.0 ppm because of the solubility of this calcium phosphate of 20ppm⁸⁾. This concentration is so high that it must be detected by STRICKLAND's molybdenum blue method.⁸⁾ Therefore, the reaction of calcium hydroxide with ferric phosphate can proceed obeying next equation the hint of which was given by STUMN and LECKIE.⁸⁾

To each 400 mg dried mud of the same sampling point was added each 5 ml of 2, 1, 0.5, 0.25 and 0.125% calcium hydroxide suspensions. These mixtures were autoclaved at 120°C for 30 min or kept at room temp for 3 days. To these mixtures was added each 20 ml 1% Na₂S solution and these mixtures were then kept at 100°C in boiling water for one hour, then filtered through fine filter paper. We detected, however, only negligible amounts of phosphate in these filtrates except two groups into which 0.125% calcium hydroxide suspension and no suspension was added, respectively. Thus, Ca₁₀(PO₄)₆(OH)₂ appears not to liberate phosphate ion when 1% Na₂S solution is added. Hence, it can say that calcium hydroxide can inhibit the elution of phosphate ion from ferric phosphate probably existing in bottom mud, even when hydrogen sulfide may be formed in the mud.

The Liberation of Phosphate Ion from Ferric Phosphate by Adding Sodium Sulfide Solution

In Table 9, the recovery of phosphate ion from ferric phosphate are given, when sodium sulfide solution was added to the suspension of ferric phosphate at various ratios.

From this table, it is clear that when the reaction between ferric phosphate and sodium sulfide proceeds at the initial molar ratios of [2: 3], [2: 6] and [2: 1.5], the recovery percentages of phosphate ion liberated from ferric phosphate amount to 83.4% after 3 hours and 84.9% after 6 hours, to 99.4% after 3 hours and 98.8% after 6 hours and to 68.4% after 6 hours, respectively. There are some differences between the actual and theoretical recovery-percentages of phosphate ion liberated from ferric phosphate. Although these recovery-percentages appear to deny the reaction in which two moles of ferric phosphate react with three moles of sodium sulfide, the cause of these underestimation and overestimation can be discussed later and the reaction presented by the equation can be justified.

From Table 9, it is clear that the addition of sodium sulfide did not help substantially the elution of phosphate ion from ferric phosphate, when calcium hydroxide was added previously to it and left for three days at room temperature. This nature of ferric phosphate is very similar to that of the phosphate in mud which stop the release of phosphate ion due to adding sodium sulfide solution, if the mud was previously treated with cal-

Table 9. Recoveries of phosphate ion liberated from ferric phosphate by adding sodium sulfide solution and those after being stood for 3 days with calcium hydroxide suspension the molar concentrations of which are 10 times as large as those of ferric phosphate

FePO ₄ ·4H ₂ O with purity of 89.9% in 750 ml pure water (g)	Ca(OH) ₂ with purity of Min. 97% in 750 ml pure water (g)	Na ₂ S·9H ₂ O with purity of Min. 90% in 750 ml pure water (g)	Molar concentration		Ratio in molar concentration	Phosphate-P liberated in molar concentration		Recovery
			FePO ₄	Na ₂ S		after 3 h.	after 6 h.	
2.9344	9.76	4.7129	0.01579	0.02355	2 : 2.98	0.01317	0.00025*1	1.9
2.9344	0	4.7129	0.01579	0.02355	2 : 2.98	0.01317	0.01317	83.4
1.9360	6.44	6.2322	0.01041	0.03113	2 : 5.98	0.01038	0.00095*1	84.9
1.9360	0	6.2322	0.01044	0.03113	2 : 5.98	0.01038	0.01031	9.2
4.5874	15.25	3.7081	0.02467	0.01852	2 : 1.50	—	0.00038*1	98.8
4.5874	0	3.7081	0.02467	0.01852	2 : 1.50	—	0.01687	2.3
4.5874	0	0	0.02467	0	—	—	0.00033*2	68.4
								1.3

*1 Detected after 3 day standing.

*2 Quickly precipitated by centrifugation, because of no protective colloid of ferrous sulfide.

cium hydroxide suspension.

Discussion

Liberation of Phosphate Ion from Bottom Mud by Adding Sodium Sulfide Solution

The addition of 1% Na₂S solution to the mud of both HAKATA and ARIAKE bays resulted in the liberation of phosphate ion into medium, even though the amounts of phosphate ion from the mud of HAKATA bay were observed to be much less than those of ARIAKE bay. In this study, we applied sodium sulfide for liberating this type of phosphate from sea bottom mud, but in natural waters hydrogen sulfide can do this action. Only such a liberation of phosphate from bottom mud can supply enough amounts of phosphate into water for phytoplankton thriving to red tide. Therefore, this phenomenon may attract the attention of scientists who are studying the mechanism of phytoplankton-blooming in waters.

If we see Table 3, it is clear that the average content of sulfide-S of the mud of HAKATA bay is 3.49 times as large as that of ARIAKE bay. The correlation coefficients between the contents of sulfide-S and total chlorophyll in the mud of both eutrophicated waters suggest that the formation of sulfide-S in these mud is deeply associated with phytoplankton deposited onto the bottom mud. The average content of total chlorophyll in the mud of HAKATA bay is 1.81 times as large as that of ARIAKE bay. Therefore, the tendency of the formation of hydrogen sulfide can be more intensive than in ARIAKE bay.

The range of tide in HAKATA bay is observed mostly less than 2 m and there are found only tiny intertidal zones. Such a situation does neither induce a rapid tidal current nor accelerate the oxidation of hydrogen sulfide in the bottom mud. This suggests that the mud of HAKATA bay can not contain so much amount of the phosphate which liberate phosphate ion into water by adding sodium sulfide solution, because hydrogen sulfide formed in the bed mud tends to liberate phosphate ion into water.

On the contrary, the range of tide in ARIAKE bay can be observed less than 4 m in the central and 5 m in the northern areas and there are located broad intertidal zones occupying 11% of the total area of this bay. These conditions induce so vigorous tidal current that disperse bottom mud into water with sufficient oxygen and accelerate the oxidation of hydrogen sulfide formed

in the bottom mud. This also suggests that the bottom mud of ARIAKE bay can contain far more amount of such a phosphate as ferric phosphate than HAKATA bay, because the less amount of hydrogen sulfide in the mud allow ferric phosphate to remain in the mud.

Liberation of Phosphate Ion from Ferric Phosphate by Adding Sodium Sulfide Solution

When sodium sulfide solution was added to hard soluble ferric phosphate, the resulted ferrous sulfide and remained ferric phosphate were expected to precipitate quickly. Nevertheless, they did not actually precipitate even when they were subjected to an intensive centrifugation. Later, we were able to know the fact that ferrous sulfide thus formed is colloidal¹³⁾. After the molar ratio between ferric phosphate and sodium sulfide was adjusted to be [2: 1.50] and the reaction was proceeded, we took the sample at first without centrifugation and analyzed phosphate-P. The recovery of this phosphate-P amounted to almost 100%. The reason was that this ferric phosphate also becomes colloidal after stirring with water and the colloidal ferrous sulfide resulted from the reaction acts as protective colloid interrupting the precipitation of colloidal ferric phosphate. Therefore, these high recoveries of near 100% or 68.4% is attributable to the incomplete precipitation of colloidal ferric phosphate due to colloidal ferrous sulfide observed in the medium even after the centrifugation. When the molar ratio between ferric phosphate and sodium sulfide was initially [2: 2.98], in the reaction system, the recovery of phosphate ion did not become 100%, but 83.4 or 84.9%. The reason is ascribable to the quick reaction of colloidal ferric phosphate with sodium sulfide and the delayed reaction of precipitable ferric phosphate with sodium sulfide. Without adding much excess amount of sodium sulfide, the period of this reaction as long as 6 hours appears insufficient.

Chemical Behavior of the Phosphate in Bottom Mud and Ferric Phosphate

The phosphate contained in the bottom mud of ARIAKE and HAKATA bays liberated phosphate ion into water by adding 1% Na₂S solution. Ferric phosphate also liberated phosphate ion by adding sodium sulfide in aqueous solution. The phosphate in the mud did not liberate phosphate ion by the addition of sodium sulfide after the treatment with calcium hydroxide. Ferric phosphate also ceased substantially the liberation of

phosphate ion by the addition of sodium sulfide after the treatment with calcium hydroxide. These similarity can allow us to regard both phosphate to be identical. Thus, the phosphate in the bottom mud can liberate phosphate ion by generated hydrogen sulfide obeying next equations of (1) to (4), or briefly (5).

The hydrogen ion resulted from these reactions can be neutralized by bicarbonate ion abundant in sea water as shown in equation(6).

When we added 0.1 N sodium hydroxide solution to the bottom mud of HAKATA and ARIAKE bays, the liberation of phosphate-P was observed in the range of 60 ± 38 mg/kg dry mud corresponding to $12.9 \pm 10.6\%$ of total P in the mud of HAKATA bay and 92 ± 132 mg/kg dry mud being equal to $26.2 \pm 20.9\%$ of total P in the mud of ARIAKE bay, respectively. In this case we added 0.1 N sodium hydroxide solution to these mud and subjected them to an autoclave at 120°C for half an hour, then filtered through fine filter paper which allows passing easily soluble sulfide but hardly insoluble sulfide. When the consequent filtrates were acidified just below the pH of the color change of phenolphthalein with sulfuric acid, many of them emitted hydrogen sulfide. This suggests that the reaction of liberating phosphate ion from these mud with 0.1 N sodium hydroxide solution does not obey equation (7), but equations (8), (2) and (3).

Productivities of Waters Depending on the Types of Ferric Phosphate-Dissolution

According to GOLTERMAN *et al.*^{10,11)}, phosphates in sediments seem not always available for algal growth and only part of these phosphates can be used by algae: ferric phosphate can be used entirely although the growth rate was smaller than with an equal amount of KH₂PO₄. Hydroxyapatite could only partly be used and Ca₈(PO₄)₂ could not be used. If this is true, phosphate ion from ferric phosphate in mud can be gradually supplied into water in ARIAKE bay and the fishery pro-

ductivity must be maintained at high level. Indeed, such a high productivity has been observed up to now in this bay. On the contrary, phosphate ion-supplied abundantly in a short period from mud into water can induce a high rate propagation of phytoplankton leading red tide. Such red tide have been repeatedly observed every summer in HAKATA bay and ruined the waters of the eastern half of this bay.

Thus, the less amount of phosphate-P eluted from the mud of HAKATA bay by adding 1% Na_2S solution must indicate the evidence that tells us the release of phosphate ion from ferric phosphate in the mud before the sampling of the mud. The high contents of total chlorophyll in the mud of HAKATA bay must show the corpses of phytoplankton thrived in water as red tide.

However, the correlation coefficient between total chlorophyll and total P of the mud of ARIAKE bay is very small as shown by $+0.173$. The reason of this low value is attributable to the high contents of ferric phosphate which can remain in the mud in the absence of soluble sulfide even after the oxidative decomposition of chlorophyll¹²⁾. If ferric phosphate are removed from the mud and organic phosphorous compounds are remained in the mud in ARIAKE bay, the correlation coefficient between the contents of chlorophyll and total P can become more larger than that observed in this bay. The reason is that these organic phosphorous compounds are more or less dependent on phosphate originated from phytoplankton with abundant chlorophyll.

Summary

Soluble sulfide such as hydrogen sulfide was made clear to be able to become the cause of the dissolution of phosphate ion from sea bottom mud. The phosphate which is capable of liberating phosphate ion from the bottom mud into water when met with soluble sulfide was examined and concluded to be ferric phosphate. Calcium hydroxide so interfered with the action of soluble sulfide as not to liberate phosphate ion from ferric phosphate in the bottom mud. The cause of the scarcity of red tide in ARIAKE bay was ascribed to the rapid tidal current which accelerate the oxidation of hydrogen sulfide in bottom mud by dispersing the bottom mud into water with sufficient oxygen.

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