# ON THE FERTILITY OF MATSUSHIMA BAY 1. SOME CHEMICAL PROPERTIES ON THE MARINE DEPOSITS

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## 1. SOME CHEMICAL PROPERTIES ON THE MARINE DEPOSITS

By

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## Introduction

Generally, marine deposits contain more or less organic material, chiefly originated from the marine plankton. In the open sea, most of the plankton become disintegrated usually after death, being attacked by bacteria and protozoa before reaching the bottom and only the undigested part is accumlated on the bottom as organic detritus. However, in shallow water such as inlets or estuaries, the sediments contain abundant organic materials derived from undecomposed plankton organisms. A part of these organic materials in the deposit are soon collected and digested mainly by filter-feeding bottom living animals. Sometimes, they are suspended again in the water as fragments by the physical action of the winds and tides, and leaving the humus, the remainder is finally converted into $CO_2$ and mineral nutrient salts which can be utilized by aquatic plants and phytoplankton. These mineralizations of organic deposits appear to be different in their velocity and their products by oceanographical conditions mainly either aerobic or anaerobic. On the other hand, it has been known that some toxic substances such as $H_2S$ are evolved from the organic deposits under the anaerobic condition, and they work to decrease the commercial productions of the bay and the coastal area.

From this view, it has been considered to be important to catch clearly the chemical nature of the organic deposits and the processes of their mineralization under various environmental conditions. As the first step, an attempt was made to determine the analytical methods for organic carbon and $\alpha$-amino acid nitrogen cotained in the organic deposits which are considered as the main elements derived from the carbohydate and protein in marine organisms. The method was applied to the organic deposits collected from Matsushima Bay.

## Materials

The muds used in the study were collected with the Ekman's sampler from Matsushima Bay from a depth of less than 4 m (in May, 1960). In this bay, there are several aquatic plants of which the eelgrass (*Zostera marina*) is most abundant and has wide distribution from spring to summer. The muds used for the analysis were passed through a sieve (0.5 mm in diameter) and then air dried. The sampling stations are shown in Fig. 1.

## Methods

### (1)  Ammonium nitrogen

Ten times of 10 per cent KCl were used for extracting the free ammonium nitrogen from the air dried muds. The suspension of mud in 10 per cent KCl was shaken for 30 minutes and then filtered through the filter paper (Toyoroshi No. 5C). The ammonium nitrogen in the KCl extract was determined by Bremner's method using Conway's apparatus (1).

The total nitrogen in the mud was obtaned by the micro-Kjeldahl procedure.

### (2)  Alpha-amino acid nitrogen

The mud was hyrolysed with 6 N HCl to measure the $\alpha$-amino acid nitrogen in the proteinous compounds of the mud and the hydrolysate was used for the determination of $\alpha$-amino acid and ammonium nitrogen and organic carbon.

The $\alpha$-amino acid nitrogen was calculated from the amount of $CO_2$ liberated by the treatment with ninhydrin according to Van Slyke's method (2). This method is based on the fact that the $\alpha$-amino acids, when boiled in water with excess of ninhydrin at pH 1 to 5, evolve quantitatively the $CO_2$ of their carboxyl groups. In this paper, the decarboxylation was carried out at pH 2.5 and $CO_2$ was measured with Van Slyke's apparatus (2).

Since the hydrolysate contains much inorganic salts, the interference of these salts towards the ninhydrin reaction was examined and the results showed that the salts had no interference as show in Table 1 and 2. Consequently, the hydrolysate could be used directly for the measurement of carboxyl nitrogen after neutralization.

Table 1.  The measurement of carboxyl nitrogen (COOH-N) in the presence of salts.

| Salts | Aqua | Satu. NaCl | 1% NaAlO$_2$ | 1% Fe SO$_4$6H$_2$O + 1% MgSO$_4$7H$_2$O |
|---|---|---|---|---|
| L–Leucine | 0.137<br>0.141<br>0.138 | 0.139<br>0.142<br>0.137 | 0.137<br>0.138<br>0.138 | 0.136<br>0.136<br>0.139 |

Note :  The theoretical value is 0.140mg.
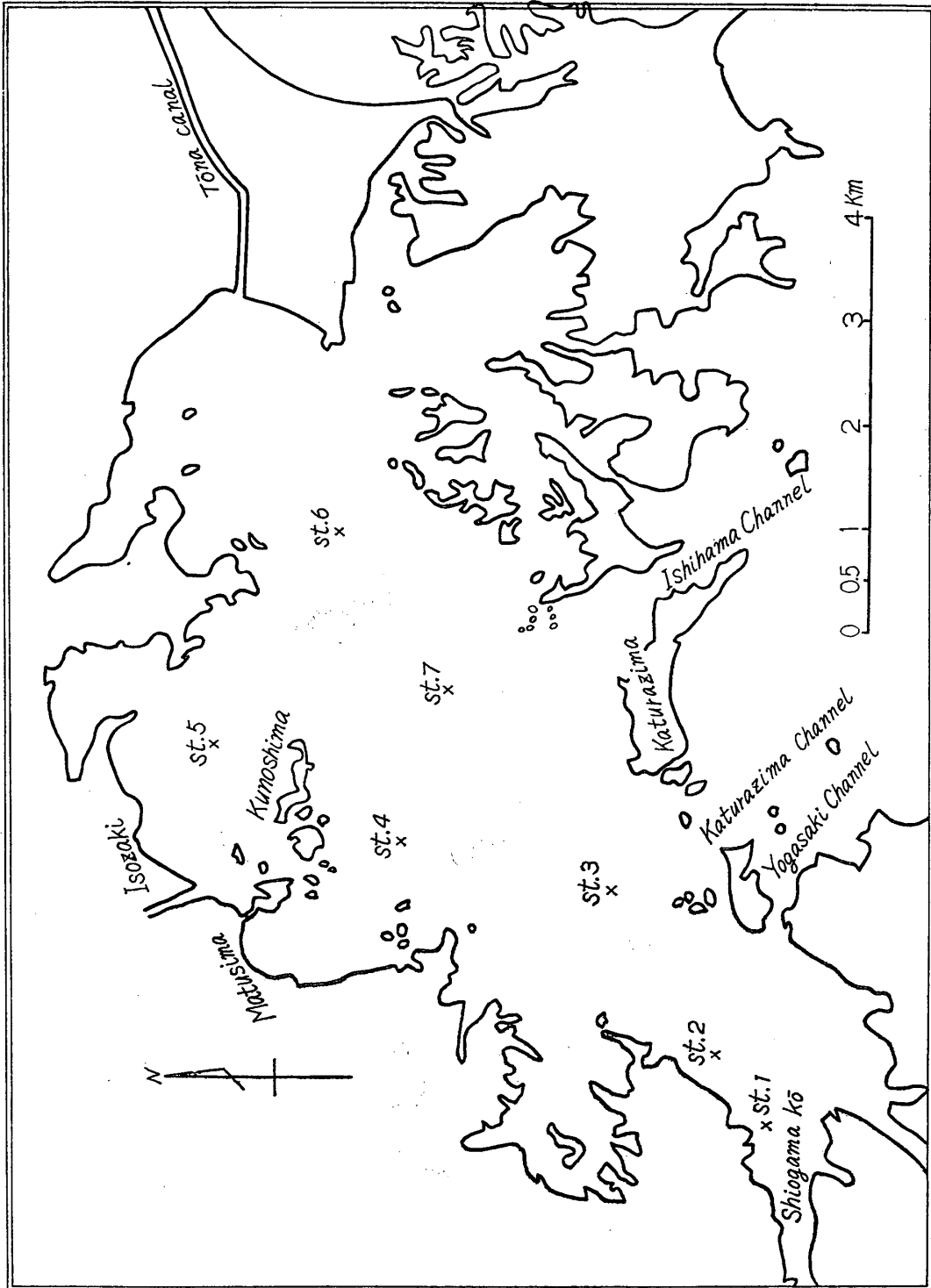
Fig. 1. Sampling station.

**Table 2.** Recovery of L-Leucine from the hydrolysate (mg)

| Sample | Addition | COOH-N | Mean values |
|--------|----------|--------|-------------|
| 6 | 0 | 0.187<br>0.185<br>0.183 | 0.185 |
| | 0.240 | 0.424<br>0.427<br>0.428 | 0.426 |

(3)  The time required for hydrolysis

The quantity of carboxyl nitrogen in the hydrolysate is likely to be fluctuated by the concentration of acid, the time of hydrolysis and physical-chemical characters of the mud.   Therefore, the relation between the total amount of nitrogen and carbon in the hydrolysate and the time of hydrolysis was examined.   Five grams of the air dried mud were hydrolyzed with 50 ml. of 6 N HCl for 4, 8, 13, 16, 20 and 24 hours.   The mixture was filtrated through the filter paper (Toyoroshi No. 5C) by a suction pump and the residue was washed thoroughly with distilled water.   The filtrate and washings were mixed and used for the measurements of the total amount of nitrogen, carboxyl nitrogen and ammonium nitrogen.

(4)  Organic carbon

The organic carbon was determined by Van Slyke's method (3) which is calculated from the amount of $CO_2$ generated by the combustion with Van Slyke's reagent.   This method is convenient and satisfactorily accurate.

For absorption of the $CO_2$, the mixture of NaOH and hydrazine was used, in which the hydrazine can eliminate the interference by halogens evolved during the combustion.

As the procedure of digestion is important to obtain the accurate value of organic carbon, Van Slyke's (4) and Kay's (5) digestion regents were compared with each other.   The degree of digestion in both methods are shown in Table 3.

**Table 3.** Comparison of Van Slyke's with Kay's catalysts on determination of organic carbon compounds. (mg/g)

| Station | 6 | 4 | 1 |
|---------|------|-------|-------|
| Van Slyke's | 27.16 | 15.86 | 25.88 |
| Kay's | 25.40 | 15.42 | 25.50 |

The table indicates that the former has higher oxidizing power than the latter.

Before the combustion, the sample was treated as follows to eliminate the inorganic carbon.   Namely, the sample was kept on a boiling water bath for

about 15 minutes after the addition of few drops of conc. $H_3PO_4$ and a small quantity of water according to Harada and Hashimoto's method (6).

## Result and Discussion

The organic content in marine deposits are usually determined by the total nitrogen and the total organic carbon but the information on the proteinous substances which are available to the benthos as a nutrition is very few. Therefore, in the present work the analysis of proteinous substances was added especially to the ordinary procedure. The amount of proteinous substances contained in the mud samples was measured by carboxyl nitrogen and ammonium nitrogen resulting from the hydrolysis of the deposits and it was compared with the total nitrogen. The results are shown in Table 4.

Table 4. Nitrogen and carbon contents in the air dried muds. (mg/10g)

| Station | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Total–N | 31.6 | 18.5 | 17.0 | 18.0 | 24.1 | 24.5 | 21.4 |
| COOH–N* | 11.3 | 10.7 | 7.52 | 9.48 | 12.7 | 12.0 | 10.5 |
| $NH_4$–N* | 4.00 | 4.01 | 3.26 | 4.04 | 4.80 | 5.18 | 3.76 |
| free $NH_4$–N | 0.48 | 0.29 | 0.27 | 0.19 | 0.37 | 0.38 | 0.26 |
| Org–C | 243.4 | 218.0 | 157.0 | 212.8 | 254.0 | 281.2 | 235.9 |
| C/N | 7.7 | 11.8 | 9.2 | 11.3 | 10.1 | 11.5 | 11.0 |

Note. * in the hydrolysate

Assuming from the amount of the total nitrogen or the total carbon, the organic content in the deposits at St. 1 was much more higher than that of St. 2—4 and at other stations, the amount gave median values. As St. 1 is located near the fish market and the estuary, the abundant organic content in deposit at this station is considered to be originated from the inflow of the excreta of the fish market and the city. The deposit at St. 3 which is located near the channel is washed by the tidal current and consequently it will be easily presumed that the deposit does not contain so much organic material. Although the lower content of organic material at St. 2 and 4 was not determined, this remains a future problem for study.

The content of carboxyl nitrogen in the deposit is 35.8 per cent of the total nitrogen at St. 1 but between 49.3 and 52.9 per cent St. at 5—7. Therefore, it is presumed that the amount of proteinous nitrogen is nearly from 36 to 53 per cent of the tolal nitrogen. However, it is noteworthy that the ratio of carboxyl nitrogen to the total nitrogen at St. 1 is lower than the other stations in spite of its higher total nitrogen content. This can be explained by that the decomposition of the deposit at St. 1 has proceeded thoroughly and much

undecomposable nitrogenous matter remained but it will be necessary to make clear the decomposing mechanism at another opportunity.

Not much free ammonium nitrogen is contained in the air dried mud as shown in Table 4. Therefore, the majority of ammonia which is increased by the hydrolysis must be derived from organic nitrogenous compounds such as amides and some amino acids as pointed out by Bremner (7) and Stevenson (8).

As the time required for the complete hydrolysis was expected to be different among samples, the hyrolysing time was examined in the three samples of St. 1, 5 and 6. The result is show in Fig. 2 from which it is noticed that the amount
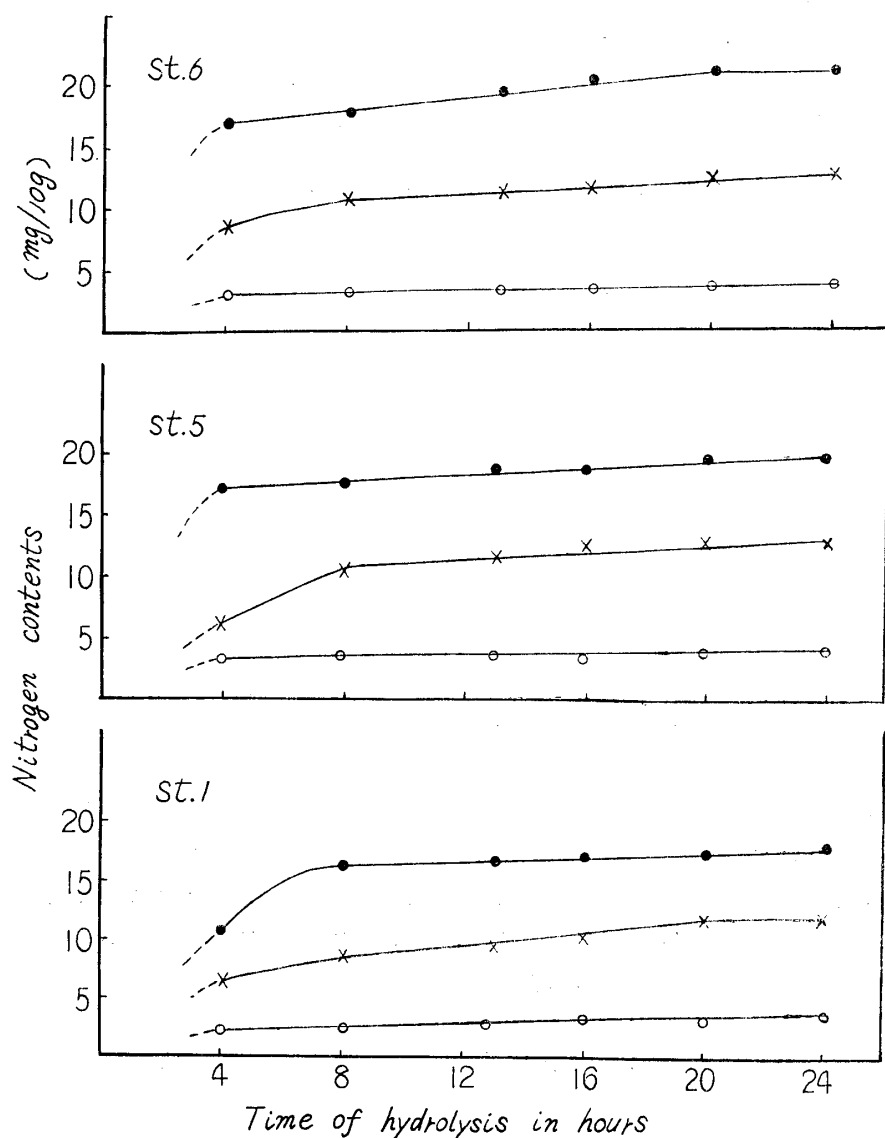


Fig. 2.  Relationship between the acid soluble nitrogen and
        hydrolyzing hours.
        • ··· Acid soluble nitrogen,  o ··· Ammonium nitrogen,
        x ··· Carboxyl nitrogen.

of the soluble part of nitrogen dissolved out from the muds by 8 hours' hydrolysis differed among the samples but by further hydrolysis it was increased and reached to nearly the maximum by 20 hours' hydrolysis. The increasing pattern of carboxyl nitrogen in the hydrolysis was also nearly the same as that of the soluble nitrogen when hydrolyzed for more than 8 hours.

Table 5 shows the relation of the amount of tolal carboxyl and ammonium nitrogen which were dissolved out by 20 hours' hydrolysis to the total amount of nitrogen in the air dried mud. According to this analysis, the amount of the

**Taele 5.** The relation of nitrogen and carbon in the hydolysate (20 hours) to that of the air dried mud.

| Station | 6 | 5 | 1 |
|---|---|---|---|
| Total-N in the air dried mud (A) (mg/g) | 2.26 | 2.41 | 3.16 |
| Org-C in the air dried mud (B) (mg/g) | 26.7 | 25.4 | 24.4 |
| $\dfrac{COOH\text{-}N+NH_4\text{-}N}{Total\text{-}N \text{ in the hydrolysate}}$ (%) | 77.8 | 84.9 | 92.0 |
| $\dfrac{COOH\text{-}N+NH_4\text{-}N}{(A)}$ (%) | 73.4 | 72.6 | 48.5 |
| $\dfrac{Total\text{-}N \text{ in the hydrolysate}}{(A)}$ (%) | 94.5 | 83.8 | 54.7 |
| $\dfrac{Org\text{-}C \text{ in the hydrolysate}}{(B)}$ (%) | 48.5 | 51.7 | 45.1 |

soluble nitrogen at St. 1, 5 and 6 was respectively 54.7, 83.8 and 94.5 per cent of the total amount of nitrogen in the air dried mud.

On the other hand, the amount of carboxyl and ammonium nitrogen in the hydrolysate is between 77.8 and 92.0 per cent of the soluble nitrogen content, so a large part of the nitrogen passed into the solution appears to be derived from the compounds like peptides.

The relation between the hours of hydrolysis and the amount of organic carbon soluble by hydrolysis with 6 N HCl is shown in Table 6. According to

**Table 6.** Relationship between the acid soluble carbon and hydrolyzing hours.

| Station | 6 | | 5 | | 1 | |
|---|---|---|---|---|---|---|
| Hours | Org-C(mg/g) | C/N | Org-C(mg/g) | C/N | Org-C(mg/g) | C/N |
| 4 | 12.8 | 7.5 | 12.4 | 7.2 | 10.5 | 9.7 |
| 8 | 12.4 | 7.3 | 12.4 | 7.1 | 10.4 | 6.4 |
| 13 | 12.4 | 6.6 | 12.4 | 6.7 | 10.8 | 6.5 |
| 16 | 12.4 | 6.2 | 12.6 | 6.8 | 11.1 | 6.5 |
| 20 | 12.7 | 6.0 | 13.1 | 6.5 | 11.0 | 6.4 |
| 24 | 13.5 | 6.3 | 13.4 | 6.7 | 11.4 | 6.6 |

this table, a large part of the soluble carbon in the air dried mud passed into the solution by 4 hours' hydrolysis, and further hydrolysis did not increase of the amount of sobuble carbon.

The organic carbon compounds in the air dried mud seem to be converted readily into the soluble form than into organic nitrogen compounds by the hydrolysis. The amount of organic carbon that passed into the solution was between 45.1 to 51.7 per cent of the organic carbon in the air dried mud, and this part of organic carbon did not fluctuate among the samples as that of nitrogen. The ratio of C/N in the hydrolysate was lower than that of the air dried mud.

As mentioned above, the composition of organic nitrogen compounds in the deposits are different considerably among the samples and this is considered as due to the difference of the origin and decomposition of the organic materials contained and to the physical and chemical environments, such as tidal current, temperature and oxgen contents. We hope to continue this research to make clear the mechanisms of decomposition and accumulation of organic materials on the sea floor and their physiological influenece upon the benthos.

## Summary

1. Contents of the organic carbon and carboxly nirogen in marine deposists were detemined by using Van Slyke's manometric method.

2. Time for hydrolysis of organic carbon and nitrogen compounds with 6 N HCl in air dried muds was examined. Most of the acid soluble nitrogen compounds was disolved out in 8 hours of hydrolysis. The amount of hydrolysates attained almost the maximum value in 20 hours. These acid soluble nitrogen compounds seemed to have originated from peptideform compounds.

Most of the acid soludle organic carbon compounds were dissolved in 4 hours of hydrolysis.

3. The amounts of organic carbon compounds in the hydrolysate were 45.1 to 51.7 per cent of the organic carbon compounds in the muds.

## Acknowledgments

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