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HEAVY METALS, CHEMICAL CHARACTERISTICS, AND BACTERIAL POPULATIONS IN THE BOTTOM SEDIMENTS OF THE HIUCHI-NADA

Koichi OKUTANI and Tomotoshi OKAICHI

Introduction

The Hiuchi-nada is situated in the Seto Inland Sea (Fig.1). Temperature in the surface layers ranged between 8 and 28°C. The salinity range of eastern part of the Hichi-nada is 17.0-18.3 ‰. Bottom temperatures range between 7.4 and 24.7°C. The mean depth of the Hiuchi-nada lies 20 m.

Pollution in the Seto Inland Sea has received a considerable attentions in recent years. In particular, pollutions from the large amounts of organic materials and heavy metals seem to be regularly discharged into the sea from industrial and agricultural sources, besides in human activity. In the Hiuchi-nada, some sources of untreated effluents from paper factories exist in the Iyomishima and the Kawanoe areas (Fig.1).

Industrial wastes from paper factory may contain large amount of suspended solids and organic matters.

As in general, bottom deposits are the result of the settling of suspended material from the overlying water, these sediments may contribute to have a significant effect on the water quality. Decomposing organic materials exert an oxygen demand(1), release nutrients during periods of turnover, and contribute hydrogen sulfide to the water and the sediments. It is known that these nutrients leads to ubnormal agal blooms, called red water.

Heavy metals may have effects on the ecosystems greater than those of more common pollutants. The information on the toxic properties of heavy metals is not fully enough but it is known that heavy metals in high concentrations are toxic to living organisms.

It was the purpose of the present study to establish the means and ranges of concentrations for some heavy metals in the sediments of different polluted areas of eastern part of the Hiuchi-nada and to search for a relation between concentrations of these heavy metals and chemical and bacteriological parameters that are use to measure pollution.

Materials and methods

Samplings

Samples were collected in the eastern part of the Hiuchi-nada in October 2 and 22, 1970. The sediments were sampled from a total of 16 stations. The location of each station is shown on the map of the sea in Fig.1.

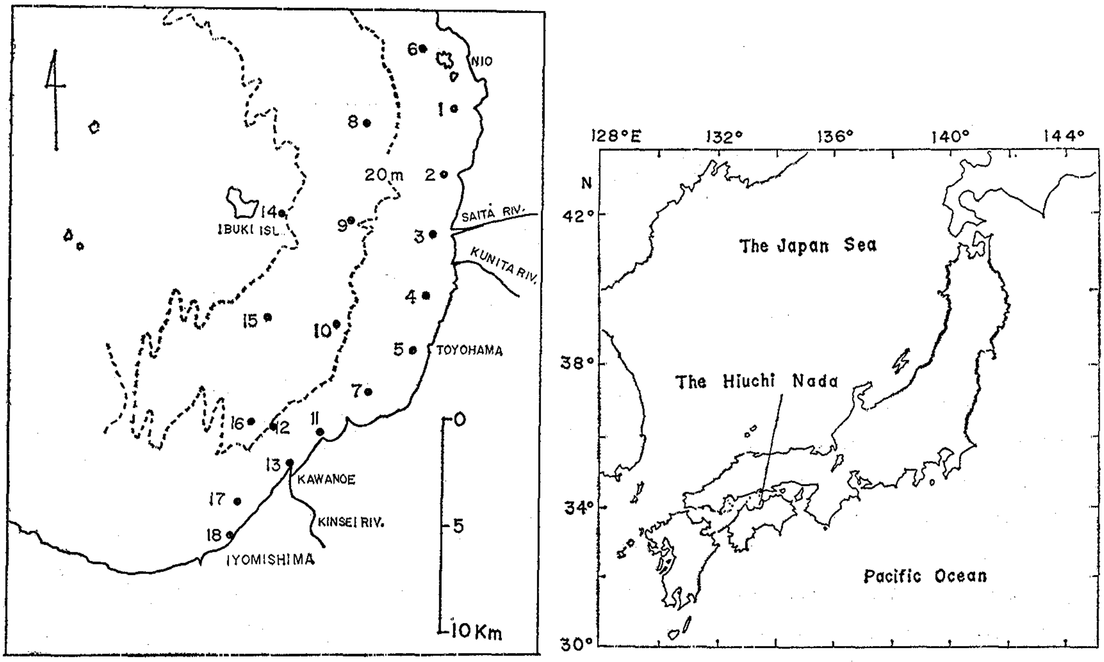


Fig.1. Location of sampling stations

Many pulp factories discharge wastes between Station 13 and 18.

Sediment samples were collected by using KK type mud sampler[2]. Core samples were immediately segmented in 3 cm each and stored as described below.

(i) For the determination of total sulfides, mud samples were treated with saturated zic acetate solution.

(ii) For the determination of bacterial populations, loss on ignition and heavy metals, mud samples were put into sterile plastic bottles and stored in the cold.

Total sulfides

The content of the total sulfides in the muds was determined by the method in which paradimethylaniline reagent was used after distillation[3]. The data were calculated as mg of H_2S liberated per 100 g of dry muds.

Loss on ignition

Mud samples were dried in an oven at 90°C for 24 hours and then heated to 450-500°C for 24 hours. The data were calculated as per cent of loss on ignition of the dry muds.

Heavy metals

The mud ash prepared as above mentioned (loss on ignition) was dissolved in 5 ml of boiling water and then 10 ml of 5N-HCl were added. This solution was boiled for 5 minutes, then the solution was filtered. Remaining precipitates on the filter were washed several times with boiling water. Filtrates were filled up to 100 ml and stored in polyethylene bottles.

Heavy metals in the solution were determined by HITACHI model 207 atomic absorption spectrophotometer. The amount of each heavy metals was estimated by using the method of additions(4).

Total blank was determined by carring out the complete procedure of digestions and analysis with no mud samples.

Bacterial populations

Total heterotrophs: The enumeration of viable heterotrophic bacteria was carried out by using Most Probable Number (MPN) method(5).

The culture medium was similar to ZOBELL's 2216E(6). The inoculated medium was incubated at 25°C for two weeks, and the growth in each tube was then estimated visually.

Chitin-decomposing bacteria: A loopful from the growth tube of total heterotrophs was then streaked on chitin-agar plate (2.5 % of agar and 0.5 % of precipitated chitin(7) in ZOBELL's 2216E, pH adjusted to 7.0).The inoculated medium was incubated at 25°C for two weeks.

When chitin-decomposing bacteria grew on the surface of this agar a clear halo was produced along the bacterial masses, because the precipitated chitin was decomposed.

Starch-decomposing bacteria: An aliquot (0.1 ml) of the growth from each tube of total heterotrophs was transffered to starch medium (0.5 % soluble starch in ZOBELL's 2216E, pH adjusted to 7.0).The inoculated medium was incubated at 25°C for two weeks.

Hydrolysis of starch was detected by iodine method.

N-acetylglucosamine-decomposing bacteria: An aliquot (0.1 ml) of the growth from each tube of total heterotrophs was then transffered to N-acetylglucosamine (NGA) medium (0.2 % NGA and BTB in ZOBELL's 2216E, pH adjusted to 7.0). The inoculated medium was incubated at 25°C for two weeks.

Decomposition of NGA was estimated by the formation of acid from the substrate in the culture.

Sulfate reducing bacteria: The enumeration of sulfate reducing bacteria was carried out by using MPN method. The culture medium was as follows;

Ca-lactate	3.0 g
Peptone	2.0 g
Beef extract	1.0 g
EDTA-Na	0.4 g
K_2HPO_4	0.05 g*
$MgSO_4 \cdot 7H_2O$	0.2 g
$FeSO_4 \cdot 7H_2O$	0.2 g
L-ascorbic acid	0.1 g*

Agar	3.0 g
Sea water	1000 ml

pH adjusted to 7.0

*Autoclaved separately.

The inoculated medium was incubated at 25°C for two weeks. Sulfate reduction was detected by the blacking of the culture.

Results

Concentrations of four heavy metals in the bottom sediments from 16 stations are given in Table 1 and Fig.2-Fig.5.

Table 1. Concentrations of heavy metals in the sediments*

Station	Cu	Pb	Mn	Zn
1	6.6	8.8	20.9	33.6
2	6.1	8.7	35.6	43.4
3	9.6	26.4	31.8	43.2
5	6.6	15.4	33.0	53.8
7	15.5	10.7	45.5	47.1
8	13.8	27.3	101.4	106.7
9	6.8	27.9	73.1	81.0
10	10.0	20.1	57.9	60.9
11	11.2	20.1	36.7	102.2
12	22.3	15.5	27.4	57.8
13	39.2	44.5	51.2	127.2
14	9.1	24.0	57.7	43.2
15	27.1	22.4	79.9	152.8
16	5.8	14.0	42.9	64.3
17	3.9	8.7	24.4	39.2
18	45.2	30.3	122.1	291.0

*PPM ind dry muds. (0—6 cm Segment.)

Copper

As shown in Table 1, the concentration of this metal was highest at Station 18, followed by Station 13. The values are about 10 times higher than those in the lowest sample.

As shown in Fig. 2, at Station 18 the concentration of this metal in the upper 0-3 cm segment was 53.7 ppm, while 36.7 ppm was found in the 3-6 cm segment. A similar correlation was possible on the sample of Station 13 (Fig.2).

Lead

As shown in Table 1, the concentration of this metal was highest at Station 13, followed by Station 18. The values are 3-5 times higher than those in the lowest sample.

As shown in Fig.3, at Station 13 the concentration of this metal in the upper 0-3 cm segment was 25.8 ppm, while 63.1 ppm was found in the 3-6 cm segment. A similar correlation was possible on the sample of Station 18 (Fig.3).

Manganese

As shown in Table 1, the concentration of this metal was highest at Station 18, followed by Station 8. The values are 5-6 times higher than those in the lowest sample.

As shown in Fig.4, at Stations 18 the concentration of this metal in the upper 0-3 cm segment was 107.5 ppm, while 137.0 ppm was found in the 3-6 cm segment.

Zinc

As shown in Table 1, the concentration of this metal was highest at Station 18, followed by Station 15. The values are 5-9 times higher than those in the lowest sample.

As shown in Fig.5, at Station 18 the concentration of this metal in the upper 0-3 cm segment was 459.7 ppm, while 122.3 ppm was found in the 3-6 cm segment. A similar

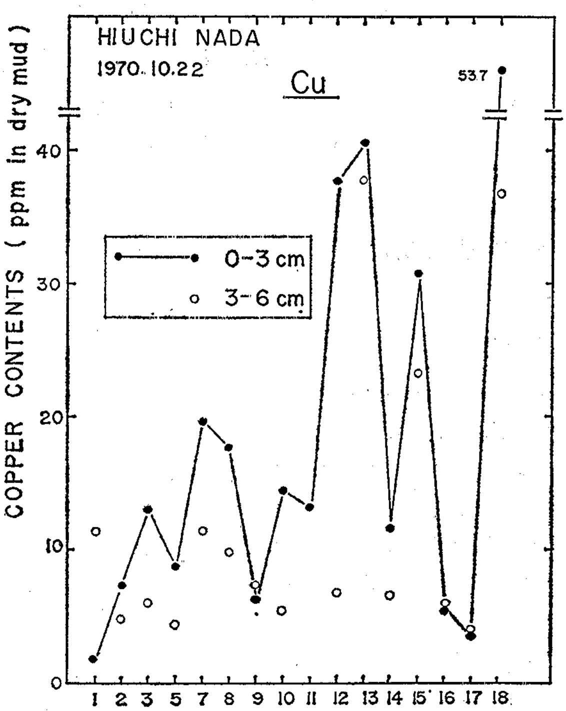


Fig.2. The concentration of copper in the bottom sediments of Hiuchi-nada

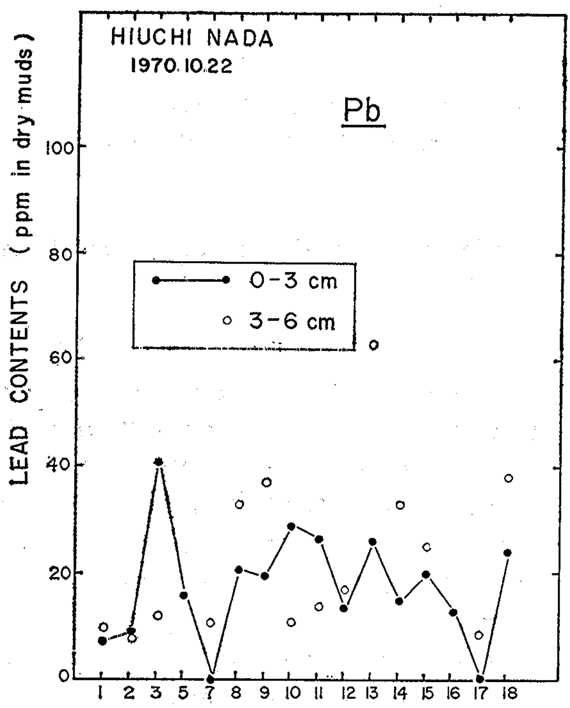


Fig.3. The concentration of lead in the bottom sediments of the Hiuchi-nada

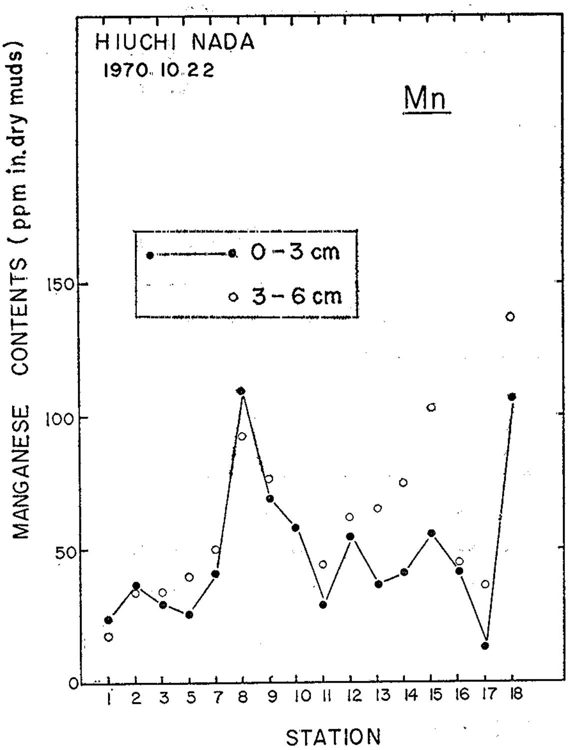
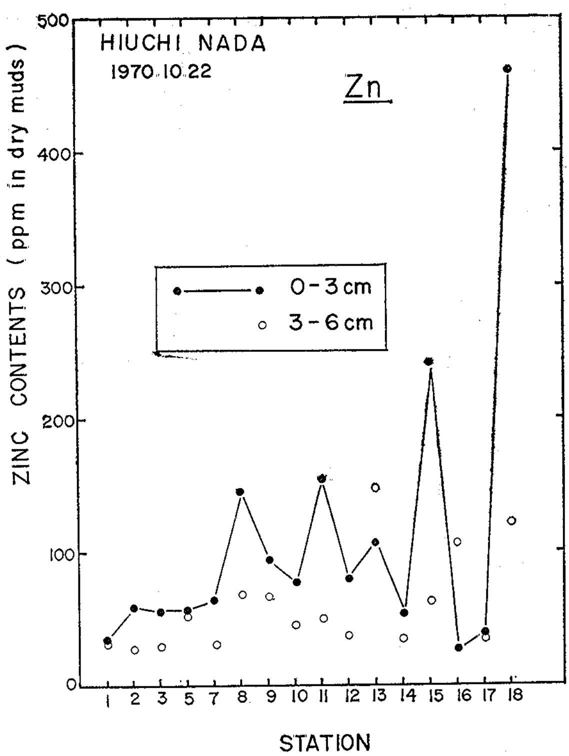


Fig.4. The concentration of manganese in the bottom sediments of the Hiuchi-nada



Fg.5. The concentration of zinc in the bottom sadiments of the Hiuchi-nada

correlation was possible on the sample of Station 15 (Fig. 5).

Total sulfides

As shown in Table 2, the content of total sulfides was highest at Station 18, followed by Station 13. The values are 60-70 times higher than those in the lowest sample.

As shown in Fig. 6, at Station 18 the content of total sulfides in the upper 0-3 cm segment was 226.7mg/100g dry mud, while 231.8 mg/100g dry mud was found in the 3-6 cm segment. At Station 13, the content of total sulfides in the upper 0-3 cm segment was 293.5 mg/100g dry mud, while 119.2 mg/100g dry mud was found in the 3-6 cm segment.

A comparison of contents of total sulfides in two dates of samplings in the upper 0-3 cm segment was shown in Fig6. Fig. 6 shows that contents of total sulfides excluding Station 18 were similar between samples from different dates in the same stations.

Loss on ignition

As shown in Table 2, the loss on ignition was hignest at Station 13, followed by Station 18. The values are 7-10 times higher than those in the lowest sample. As

Table 2. The total sulfides and loss on ignition in the bottom sediments *

Station	Total suldides	Loss on ignition
1	4.5 $mgH_2S/100g$ dry mud	3.9% of dry mud
2	4.9	6.0
3	6.0	6.4
5	4.2	5.2
6	4.8	—
7	11.2	8.2
8	11.2	10.7
9	4.5	9.3
10	5.1	8.1
11	6.6	7.9
12	6.1	9.1
13	206.4	37.3
14	3.2	9.8
15	5.0	9.9
16	3.4	6.4
17	3.5	4.0
18	229.3	28.7

* 0-6 cm Segment.

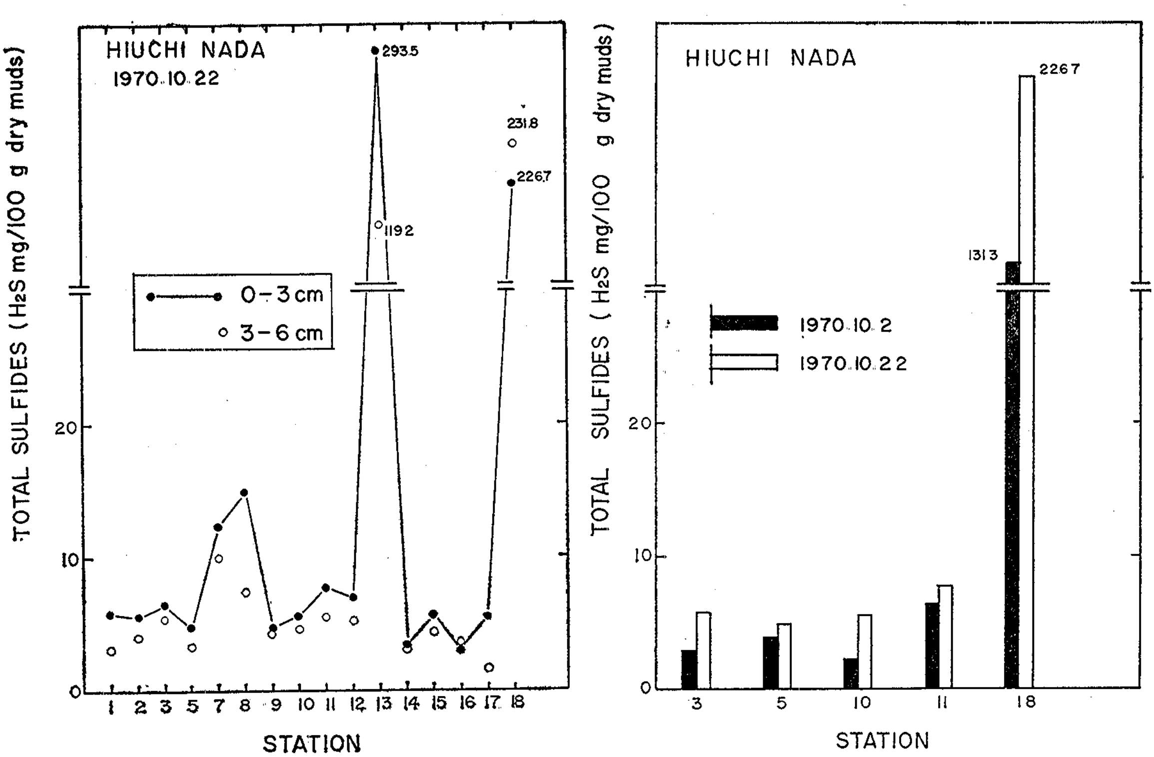


Fig.6. The content of total sulfides in the bottom sediments of the Hiuchi-nada

shown in Fig. 7, at Station 13 the value in the upper 0-3 cm segment was 37.6%, while 37.0% was found in the 3-6 cm segment. A similar correlation was possible on the sample of Station 18.

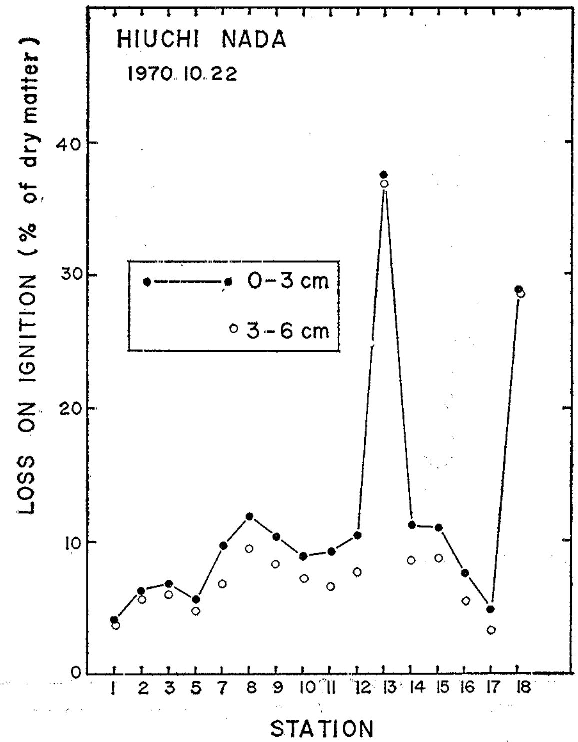


Fig. 7. The value of loss on ignition in the bottom sediments of the Hiuchi-nada

Bacterial populations

Five stations were analyzed for bacteria. The overall results of the analysis are shown in Table 3.

In the surface layer of the bottom muds, 10^4-10^6 cells per gram of wet mud of the heterotrophic bacteria and 10^2-10^3 cells of the chitin-decomposing bacteria were distributed.

The populations of chitin-, starch-, NGA-decmposer and sulfate-reducer were highest at Station 18.

Starch-, NGA-decomposer and sulfate-reducer ranged 10^4-10^5, 10^3-10^6 and 10^3-10^5 cells per gram of wet mud respectively. These ranges excluding sulfate-reducer in Station 18 are similar to those reported for marine samples.

The populaiton of sulfate-reducers in Station 18 was higher than that reported for marine samples. The total heterotrophs were highest at Station 18, followed by Station 5 and 16. The values are 10-100 times higher than those in other two stations tested in the present experiments.

Table 3. Bacterial populations in the bottom sediments. *

	T—B	C—B	S—B	NGA—B	H_2S—B
St. 3	7.0×10^4	9.3×10^2	4.6×10^4	7.0×10^4	1.3×10^4
St. 5	9.2×10^5	4.5×10^2	2.6×10^4	3.9×10^4	3.5×10^4
St. 10	5.4×10^4	2×10^2	1.1×10^4	7.0×10^3	7.9×10^3
St. 16	1.4×10^5	4.5×10^2	1.7×10^4	7.0×10^4	3.5×10^4
St. 18	2.4×10^6	1.3×10^3	3.5×10^5	1.6×10^6	2.8×10^5

* Bacterial number (cells/g wet mud, 0—3 cm segment).

T—B= Total Heterotrophic Bacteria.
C—B= Chitin—decomposing Bacteria.
S—B= Starch—decomposing Bacteria.
NGA—B= NGA—decomposing Bacteria.
H_2S—B= Sulfate—reducing Bacteria.

Discussion

The concentration of heavy metals varied according to stations. The values also varied among segments of the same core of muds. The high concentration of heavy metals was found in Station 8 (Mn, Zn), Station 11 (Zn), Station 13 (Cu, Pb, Zn), Station 15 (Zn) and Station 18 (Cu, Pb, Mn, Zn) as compared with that in other stations tested.

Table 4. Relation between the concentration of heavy metals and the content of total sulfides in the bottom sediments*

Station	Cu	Pb	Mn	Zn
1	1.4	1.9	4.6	7.4
2	1.2	1.7	7.2	8.8
3	1.6	4.4	5.3	7.2
5	1.5	3.6	7.8	12.8
7	1.3	0.9	4.0	4.2
8	1.2	2.4	9.0	9.5
9	1.5	6.2	16.2	18.0
10	1.9	3.9	11.3	11.9
11	1.6	3.0	5.5	15.4
12	3.6	2.5	4.4	9.4
13	0.1	0.2	0.2	0.6
14	2.8	7.5	18.0	13.5
15	5.4	4.4	15.9	30.5
16	1.7	4.1	12.6	18.9
17	1.1	2.4	6.9	10.3
18	0.1	0.1	0.5	1.2

*PPM/mg H_2S

It is well known that these metals often consist in sulfide and the sulfide of the metals are extremely insoluble.

As shown in Table 4, only the concentration of copper in the bottom sediments was correlated with the content of total sulfides. For copper the ratio was 1.2-1.9 with the exception of Station 12-15, and Station 18.

To aid in classifying the bottom sediments by means of the concentration of heavy metals the Zn : Mn ratios were considered. For the ratio of Zn to Mn was 1.0-1.6 with the exception of Station 11-15, and Station 18.

Is there any correlation between the concentration of heavy metals and the pollution in the bottom sediments of eastern part of the Hiuchi-nada?

It is well known that bottom sediments of coastal regions of the receiving sewage or industrial waste which contains a large amount of organic matter, hydrogen sulfides and metallic sulfides are often found in abnormally high concentrations.

The total heterotrophic bacteria in the Hiuchi-nada sediment was10^4-16^6cells per gram of wet mud (Table 3). 0.1-1 per cent of the total heterotroph was the chitin-decomposing bacteria. This figure is lower than the average count reported from the marine sediments.

Table 3 shows that there is some quantitative variation in the distribution of the bacterial flora in the bottom sediments. At Station 10 which was distant from the coast the bacterial populations were low. At Station 18 the bacterial counts were very high. The indication may be that the high populations of bacteria in the Station 18 are significant feature of the bottom sediments and results of the presence of favorable metabolic substrates for their growth.

Very high populations of sulfate-reducers, contents of total sulfides and organic matter(loss on ignitin) in the sediments were found at Station 13 and 18 which were located in the Kawanoe and the Iyomishima areas. It is probable that Station 13 and 18 receive a considerable amounts of industrial waste containing organic matter from those pulp factories and city sewage which stimulate the growth and physiological activity of bacteria, especially sulfate-reducing bacteria and subsequently causes the vigorous production of sulfides in the bottom sediments.

It is considered that the sulfide production in the sediments was principally limited by the concentration of available organic matter present(8). It is probable that the changes in the contents of total sulfides between sampling stations or within a core represent differences in the amount and quality of organic matter deposited. Thus, Station 13 seems to be more polluted than Station 18 from the data in the 0-3 cm segment, but from the data in the lower 3-6 cm segment Station 18 shows to be more highly polluted than Station 13 (Fig.6).

With the exception of Station 13 and 18, the total sulfide contents were high at Station 7 and 8. It seems probable that Station 7 receives organic matter required by sulfate reducers from the land because the station locates near the coasts.

At Station 8 which was distant from the coast both the concentration of total sulfides and the value of loss on ignition were high. It is not probable in general, however, that Station 8 receives directly organic matter from the coast, because in the Station 1 and 2 which were located near the coast lower concentrations of the total sulfides were found than those at Station 8. Similar phenomena were observed in the sediment at Station 8 about the concentration of heavy metals (Table 1). A important question that must be resolved is the sources of organic matter and heavy metals in the Station 8. The wind drift and water currents of surface water indicate that southern part of water (The Iyomishima and the Kawanoe areas) may be transported towards the Station 8[9]. The accumulation of organic matter may also be related to the region of transporting by bottom currents or due to bottom topography. The reason for the low content of heavy metals, organic matter and total sulfides at the Station 17 which was near the Station 18 may be related to the region of scouring by bottom currents or may be due to bottom topography.

Summary

Bottom sediments in the eastern part of the Hiuchi-nada were analyzed for heavy metals, total hydrogen sulfides, loss on ignition and heterotrophic bacteria.

The following heavy metals and estimated ranges of concentrations were found: copper, 3.9-45.2 ppm in dry mud of the 0-6 cm segment of core; lead 8.7-44.5 ppm; manganese, 20.9-122.1 ppm; zinc, 33.6-291.0 ppm.

The content of total sulfides ranged from 3.2 to 229.3 mg H_2S per 100 g of dry mud (0-6 cm segment of core).

The value of loss on ignition ranged from 4.0-37.3 % of dry mud (0-6 cm segment of core).

Of all 16 stations only five stations were analyzed for bacteria. Sulfate-reducer in Station 18 was higher than that reported for marine samples.

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燧灘底土堆積物の化学的性質，重金属量および微生物数について

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燧灘東部海域（Fig 1）の底土堆積物について重金属量，硫化物量，灼熱減量および微生物相の測定を行ない重金属の蓄積と底質汚濁との関連性について追求した．その結果，次の事を明らかにした．

(1) 泥柱0-6 cm中の重金属量，硫化物量および灼熱減量は乾泥に換算すると次に示すごとくなる．すなわち，銅，3.9-45.2 ppm；鉛，8.7-44.5 ppm；マンガン，20.9-122.1 ppm；亜鉛，33.6-291.0 ppm．硫化物量，3.2-229.3 mgH_2S/100 g泥．灼熱減量，4.0-37.3%．

(2) 微生物相は表層底土 0—3 cmについて行ない次に示す結果を得た．

全有機栄養細菌，10^2—10^3個/g；キチン分解細菌，10^2—10^3個/g；でんぷん分解細菌，10^4—10^5個/g；N—アセチルグルコサミン分解細菌，10^3—10^6個/g；硫酸塩還元細菌，10^3—10^5個/g．重金属，硫化物，灼熱減量および微生物数はいずれも試料採取地点の変化によって変動し Station13 又は18の如く，人為的汚染が大きい地点ではこれらの値も著しく高いことが認められた．

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