II. Water Quality

4. Other variables

4-1. COD (by Takanobu Inoue and Kazuhiro Komatsu)

1. Sampling and pretreatment

Water samples were collected in a column water sampler and filtered through glass fiber filters (GF/F, combusted under 400^{o}C before use). Both filtered and unfiltered water were analyzed.

2. Analysis

Water samples were poured into a 300ml conical flask and the volume was increased to 100 ml by adding water (dilution of the sample should be enough so that about a half of N/40 potassium permanganate solution can remain after the next procedure). Once 10 ml sulfuric acid (1+2) and 10ml N/40 potassium permanganate were added, the flask was shaked and immediately put into a boiling water bath to heat up for 30 minutes. A liquid level of samples should be below the surface of boiling water, and the bottom of the flask should not touch the bottom of the bath. After the flask was taken out from the bath, 10 ml N/40 sodium oxalate was added and subsequently, the flask was shaked. Titration was conducted at temperatures between 55 and 60 ^{o}C until the color of the solution turns slightly red due to the N/40 potassium permanganate solution (wait for 30 seconds at this point). In parallel, 100 ml of distilled or deionized water was poured into a conical flask, and the same procedure was conducted. CODMn (mgO/l) is calculated by following equation:

$$CODMn = (a-b)\times f\times 1000 / V\times 0.2$$

CODMn: Chemical Oxygen Demand by potassium permanganate at 100^{o}C
a : Volume of N/40 potassium permanganate solution required in titration (ml) used for samples
b : Volume of N/40 potassium permanganate solution required in titration (ml) used for blank
f : Factor of N/40 potassium permanganate solution
V : Volume of the water sample (ml)
0.2 : Equivalent oxygen value (0.025×8mg) of N/40 potassium permanganate solution

4-2. Pigment (by Kazuo Matsushige and Kazuhiro Komatsu)

1. Sampling and pretreatment

Water samples at 2 m below the surface were collected in a column water sampler (2 m length, 6

cm diameter), and poured into a stainless steal vat with a grip. Polyethylene bottles were rinsed and filled up with water samples, closed with caps and brought back to the laboratory in an ice box. Water samples were filtered through glass fiber filters (GF/F, combusted under 400^{o}C before use), and the filters were kept in a freezer under -20^{o}C.

2. Analysis

(1) Chlorophyll-a:

10 ml methanol was added on the glass filters on which the suspended substances had been filtered, and then soluble substances were extracted by keeping it for 12 hours under 3^{o}C. Then, concentration of chlorophyll-a was measured by absorption spectrum method. The obtained solution was once stirred, and subjected to centrifuge for 10 mins under rotation of 3,000 rpm. The supernatant clear part of the solution was applied to high quality spectrograph with slit width less than 1 nm. Cell of 1 cm was used, and absorption coefficients at 750 nm, 665 nm, 645 nm and 630 nm were measured. Concentration of chlorophyll-a was obtained by the following equation:

$$\text{Chl-a } (\mu g\ l^{-1}) = (11.6E_{665} - 1.31E_{645} - 0.14E_{630}) \times v/(V \times l)$$

E_{665}, E_{645}, $E_{630:}$ Values of the absorption coefficients at 665 nm, 645 nm and 630 nm subtracted from those at 750 nm

V: Filtered volume of water samples(l)

l: Length of the cell used (cm)

v: Volume of methanol used (ml)

(2) Pheophytin: * Currently this item is not measured.

Acetone of 90% was added to the glass filters on which the suspended substances had been collected, and the filters were grinded down into a milky solution. The solution was put into a centrifuge tube by washing out with acetone of 90%. The tube was kept for about 2 hours under cold and dark conditions. After applying to centrifuge for 10 min with 3,000 rpm, the amount of the top clear part was recorded. The absorption coefficients at wavelengths of 750 nm and 665 nm were read for 90% acetone solution. After leaving it for 3 mins, the measurement was repeated. The concentration of pheo-pigment was determined using the following equation:

$$\text{Pheo-pigment } (\mu g\ l^{-1}) = 26.7(1.7E_{665a} - E_{665}) \times a/(V \times l)$$

$E_{665:}$ Value of the absorption coefficient at 665 nm subtracted from that at 750 nm.

$E_{665a:}$ Value of absorption coefficient at 665 nm subtracted from that at 750 nm for the solution, to

which 2N hydrochloric acid has been added.

V: Filtered volume of the sample water (l)

l: Length of the cell used (cm)

a: Total volume of acetone in the supernatant clear solution (ml)

(3) Phycocyanin: * Currently this item is not measured.

10ml Phosphoric acid buffer solution of 10 mM (pH 7.0) was added to the glass filters on which the suspended substances had been collected, and phycocyanin was extracted by keeping it for 12 hours under 3°C. After applying to centrifuge for 10 min under 3,000 rpm, the supernatant clear part was subjected to the fluorescence detector.

The high-performance liquid chromatograph system consisted of a Hitachi 655 Pump, Rheodyne Injector, Hitachi F-1000 fluorescence spectrophotometer and Shimazu C-RIA Inkdelator. We used a gel filter column (7.5 mm inner diameter and 60 cm lengths) of TSK-GelSW3000 or SW2000 (Tosoh Co., Ltd.). No special pretreatment was conducted, and the 200µl of the supernatant part of the extracted solution of phycocyanin was directly applied. As to the fluid phase, we used the 10 mM phosphoric acid buffer solution, which was used in the extraction of phycocyanin. The flow speed was 1.0 ml/min. The excitation and radiation wavelengths were 605 nm and 640 nm. These wavelengths are near the maximum peaks of phycocyanin.

4-3. SS (Suspended Solid) (by Kazuo Matsushige and Kazuhiro Komatsu)

1. Sampling and pretreatment

Water samples at 2 m below the surface were collected in a column water sampler (2 m length), and poured into a stainless steal vat with a grip. Polyethylene bottles were rinsed and filled up with water samples, closed with caps and brought back to the laboratory in an ice box. Water samples were filtered through glass fiber filters (GF/F, combusted under 400°C prior to the use).

2. Analysis

Samples were dried for 2 hours under temperatures between 105 and 110°C. The weight of SS was calculated by subtracting the weight of the filters. The filters were used for the measurement of POC and PON after weighing.

4-4. POC, PON, C/N (by Kazuo Matsushige and Kazuhiro Komatsu)

1. Sampling and pretreatment

Sampling and pretreatment procedure was the same as SS. The filters used for weighing SS were used for this analysis.

2. Analysis

CO_2 and N_2 gases were released from the sample filters by the dry combustion method using a YANAKO CHN-coder MT-5. The amount of each gas was measured by the heat-conductivity detect method, and then the amounts of carbon and nitrogen were calculated (C/N is the value of POC divided by PON).

4-5. DOC (by Akio Imai)

1. Sampling and pretreatment

Water samples were collected in a 2m acrylic column water sampler and poured into heat-treated (under 450^{o}C for 4 hours) glass jars with Teflon-lined caps. Water samples were filtered through glass fiber filters (GF/F, combusted under 450^{o}C for 4 hours).

2. Analysis

Filtered samples were adjusted to a pH of 2.0 by adding 2M HCl and inorganic carbon was removed by aeration of carrier gas (pure air). Density of DOC (Dissolved Organic Carbon, NPOC) was measured with a total organic carbon sensor quipped with a platinaum catalyst with high-sensitivity (Shimazu TOC-500 until March 1995, Shimazu TOC-5000 after March 1995). We measured at least three times for each sample to calculate mean values. Precision of analysis was within the range of 2 %.