II. Water Quality

2. Nutrients

(by Yukihiro Nojiri and Kazuhiro Komatsu)

1. Measurement methods

(1) Sampling and pretreatment

Lake water was collected in a column water sampler of 2 m length. The water sample was well mixed in a stainless steel bucket, and aliquots were placed in polypropylene bottles with ice. On the same day, the samples were filtered through Whatman GF/F glass filters (combusted under 400^{o}C before use) promptly after they were carried to laboratory. Filtered water samples were kept in dark and cool conditions prior to the analysis. Analysis of dissolved nutrients was usually conducted on the next day of sampling. The water samples, subjected to digestion, were subsampled into digestion bottles on the same day of sampling. Oxidation reagent was added and the analysis was conducted within a few days.

(2) Analysis

We measured following 8 items: a) nitrate-nitrogen (NO_3-N) + nitrite-nitrogen (NO_2-N), b) nitrite-nitrogen (NO_2-N), c) ammonium nitrogen (NH_4-N), d) dissolved total nitrogen (DTN), e) total nitrogen (TN), f) phosphate-phosphorus (PO_4-P), g) dissolved total phosphorus (DTP), and h) total phosphorus (TP). Filtered water samples through Whatman GF/F were used for the measurement of DTN, while unfiltered water samples were treated in an autoclave under 120^{o}C for 30 mins after adding potassium peroxodisulfate under alkaline pH for the measurement of TN. Filtered water samples through Whatman GF/F were used for the measurement of DTP, while unfiltered water samples were treated in an autoclave under 120^{o}C for 30 min after adding potassium peroxodisulfate under acidic pH for the measurement of TP.

In this data book, analytical results are given for the following 7 items: NO_3-N + NO_2-N (a), NO_3-N (a–b), NO_2-N (b), NH_4-N (c), DON (d–a–c), DTN (d), TN (e), PO_4-P (f), DTP (g), and TP (h). Either DTN or TN was analyzed until February 1992, while both items were analyzed after March 1992. The units used in this data book are μg l^{-1}(ppb) for nitrogen and for phosphorus.

2. Measuring equipments

1977 to March 1997: AAII Auto-analyzer (Technicon Co.Ltd.)

See Nojiri (1987) and Otsuki et al. (1993) for selection of reagents

Since July 1995: AACSII Auto-analyzer (Bran+Luebbe Co. Ltd.)

Some of the reagents used for colorimetric analysis were changed.

Indophenol method for auto-analyzer was applied for ammonia analysis.

* Two different types of analyzers were used in parallel for comparison and there was no significant difference. Only AACSII type was used after April 1997.

References

Nojiri, Y. (1987): Progress in water-quality analysis, J. Japanese Society of Ground Water, 29: 107-111 (in Japanese).

Otsuki, A., H. Goma, M. Aizaki and Y. Nojiri (1993): Seasonal and spatial variations of dissolved nitrogenous nutrient concentrations in hypertrophic shallow lake, with special reference to dissolved organic nitrogen, Verh. Internat. Verein Limnol., 25: 187-192.

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3. *In-situ* observations

3-1. pH (by Morihiro Aizaki)

In situ measurements of pH were conducted using pH meters with glass electrodes. Although we used several types of pH meters, measured data were accurate enough to treat them as a single time-series data.

3-2. Water Temperature (by Takehiko Fukushima)

Water temperatures were measured with thermistor thermometer, Hydrolab 8000 (Toho Dentan Co. Ltd.), and thermistor thermometer attached to digital DO meter, model 58 (YSI Co. Ltd.).

3-3. Electric conductivity (by Takehiko Fukushima)

Water samples were brought back to our laboratory and measured at about 25 ^{o}C with Electric Conductivity Meter (Toa-Dempa Co. Ltd.) using a 1 cm cell. The water temperature was measured in parallel, and the measured conductivity was corrected to that at 25 ^{o}C.

3-4. Dissolved oxygen (by Takehiko Fukushima)

Dissolved oxygen (DO) was measured with DO meter Hydro lab 8000 (Beckman Co. Ltd.) and digital DO meter model 58 (YSI Co. Ltd.).

3-5. Secchi disc transparency and underwater light intensity (by Noriko Takamura)

1. Measurement methods

Transparency has been measured at all 10 stations, while underwater light intensity was measured at Sts. 3, 7, 9 and 12. A Secchi disc (Rigo Co. Ltd.) with a diameter of 30cm was used for transparency measurement. The disc was lowered down in water until it was no longer visible from the surface and at this point the depth was measured. Under light intensity was measured at depths of surface (0m), 0.25m, 0.50m, 0.75m, 1.0m, 1.5m, 2.0m, 3.0m, 4.0m using a sensor. In actual measurement, a sensor was set on the boat to get a reference value. Measurements at all depths were conducted while light intensity was relatively stable, since the measurements on the boat and in

water could not be at the same time. According to Beer-Lambert law, the extinction of underwater light intensity is expressed as:

$$I_z = I_0 e^{-kZ}$$

I_z: Underwater light intensity at Zm
I_0: Underwater light intensity at 0 m
k: Extinction coefficient (m^{-1})

A water depth where 1% of light intensity on the surface can reach is represented as the depth of euphotic layer (Ze m). Ze = 4.6/k, and therefore, k is 4.6 at the depth of 1m.

2. Measuring equipments (underwater light intensity)
1977 - 8 June 1981: Illuminometer (λ, LI-185)
24 June 1981 - March 1983: Quantum sensor (Licor, LI-192S)
April 1984 - March 1989: Quantum sensor (Biosphaerical QSP-170)
Since April 1989: Quantum sensor (LI-192SA/B), Multichannel data logger (Licor LI-1000)

3-6. Water depth (by Tomiji Hagiwara)

Before March 1996:

Water depths were measured with scales marked on ropes (for measuring a temperature, DO and pH) which had a lead attached at the end.

After April 1996:

An aluminum disk of 15.5 cm diameter was attached to the lead to prevent it from penetrating into the mud. Also, a fishfinder using echo sounding was used in combination with the scaled ropes.

3-7. Position of station (by Tomiji Hagiwara)

Before March 1994:

More than two landmarks were set on land along the lake bank, and the position was determined from the cross-point of lines drawn in the observed orientations from these landmarks.

After April 1994:

Global Positioning System (GPS) was used. The current position for observations is the center of scattered points, determined by the former method and plotted by GPS every month between 1994 and 1995.

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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 %.