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Technical Manual
for
Inland Aquatic Environment Monitoring in East Asia
-2010

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1. Introduction

1.1. Background

In Northern Europe and North America, pH of lake water decreased in the 1970's compared with that of the 1930's and damages resulting from this decrease, such as declining fish populations, was reported. The cause of this pH decline is believed to have been deposition of acidic substances into lakes in excess of their neutralization or buffering capacity. According to the results of discussions at the specialist meeting of the Stockholm Conference on Acidification of the Environment held in 1982, acidification was acknowledged in many lakes, in Sweden, Norway, Canada and the United States that are highly sensitive to acid deposition (i.e., lakes having low alkalinity and receiving considerable deposition of sulfate ions over about 0.5 gS m^{-2} in their catchment basins) (Hileman, 1983).

In East Asia also, acidification of lakes and rivers/streams have been recently reported. Yamada et al. (2007) reported that river and lake waters had been acidified since the mid-1990s in the Lake Ijira catchment, one of the EANET monitoring sites, in Gifu Prefecture, Chubu region (central region) of Japan, where the rate of acid deposition was among the highest of all the monitoring sites in Japan. In the Chubu region of Japan, more widespread acidification of river waters has been reported. A significant long-term declining trend in river water pH was found in several watersheds in Nagano, Niigata and Gifu prefectures. Especially in Niigata, the pH declining trend was observed only in the areas receiving drainage from granitic rocks, and the acid neutralizing capacity of the river waters was in fact low in those areas (Matsubara et al., 2008). Niigata receives also the highest level of acid loading from the atmosphere in Japan. Effects of acid deposition or nitrogen deposition on inland water was observed also in other regions of Japan; e.g. Lake Sawanoike in Kyoto Prefecture (Yoshikawa et al., 2000); streams in Yakushima Island in Kagoshima Prefecture (Ebise and Nagafuchi, 2005); and streams in Kureha Hill, Toyama Prefecture (Kawakami et al., 2001). Moreover, temporary (short-term) changes in water chemistry in acid-sensitive regions were reported during periods of high flow discharge (water level), including during snowmelt and rain/storm events (e.g. Komai et al. 2001).

In Russia, the acidification of surface waters is typical of the regions, in which the geological conditions determine the low content of total dissolved solids in the waters, their low saturation in cations, and high vulnerability to the influence of acidifying substances. Surface waters of the taiga zone, which occupies a large area of the Russian territory, contain high concentrations of humic substances, causing an acidic water conditions. Almost 70% of the lakes in the Russian taiga zone have the waters with pH values less than 7.0 (Zalicheva et al., 2006). More than 10% of the 460 small lakes

studied in the northern European part of Russia are acidified; up to 30% of them are in critical condition. In this region, up to 80% of the atmospheric precipitations have pH <5.0, which is a result of both transboundary transport of pollutants from Western Europe and emissions of metallurgical plants "Severonikel" and "Pechenganickel" (Moiseenko, 2009). Strongly acid and acid sulfate waters (pH 3.6-5.5) are spread in lowland bogs located in the zone affected by the Norilsk mining and metallurgical complex, the largest one in the north of Western Siberia. There is an acidification of water bodies in the basin of the Lower Volga River (pH in the water has decreased from 7.4-7.6 to 6.0-6.2). Reduction of acid neutralizing capacity (ANC) of surface waters is observed in the mountain lakes and rivers of the Caucasus (Moiseenko, 2010) and of Southern Baikal (Khodzher et al., 2005, Sorokovikova et al, 2005; 2009). For many decades atmospheric precipitation with low acidity (pH 4.5-4.8) has been falling to the catchment area of Southern Baikal tributaries (East Siberia). The chemistry of these riverine waters is formed under severe climatic conditions of the highland. Most catchment areas of the rivers are composed of massive crystal rocks, on which podzol and brown taiga soils with acid reaction develop. The rivers are fed mainly by atmospheric precipitation. Water of these rivers is of low buffer capacity with pH ranged between 5.7 and 7.8. Resistance of river waters to acidification gradually decreases under the influence of acid atmospheric precipitations. Now, ANC values in some riverine waters dropped to 151 μeq L^{-1} compared to 778 μeq L^{-1} recorded in the 1950-1960s.

According to the Periodic Report on the State of Acid Deposition in East Asia (EANET, 2006), although no obvious acidification of inland aquatic environment was found for the five years from 2000 to 2004 in the EANET countries, lakes or rivers with low alkalinity below 200 μeq L^{-1} were found, which may be susceptible to acid deposition. In East Asia, rapid industrialization has increased emissions of air pollutants, and therefore, it may be possible that effects of acid deposition on inland aquatic environment will become obvious also in other countries than Japan and Russia in near future.

Therefore, it is necessary to conduct continuous monitoring of water bodies and aquatic fauna and flora. Moreover, inland water can be considered as a result of biogeochemical processes in the watershed/catchment as shown in Fig. 1.1 (NAPAP, 1990). Rainwater precipitated in the watershed is partly flowed into the stream or into the lake through hydrological processes in the watershed. Dissolved materials/ions deposited by wet or dry are transported with the water flow in the watershed and receive the biogeochemical processes, such as plant uptake, microbial consumption/transformation, cation/anion exchange on clay mineral surface, and mineral weathering. Therefore, water chemistry of streams and lakes must reflect such processes. Major processes hydrological flowpaths in the watershed are shown in Fig. 1.2 (NAPAP, 1990). Effects of acid

deposition on inland water should be evaluated taking the biogeochemical processes on the watershed/catchment scale into account (refer the chapter 8.1).

1.2. Objectives

Inland aquatic environment monitoring is conducted mainly for the following objectives:

i) To accumulate baseline data on inland aquatic environment and to evaluate the current situation
ii) To detect possible impacts of acid deposition on inland aquatic environment in early stages

1.3. Major revised part

Technical Manual for Inland Aquatic Environment Monitoring-2010 was prepared as the revised version of the *Technical Manual for Monitoring on Inland Aquatic Environment in East Asia*, which was adopted at the Second Interim Scientific Advisory Group Meeting of EANET in March 2000. Taking the latest scientific information and current situations of the EANET monitoring sites into account, the *Technical Manual-2010* was developed by the Expert Group as shown in the list of contributors.

The *Technical Manual-2010* includes 1) criteria for selection of monitoring sites, 2) standard sampling and monitoring methods for lakes and rivers, 3) standard monitoring parameters (both mandatory and optional) and analytical methods, and 4) data quality assurance/quality control, and data reporting and evaluation.

The basic procedures in the *Technical Manual-2010* were mostly same as the previous version, although the following subjects were adopted as major revised part:

- Rivers and streams can be selected as the monitoring site more freely. Consequently, modification on sampling frequency and measurement parameters was proposed.
- Clearer criteria on selection of lakes and rivers was described, including size of the catchment, priority among natural lake, rivers/streams, and reservoir/dam, etc.
- Parallel measurements by the end-point pH 4.8 method and the Gran's Plot titration method were recommended for alkalinity.
- The reporting forms are included in the same book.

Moreover, analytical procedures were described in further detail, and definition and meaning of the parameters were clarified for surveyors and analysts.

This manual will be reviewed and revised as appropriate, taking account of the latest scientific information and experiences accumulated in East Asia.

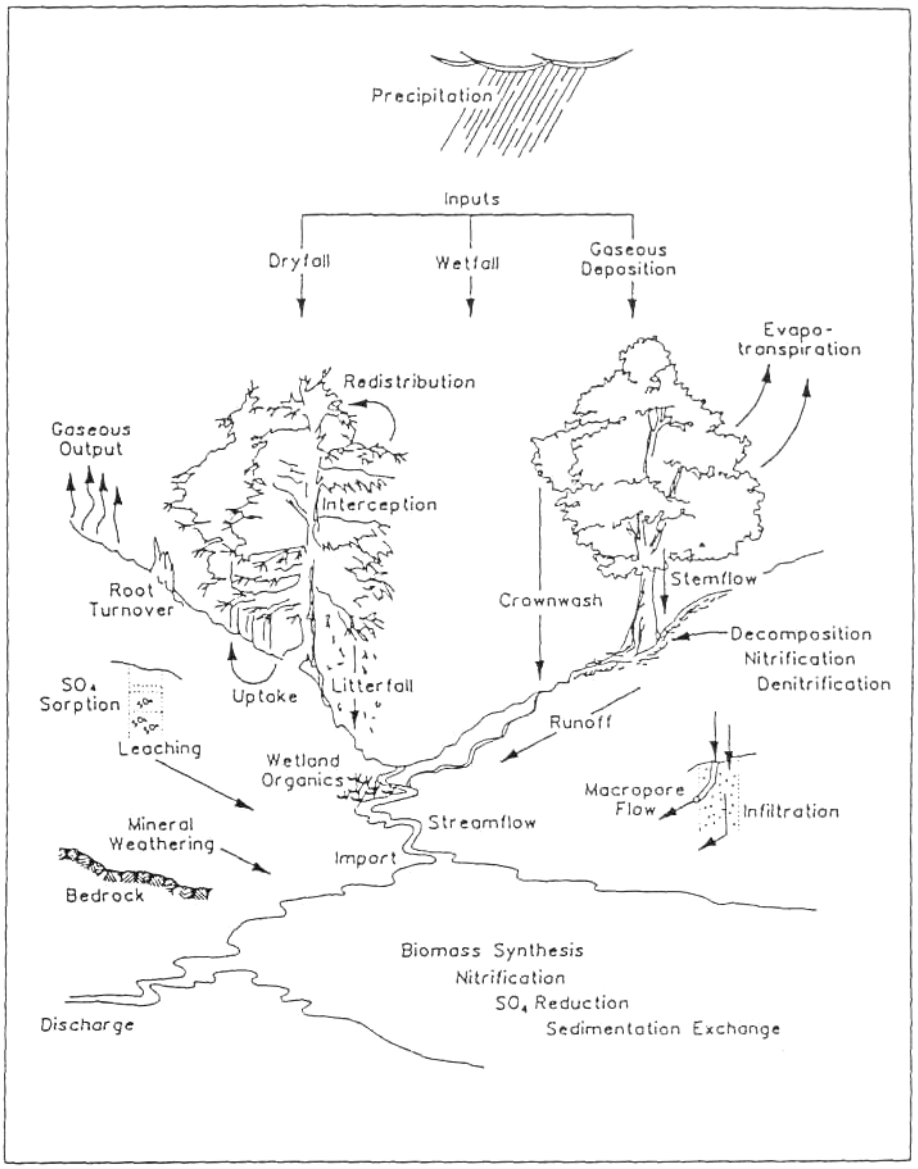


Fig. 1.1. Natural watershed and aquatic processes influencing the effects of acidic deposition on surface water chemistry (NAPAP Report No.14, 1990)

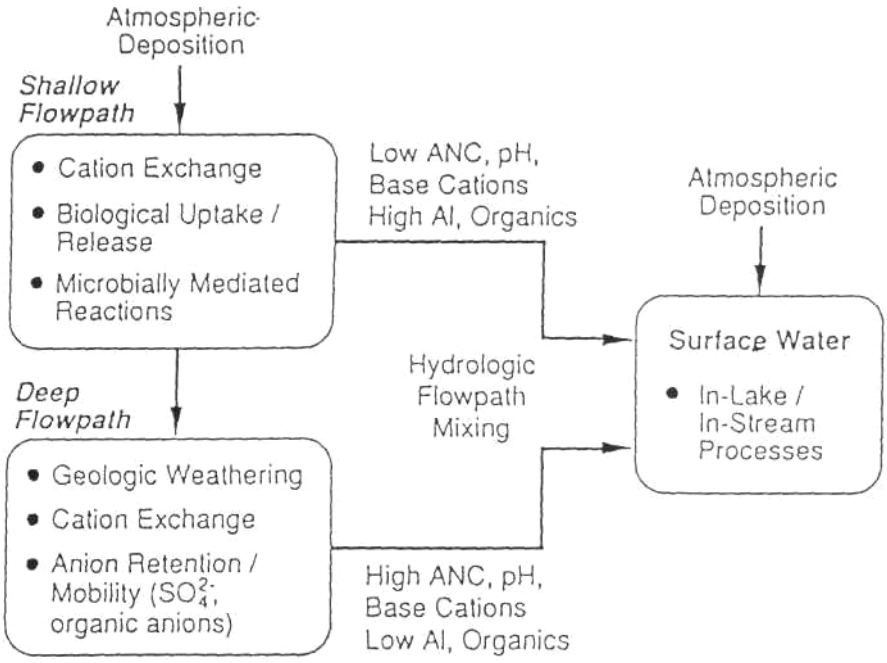


Fig. 1.2. Conceptual framework of major processes and hydrologic flowpaths that influence surface water chemistry (NAPAP No. 10, 1990)

2. Monitoring sites and parameters

2.1. Selection of monitoring sites

2.1.1. Classification of sampling site

EANET monitoring sites are classified into two basic categories, namely deposition monitoring sites and ecological survey sites. Deposition monitoring sites are sampling sites to collect fundamental data on the temporal and spatial distribution of acid deposition, and are further classified into three sub-categories: remote sites, rural sites, and urban sites for the objectives of the monitoring. Ecological survey sites are those to provide basic data for assessing the effects of acidification on terrestrial ecosystems, and further classified into two sub-categories: basic survey sites, and ecosystem analysis sites. All sites in each country should be classified according to these categories.

a. Deposition monitoring sites

Deposition monitoring sites in this network should be classified into three sub-categories: remote sites, rural sites and urban sites according to the objectives of the monitoring. Wet deposition monitoring, and desirably dry deposition monitoring as well, should be carried out at these sites.

b. Ecological survey sites

Ecological survey sites should be classified into two sub-categories: basic survey sites and ecosystem analysis sites, according to the objectives of the monitoring. Soil and vegetation monitoring and monitoring for inland aquatic environment should be carried out at these sites.

1) Basic survey sites

Basic survey sites are to be established at the deposition monitoring sites or in their vicinity in order to accumulate basic data on soil, forests and inland aquatic environment, and trends in properties. At these sites, chemical and physical analysis of soil, description of trees, survey of understory vegetation, forest decline, and inland aquatic environment should be carried out.

2) Ecosystem analysis sites

Ecosystem analysis sites are to be established for the assessment of acid deposition impacts on whole ecosystems through application of, for instance, terrestrial ecosystem analysis and/or catchment analysis. The location of these sites should be selected in areas where terrestrial ecosystems are sensitive to changes in atmospheric acidity. Some of these sites should also be located in ecologically conserved areas. At these sites, elemental dynamics in ecosystems should be surveyed, and environmental capacity for acid deposition should be estimated. Acidification models may also be

developed for these sites.

2.1.2. Criteria for site selection of lakes and/or rivers (streams)

Lakes will be selected as monitoring sites. If appropriate lakes are not available, rivers (streams) that are potentially susceptible to acidification and have little artificial influence should be selected.

Because the sampling point should be representative in the water bodies, it should be confirmed within half a year from the start of sampling, that the sampling site represents the water quality of the water body, by analyzing relevant items of surface water in several points (more than five sites including the center of the water body). In the case that there are islands at the center of site, the detailed survey is needed to decide a representative point in the site. It is desirable that the monthly and ten-days period variations be investigated to evaluate the representativeness of a sampling site (more than 4 times, in each season). For the time being, on-site measurement of water temperature, electric conductivity and pH values can be deemed as a substitute method for these investigations.

a. Criteria of lakes

It is recommended that harmonic lakes which are considered to be potentially susceptible to acidification should be selected. Natural lakes have higher priority for selection of sites than artificial lakes. If the management such as dredge is carried out, effects of the management should carefully be investigated. Oligotrophic or Mesotrophic of harmonic lake is recommended (Table 2.1). If there is no harmonic lake, dystrophic lakes could be selected for monitoring. However, in this case, appropriate monitoring methods should further be investigated.

It is desirable to choose monitoring lakes which are harmonic type with low BOD, COD, or TOC (inorganic acidic lakes, organic acidic lakes or alkaline-based eutrophic lakes is not good for the monitoring), preferably having a maximum depth of approximately 10 m or less, a water retention time of 1 year or less, water area from 1 hectare to 100 hectares, low alkalinity (less than 200 $\mu eq\ L^{-1}$) or electric conductivity (less than 10 $mS\ m^{-1}$), minimal anthropogenic water pollution and no coverage of the surface with aquatic plants.

The lakes's catchment area is desirable to be not so big. It is also desirable that the catchment is covered by acidic or neutrality bedrock geology, nature protection (conservation) areas and natural vegetation. The access from the site to the laboratory is desirable to be short for preventing change of the sample qualities.

Table 2.1. Classification of harmonic lakes by trophic level (OECD, 1982)

Classification	TP mg m^{-3}	Chlorophyll-a mg m^{-3}		Transparency m	
		mean	max	mean	min
Extreme oligotrophic	≤ 4.0	≤ 1.0	≤ 2.5	≥ 12.0	≥ 6.0
Oligotrophic	≤ 10.0	≤ 2.5	≤ 8.0	≥ 6.0	≥ 3.0
Mesotrophic	10 ~ 35	2.5~8	8~25	6~3	3~1.5
Eutrophic	35 ~ 100	8~25	25~75	3~1.5	1.5~0.7
Hypereutrophic	≥ 100	≥ 25	≥ 75	≤ 1.5	≤ 0.7

Pleliminary chemical analysis is recommended for site selection on items as follows,

●Water temperature (W.T.), ●pH, ●electric conductivity (EC), ●transparency, ●water color, ●alkalinity, ●dissolved oxygen (DO) and ●dissolved organic carbon (DOC) (if impossible, chemical oxygen demand (COD))

●Cations: NH_4^+, Na^+, Ca^{2+}, Mg^{2+}, K^+ and total dissolved Al

●Anions: SO_4^{2-}, NO_3^- , NO_2^-, Cl^- and PO_4^{3-}

b. Criteria of rivers (streams)

Rivers or streams that are potentially susceptible to acidification may be selected, where the impacts of human activities such as deforestation, slash-and-burn farming, stock-farming or cultivation is not being conducted or planned in the future in the upper stream area of the water sampling site. The river/stream's catchment area is desirable to be not so big. It is also desirable that the catchment is covered by acidic or neutrality bedrock geology, nature protection (conservation) areas and natural vegetation.

Especially, to prevent the influence of other pollutions and storm runoff, streams have higher priority than rivers in the site selection. In the case of selecting rivers, the upper streams of a river or first-order streams (as stream order) is desirable for the areas with storm events. At upper reach of the stream area, monitoring should be done at one point, and measurement of the flow is desirable.

It is desirable to choose monitoring rivers (streams) which are natural rivers (streams), having low alkalinity (less than 200 μeq L^{-1}) or electric conductivity (less than 10 mS m^{-1}) with low BOD, COD, or TOC. The recommendations for catchment properties and accesssibility are the same as the lakes.

In the case of river (streams), flow volume and ion concentrations change dramatically with intense rainfall. Therefore, sampling should be carried out when there is no or small rainfall (below 10 mm per day) within 2 days before monitoring for average samples. Samples should also be collected during flood and after intensive rainfalls or

snow melting, if possible. This will allow us to get more reliable information already on the stage of a plot selection. On this stage, the most important parameters to be measured are the temperature, electric conductivity, and pH values.

Preliminary chemical analysis is recommended for site selection on items as follows,

●Water temperature (W.T.), ●pH, ●electric conductivity (EC), ●transparency, ●water color, ●alkalinity, ●dissolved oxygen (DO) and ●dissolved organic carbon (DOC) (if impossible, chemical oxygen demand (COD))

●Cations: NH_4^+, Na^+, Ca^{2+}, Mg^{2+}, K^+ and total dissolved Al

●Anions: SO_4^{2-}, NO_3^-, NO_2^-, Cl^- and PO_4^{3-}

Recommended criteria for site selection are summarized in Table 2.2.

Table 2.2. Recommended criteria for site selection

Recommendation items	Lakes	Rivers (streams)
Alkalinity	less than 200 μeq L^{-1}	
EC	less than 10 mS m^{-1}	
Trophic level	oligotrophic	-
BOD (COD), TOC	low	
Retention time	Less than 1 year	-
Depth	Less than 10 m (max)	Less than 2 m (cross-sectional mean)
Discharge	-	< 5 m^3 s^{-1}
Water area	1~100 ha	-
Surface situation	No coverage of aquatic plants	
Human activities	No or minimal	
Recommendation items for the catchment of the site		
Location of river	Rivers in the mountain areas	
Catchment Area	< 500 ha	
Bedrock geology	Acidic or neutrality	
Vegetation	Natural	

2.2. Collection of information concerning monitoring site

An inventory of lakes (including man-made reservoirs) based on their limnological significance and/or with water area of larger than 1 ha in the area in question should first be prepared. The monitoring site should be selected from the inventory, based on the criteria for site selection. Then the following information on both the selected site and its watershed/catchment should be collected as much as possible from the past to the present. The standard format and an example of information on the site and its watershed/catchment are shown in Table 2.3 and 2.4 respectively. A colored photograph

of the site is useful (Photo.1). An aerial view of the site is most preferable.

2.2.1. Lakes

a. Characteristics of lakes

● location and location map, ●elevation, ●origin, ●area, ● shore line length,
● lake hydrologic type (seepage, closed, drainage, and reservoir)
● lake trophic type (oligotrophic, mesotrophic, eutrophic and dystrophic with indication of OECD criteria etc.), ●water depth (mean and maximum),
●water volume, ●bathometric map, ●range of annual water level fluctuation,
●residence time of water, ●lake utilization (irrigation, domestic water, electric power, fish culture, sightseeing, and others)

b. Watersheds/catchments

● area, ●elevation and topography, ● surface geology, ●soil types, ●vegetation,
●land use, ●population, ● numbers and discharge of streams (inlets and outlets),
● numbers, discharge and water qualities of spring or ground waters around the shore
● wind direction and speed (mean and prevailing), ●precipitation, ●solar radiation

c. Living organisms in lakes

● chlorophyll pigments, ● fauna, ● flora, ● biomass of bacteria and phytoplankton,
● primary productivity of phytoplankton, ●zooplankton, ●fish, ●benthic organisms

d. Sediment in lakes

Physico-chemical properties:
● texture, ●grain size, ●volumetric water content(bulk density)
● electoric potential of hydrogen (Eh), ●organic carbon, ●SO_4^{2-} , ● NO_3^-
● sulfur stable isotope ratio of sulfate (if available), ●sedimentation rate (if available)
Biological properties:
● Diatom species

2.2.2. Rivers (streams)

a. Characteristics of rivers (streams)

● location and location map, ●elevation, ●origin, ●area,
●range of annual discharge fluctuation (at the sampling site),
●river utilization (irrigation, domestic water, electric power, fish culture, sightseeing, and others)

b. Watersheds/catchments of rivers (streams)

● area, ●elevation and topography, ●surface geology, ●soil types, ●vegetation,
● land use, ●population, ●numbers, discharge and water qualities of spring or ground waters around the river, ●precipitation, ●solar radiation,

●wind direction and speed (mean and prevailing)

c. Living organisms rivers(streams)

● fauna, ●flora, ●fish, ●benthic organisms

d. Sediment in rivers (streams)

Physico-chemical properties:

● texture, ●grain size, ●volumetric water content(bulk density),

●electoric potential of hydrogen (Eh), ●organic carbon, ●SO_4^{2-}, ● NO_3^-,

● sulfur stable isotope ratio of sulfate (if available), ●sedimentation rate (if available)

Biological properties:

● attached algae (diatom species), ●chlorophyll pigments

2.2.3. Standard format

Each laboratory makes the effort for submission following standard format for the lake or the river (Table 2.3) every year. There is an example of information concerning monitoring site (Table 2.4).

Table 2.3. Standard format for the site properties (research year)

Country			
Location			
Kind	1. Lake 2. River (stream) 3. Other ( )		
Site name			
Altitude	m above sea-level		
Site Classification	1. Urban 2. Rural 3. Remote		
Latitude		Longitude	
Origin (for lakes/ponds)			
Nearest Wet deposition monitoring site		(km)	
Living organisms			
Catchment Area	km^2 (based on the sampling site)		
Catchment elevation and topography		m~ m	
Surface geology			
Soil types			
Vegetation			
Land use			
Population			
Lake area	m^2	Lake shape	
Shore line length	m		
Lake trophic type			
Water depth(mean)	m	(maximum)	m
Water volume	m^3		
Annual water level fluctuation		m ~ m (mean m)	

Residence time of water			
Lake utilization			
Number of inflow river		Number of outflow river	
River length			
River water depth (mean)	m	Minimum & maximum	m
Flow discharge (m^3 sec^{-1})	Mean Minimum Maximum		
Drought or freeze	1. Nothing 2. Existence(~)		
Lake or river (flows into)			
Precipitation (mm)	Annual and monthly data		
Evaporation (mm)	At least annual		
Solar radiation			
Wind speed	mean		
Prevailing Wind direction			
Annual air temperature			
Relative humidity			
Nearest meteorological station			
Soil chemical properties in the catchment area			
Bottom sediment			

Table 2.4. The example of information concerning lakes

Lake Name: Ijirako Lake (Pond)

Lake Characteristics

Country: Japan
Location: Yamagata-shi, Gifu Prefecture, 35°34′N, 136°56′E, refer to attached Map
Altitude: 110m above sea-level
Origin: Artificial (dam-made lake)
Area and shape: 0.1 km^2, refer to attached Figure
Shore line length: 1.8 km
Lake hydrologic type: Reservoir
Lake trophic type: Oligotrophic to mesotrophic
Water depth (mean and maximum): 5.4 m and 10.9 m
Water volume: 540× 10^3 m^3
Annual water level fluctuation: 0 to 740 mm (mean 220 mm)
Precipitation: 1985mm/year (1983-1988)
Solar radiation: Daylight time 173 hr. $month^{-1}$ (1983-1988)
Wind speed (mean and dominant): 1.8-2.8m s^{-1}
Wind direction(dominant) : SE,S(summer),NW,N(winter)
Residence time of water : 23 days
Lake utilization : Irrigation, sightseeing and fishing

Watershed

Area: 5.4 km^2

Elevation and topography: 110 – 696 m above sea-level

Superficial geology: Chert

Soil types: Brown forest soil

Land use: Forests(99.6%)

Vegetation: Coniferous trees (Japanese red pine, cedar,cypress, and red pine)

Population: None

Numbers of streams: 2 inlets ; 8300× 10^3 m^3 $year^{-1}$

Ground water: (No spring water: 1 well with pumping-up rate 4 × 10^3 m^3 $year^{-1}$)

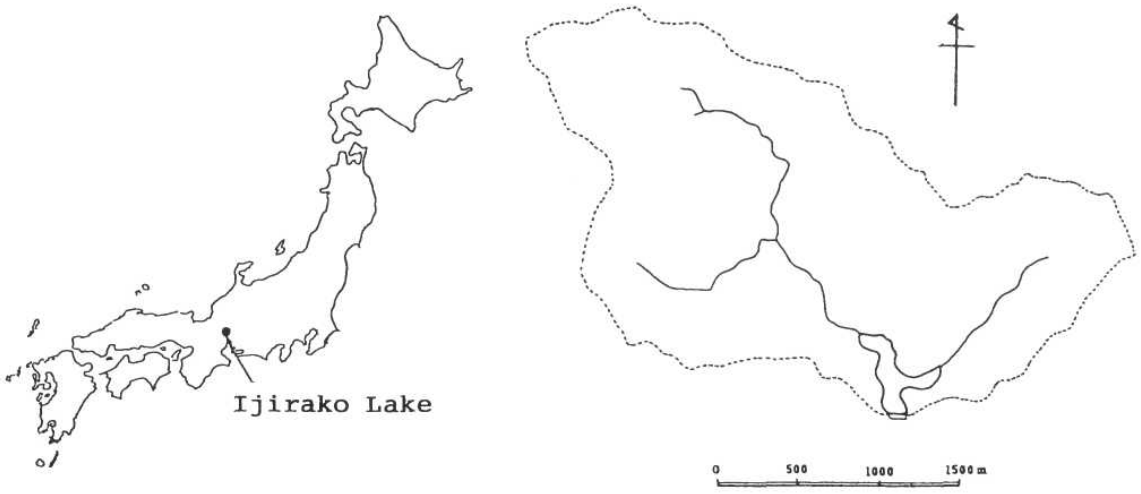
Sediment

Water contents: 42.6%, Ignition loss: 11.8%

Texture: Grayish clay, Particle size: below 0.074mm (72% wt.)

Benthos: *Tubifex* sp., *Chironomus* sp.

Data Source; Gifu Prefecture (1989,1990), Murase *et al*. (1990,1991)



Lake Area: 0.1km^2

Watershed Area: 5.4km^2

Fig. 2.1. Location of Ijirako Lake and Its Watershed

Fig. 2.2. A view from the lakeside of Ijirako Lake (Photo: by H. Murase)

2.3. Measurement parameters and frequency of monitoring

Measurement parameters should be classified into two categories: mandatory and optional parameters presented in Table 2.5. Frequency of monitoring depends on parameters and items. The information on the definition and/or meaning of the respective parameters were summarized in Appendix 3

2.3.1. Lakes

1) Mandatory parameters

Frequency: 4 times a year

- Water Temperature (W.T.)
- pH
- Electric Conductivity (EC)
- Alkalinity (Gran's plot titration and/or the pH 4.8 endpoint)
- Major cations: NH_4^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+
- Major anions: SO_4^{2-}, NO_3^- and Cl^-
- Transparency
- Water color
- Dissolved organic carbon (DOC) or Total organic carbon (TOC)
- Other anions: NO_2^- and PO_4^{3-}
- Chlorophyll a
- Total P
- Total N
- Dissolved oxygen (DO)

Frequency: every 3-5 years

- Sediment: SO_4^{2-}, NO_3^- and NH_4^+ (in pore water)

2) Optional parameters

Frequency: 4 times a year

- Total dissolved Al
- Reactive Al (if total dissolved Al > 200 μg L^{-1})
- Chemical oxygen demand (COD)
- Phytoplankton (Diatom species)

Frequency: every 3-5 years

- Living organisms other than phytoplankton
- Sediment (Pb, Pb-210 and stable isotope of S)

2.3.2. Rivers (streams)

1) Mandatory parameters

Frequency: every one month or two months

- Water Temperature
- pH
- Electric Conductivity (EC)
- Alkalinity (Gran's plot titration and/or the pH 4.8 endpoint)
- Major cations: NH_4^+, Ca^{2+}, Mg^{2+}, Na^+ and K^+
- Major anions: SO_4^{2-}, NO_3^- and Cl^-
- Water color
- Dissolved organic carbon (DOC) or Total organic carbon (TOC)
- Other anions: NO_2^- and PO_4^{3-}
- Total P
- Total N
- Suspended solids (SS)

2) Optional parameters

Frequency: every one month or two months

- Hydrological flow (at sampling time)
- Total dissolved Al
- Reactive Al (if total dissolved Al > 200 μg L^{-1})
- Chemical oxygen demand (COD)

Frequency: 4 times a year

- Epilithic algae (diatom species)

Frequency: once 3-5 years

- Living organisms other than epilithic algae

Table 2.5. Mandatory and optional parameters

Mandatory Parameters	Optional Parameters	Frequency
W.T., pH, EC, Alkalinity, Major cations, Major anions, Transparency*, water color, DOC or TOC, NO_2^- and PO_4^{3-}, Chlorophyll a*, Total P, Total N, DO*, SS**	Hydrological flow**, Total dissolved Al, Reactive Al (if total dissolved Al > 200 μg L^{-1}), COD, Phytoplankton (diatom species)* Epilithic algae (diatom species)**	4 times a year for lakes Every one or two month(s) for rivers
Sediment (SO_4^{2-}, NO_3^- and NH_4^+ in pore water)*	Other living organisms, Sediment (Pb, Pb-210 and stable isotope of S)*	Once 3-5 years

*, parameter for lakes only; **, parameter for rivers only.

The minimum frequency of water sampling in each region is presented on Table 2.6.

Table 2.6 Minimum frequency of sampling times in each region*

Region	Water sample in lakes, and rivers	Sediment in lakes	Attached matter in rivers
Tropical	Lake: 4 (wet, dry, 2 between in one year) River: 12 or 6 at least (every month or two months at least)	1 (in 3-5 years)	4 (wet, dry, 2 between in one year)
Temperate	Lake: 4 (high water level, low water level, 2 between in one year) River: 12 or 6 at least (every month or two months at least)	1 (in 3-5 years)	4 (high water level, low water level, 2 between in one year)
Boreal	Lake: 4 (high water level, low water level, 2 between in one year) River: 6 (no-frozen season)	1 (in 3-5 years)	4 (high water level, low water level, 2 between in one year)

* This table is prepared for items measured more than 4 times and once in 3-5 years. The iced stream in frigid region and dried stream in tropical region should be avoided for sampling.

Dry-day runoff and storm runoff

The concentration of water quality in mountainous streams is hydrologically influenced by a magnitude of preceding storm event and a length of preceding dry days. Furthermore, it is water-chemically affected by a course of flowing-path and a retention time in the path.

Generally, the stages and conditions of stream flow are hydrologically classified by flow rate and water level. The runoff loadings of pollutants and nutrient salts are classified to two stages, that is, **a dry day stage** and **a stormy day stage** in the research field of water pollution.

During the rising stage of stream flow the dissolved components of water quality are diluted with swollen stream water by a storm event and a melting-snow event, but the particulate components become higher concentrations by erosion of surface soil layer and stream bed in the water-course. In the flooding stage, the concentrations of dissolved components become low, though the surface runoff components of mainly "new rainwater (this rain event)" with low concentration due to its short detention time (that is, reaction time) on the ground surface dilute the interflow and groundwater runoff components of "old rainwater (of preceding rain events)" with high concentration owing to their long detention time in the soil and basic rock layers.

However, in the case when the component of surface runoff is small negligibly as compared to the component of interflow runoff in the first half of a storm event with long preceding dry days (*i.e.* if the rain intensity exceeds the infiltration capacity of the ground surface in the catchment), the dissolved components of stream water become higher due to the higher concentration of interflow runoff component (that is, old rainwater) owing to its long detention time in the soil layer.

Therefore, to measure the quantitative analysis of neutralizing ability in a catchment to acid deposition load, we must take strictly account of both runoffs of **dry-day runoff** and **storm runoff** in the hydrological condition.

3. On-site measurement and sampling

3.1. On-site measurement

Methods for measurements of water quality at the site are as follows.

Water temperature (W.T.): The measurement of W.T. should be conducted by using a thermometer and/or a portable pH or EC meter with a temperature sensor.

pH: The measurement of pH at the site should be carried out by using a portable electrometric pH meter with glass electrode as reference data (Fig. 3.1). Before determination at the site, the pH meter must be calibrated at pH 4, 7 and/or 9 by using standard solutions for the instrument. When not in use for long time, the glass electrode should be kept clean.

Electric conductivity (EC): The measurement of electric conductivity at the site should be carried out by using a portable EC meter as reference data (Fig. 3.2). When the electric conductivity is measured at the water temperature of the site, it may be corrected to the value at 25°C. The equation for correcting is as follow.

$$(EC(t)) = (EC(25)) \times [1 + 0.022 \times (t-25)]$$

where, t : water temperature at the site

DO (dissolved oxygen): The DO is measured vertically (if possible, in some vertical points) by portable DO meter at the water sampling point instead of Winkler-modified method. Before using it, it should be calibrated. For further procedure refer to 5.1.15.1.

Water color: It is recommended to measure water color by using Forel's and Ule's color standards and so on for more objective measurement instead of measurement by your eyes. The water color of surface water should be measured from the center of lake (on boat) or main flow of river.

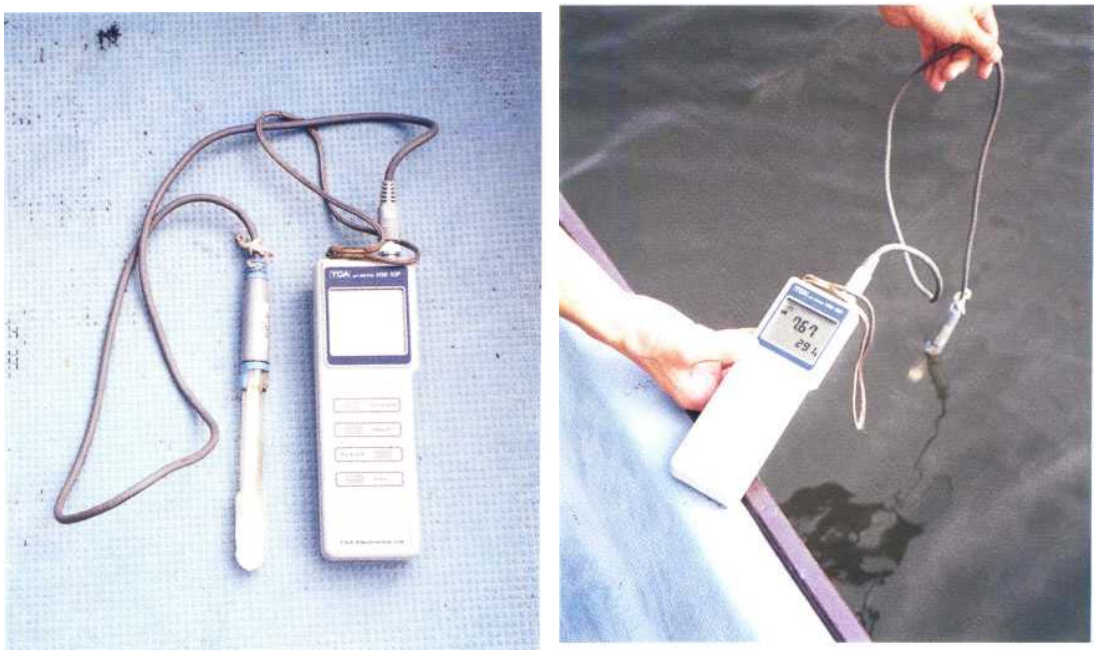


Fig. 3.1. Portable pH meter

Fig. 3.2. Portable electric conductivity meter

3.2. Collection of samples

3.2.1. Lake water

Surface water is sampled directly at the center of the lake or another representative point of the lake. Use of GPS, anchors or ropes for fixation of the boat is recommended. For the surveyors' safety, life jackets or lifelines should be weared. As reference data,

the measurements of W.T., pH and EC are recommended to be conducted at the site by using portable instruments. The water sample for determining EC, pH and alkalinity should be taken in glass or polyethylene/polypropylene bottles without air. The water sample for later chemical analysis should be filtered and put in a tightly stoppered glass or polyethylene bottle, and be stored in a cool and dark place. The bottles for chemical analysis should be cleaned well beforehand using 10% HCl solution.

Surface water should be sampled directly by a clean polyethylene bucket or a dipper at the representative point of the lake. The duplicate samples should be collected at the same point. The water sample should be taken full up in two well-washed 1 L glass or polyethylene (polypropylene) bottles without air after washing twice by sample water. It is desirable that the samples for analysis of chemical components other than pH, EC and alkalinity are filtered at the site when the samples will not be measured for longer time than several hours after sampling (Whatman GF/C,GF/F or Millipore GF filter whichpore size is about 1 μm with 47 mm diameter, dried at 100 ± 5°C for 2 hours).

Water sampling and fixation for DO analysis: (Winkler-modified method)
Water for measuring dissolved oxgen (DO) concentration should be collected vertically using a Van Dorn water sampler.

Insert the tube of the water sampler into the bottom of the oxygen bottles (two bottles for one depth). Allow the water to overflow carefully to two or three times its volume without introducing air bubbles. Gently remove the tube while letting the flow continue, then stopper the bottles. Add 0.5 ml of manganese sulfate solution by inserting the tip of a pipette into the bottle, and then add 0.5 ml of alkali-iodine azide reagent in the same way. After carefully stoppering the bottle without retaining air bubbles, mix by inverting the bottle about thirteen times. Store the bottles in buckets of lake water, and transport them to a laboratory in dark condition. For further procedure refer to 5.1.15.2.

If possible, DO should be measured vertically by portable DO meter at the water sampling point instead of Winkler-modified method.

Transparency: It is measured by secci disk at the sampling point (on boat).

It is recommended to measure the lake water level for estimating the hydrological condition of lake at the sampling time.

3.2.2. River (stream) water

Surface water is collected directly at the center of main flow without floating material. For the surveyors' safety, life jackets or lifelines should be weared. As the reference data, the measurement of water temperature, EC and pH are conducted at the site by using

portable instruments. The treatment of water samples and on-site measurement is carried out in the same way as for lake water. It is recommended to measure discharge as the hydrological condition at the sampling time (refer to Appendix 3).

3.2.3. Phytoplankton and epilithic algae (diatom)

Column sampling:

Small cells of phytoplankton species, much smaller than the mesh openings of usual plankton nets, are abundant in lake water, a simple column sampler having 30 to 50 mm diameter and 1-1.5 m length (Fig. 3.3) is recommended for sample collection. The water sample is collected from the surface water layers. It is transferred into a clean bucket, then dispensed into polyethylene bottles with appropirtae volume of 100 mL – 2L, depending on phytplankton density. Add Lugol's solution* to the sample water to make a 1% solution or neutral formaldehyde solution to water giving 2 - 3% solution for preservation of phytoplankton. Bottles are transported to the laboratory under dark conditions. For further procedure refer to 5.2.

*Lugol's solution: Dissolve 5 g of iodine, 10 g potassium iodine and 10 mL glacial acetic acid in 100 mL distilled water.

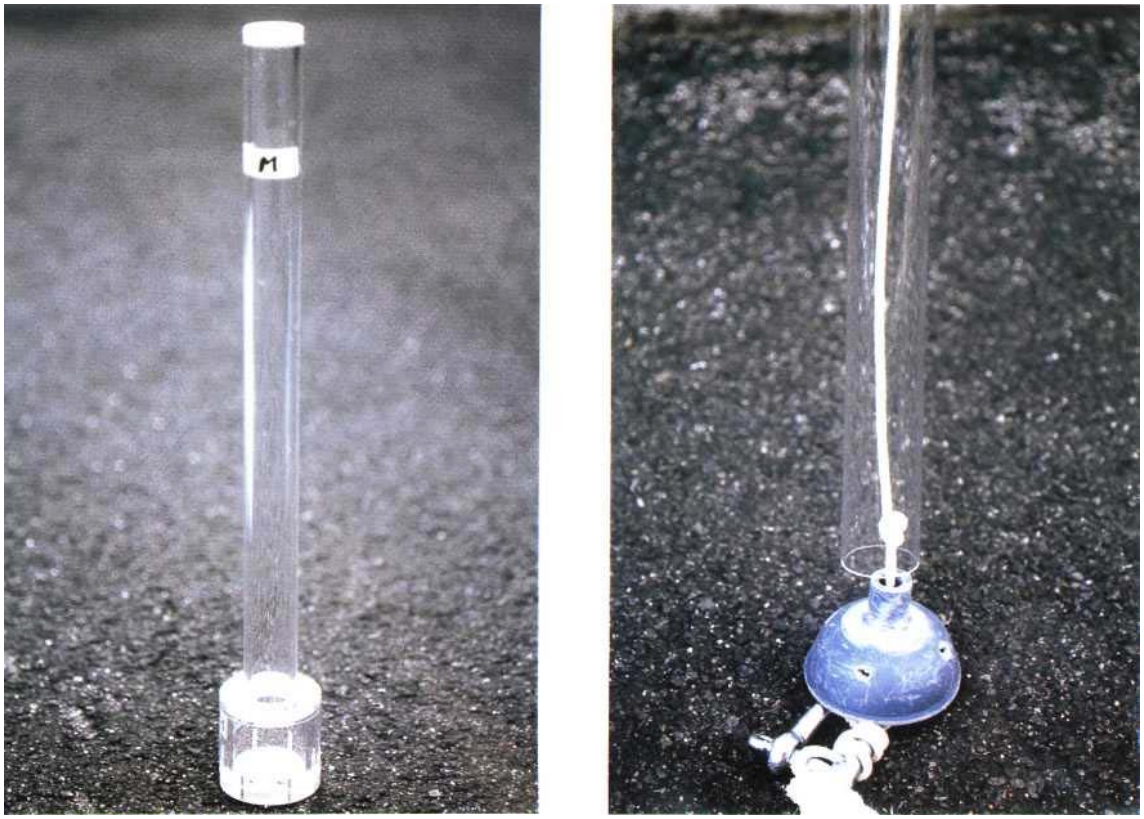


Fig. 3.3. Column sampler

Epilithic algae (Diatom) (from river)

Stable submerged stones with about 15 - 20 cm are picked out from more than five

places in the middle of rapid main flow. Then attached substances containing algae to the upper surfaces of the stones are stripped off with a stiff nylon brush (teeth brush) in a shallow plastic pan, rinsing the brusshing side of stones with a small amount of clean water in a washing bottle. If the amount of algae is not sufficient, samples should be gathered from several stones. Water containnig attached algae should be fixed by adding neutral formalin* to make about 3% solution. For further procedure refer to 5.2.
*Neutral formalin: Saturate 37% formaldehyde solution with magnesium carbonate

3.2.4. Sediment and attached matter

a. Lakes

It is desirable, if possible, to estimate past changes in water quality by examining samples of lake sediment for the information recorded within. Analysis of the sediment core samples is used to provide clues to assess the yearly trends of lake acidification when data of the past water quality are not available.

A lake sediment core of 150 to 300 mm length is obtained at one location at the center of lake by using a core sampler (Fig. 3.4 and 3.5) with at least 50 mm diameter. For further procedure refer to 5.3.

Fig. 3.4. Sampling of bottom mud core sample by using a simple core sampler (left) and put on a rubber cork in the bottom side of a column in water (right)

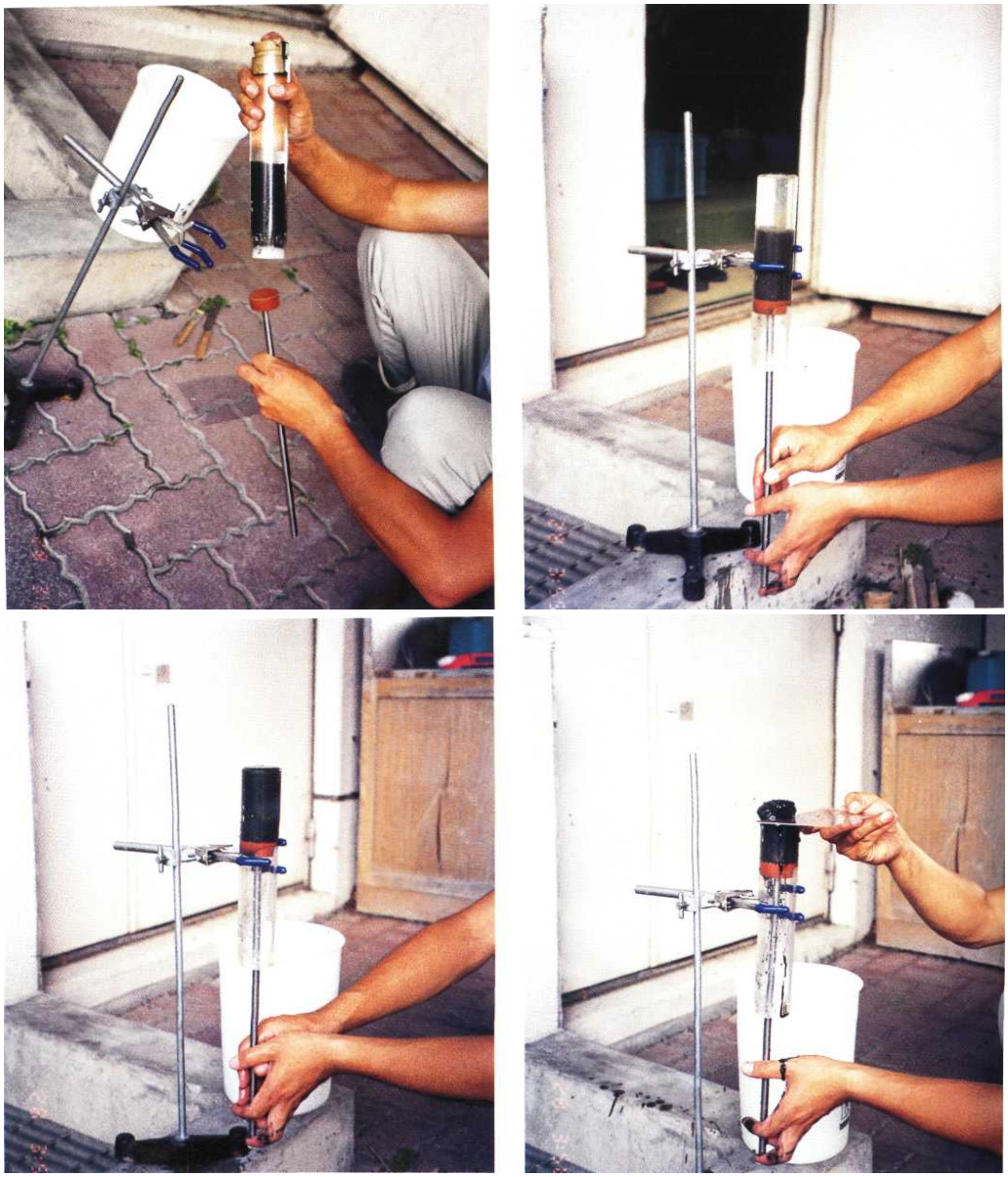


Fig.3.5. Treatment procedure for cuttin sample in laboratory

b. Rivers

Attached matter (river):

The matter attached to the stones or rocks of a river bed can be used for chemical analysis and algal species composition analysis. Fixation method of samples is described in 3.2.3. The attached matter scraped off (5 × 5 cm) with water is separated into two parts; one for species composition analysis of diatoms, and the other for chemical analysis of the attached substances . For further procedure of species composition analysis refer to 5.2.

The sample for chemical analysis should be stored in a cool and dark place. After coming back to a laboratory, the samples should be analyzed as soon as possible.

4. Transportation and storage of samples

4.1. Water samples

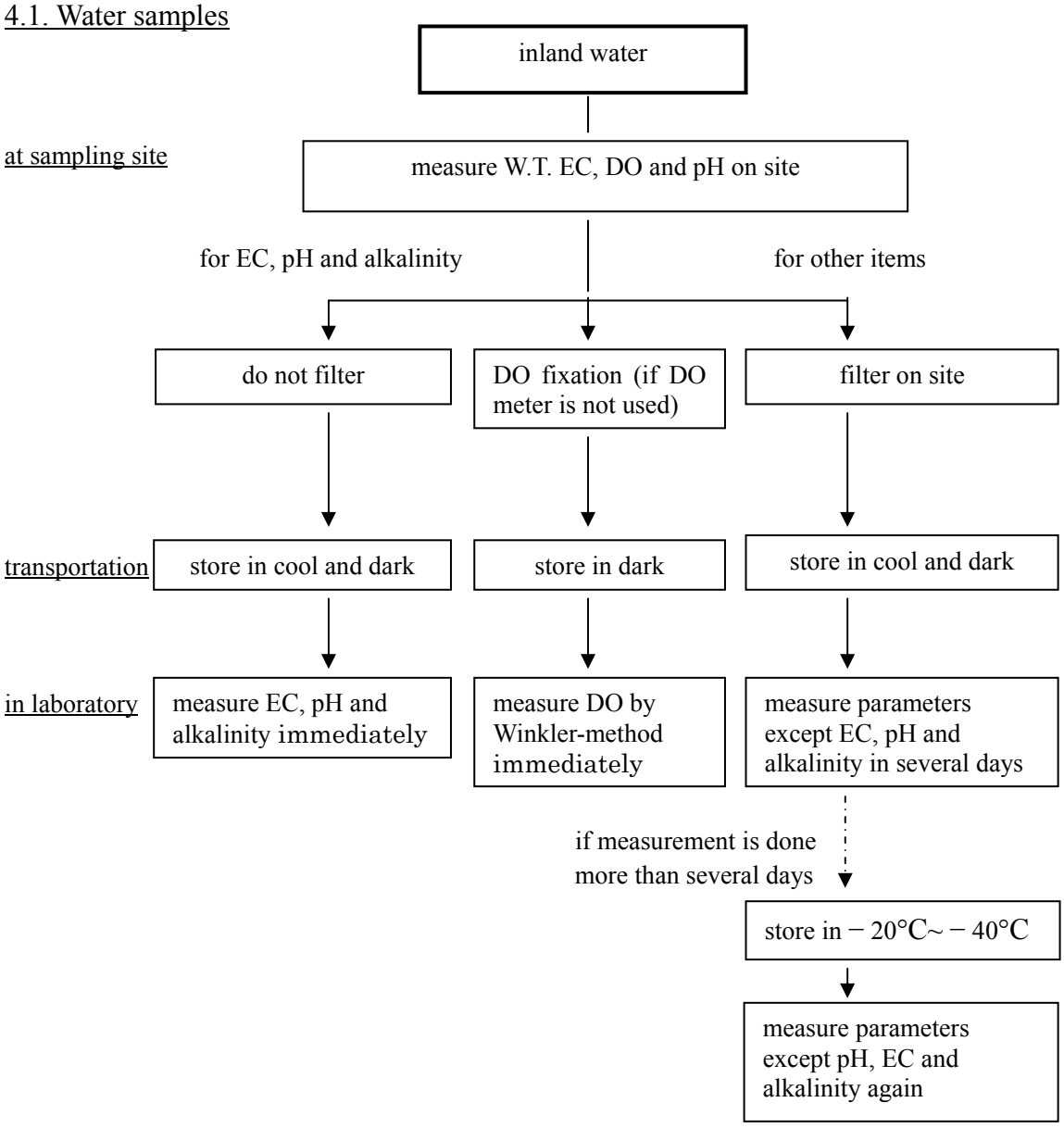


Fig. 4.1. Treatment procedure of inland water samples

The water samples for analysis of chemical components other than pH, EC and alkalinity should be filtered at the sampling site, and are done with a glass fibre filter (Whatman GF/C or Millipore GF filter which pore size is about 1 μm with 47 mm diameter, dried at 100 ± 5°C for 2 hours in an oven: preliminary filtration for SS analyses). For the chemical components, aliquot of the water samples should be filtered again via membrane filter of 0.45 μm pore size (with 47 mm diameter). The water samples should be carried to the laboratory in a cool and dark box with cooling gel. If

the time of transfortation is within several hours, the water samples could be filtered in laboratory immediately after arrival at the laboratory.

The measurement of parameters of pH, EC and alkalinity must be carried out as soon as possible after the water samples arrive at the laboratory in the same day. The non-filtered sample should be stored in refrigerator less than 5°C for measuring the sample again. The filtered water samples should be measured within several days after arriving at the laboratory.

If the filtered water samples must be stored for more than several days in the laboratory, they should be frozen in a freezer at −20 °C to −40 °C. It is recommended that a part of these samples at least more than 100 mL should be in storage in freezing condition for one year because of measuring some ions again. Fig.4.1 shows the treatment procedure of water samples from sampling site to the laboratory. Transport and storage situation and its photo should be recorded.

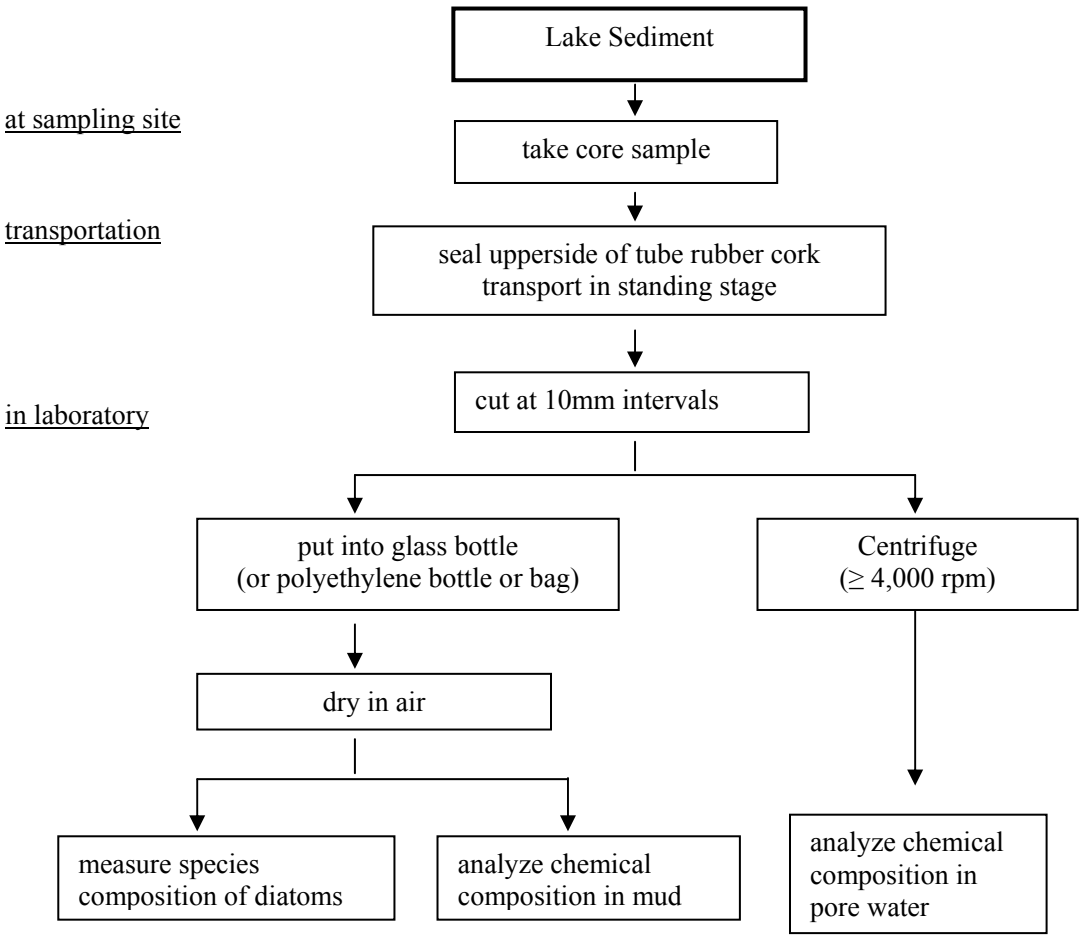


Fig. 4.2 Treatment procedure of lake sediment sample

4.2. Sediment

The sediment sample is divided into strata as appropriate based on its condition and structure, and applied to determine the chemical composition and the species composition of diatoms in the various strata. Each sediment sample is cut to 10 mm thickness by using a knife that does not contaminate the sample. Each sample is air-dried and stored in clean glass or polyethylene bottles or bags (Photo.6). The sediment sample should be stored in refrigerator less than 5 °C for measuring the sample again. Treatment procedure of the sediment sample is shown in Fig. 4.2.

4.3. Plankton (diatom)

The collected plankton should be dispensed into polyethylene bottles and transported to the laboratory under dark conditions.

Identification of diatom species should be carried out by a specialist in the group. If the lake monitored is at risk of acidification and identification of diatoms is not possible, it is desirable to store fixed samples or diatom slides. Taking microscopic photographs of algal samples is also recommended. Fig. 4.3 shows the treatment procedure for the attached matter of rivers.

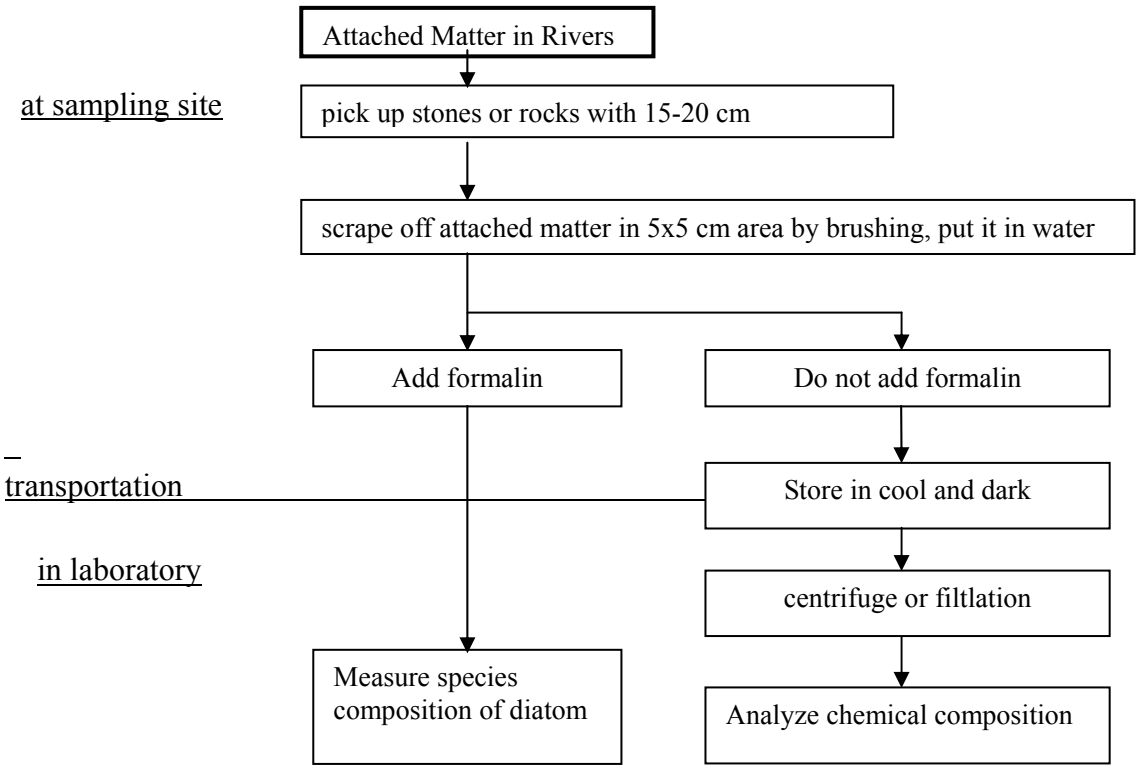


Fig. 4.3. Treatment procedure for attached matter in rivers

5. Analysis in laboratory

5.1. Water

The analytical methods for water quality are listed in Table 5.1. Analytical procedures for these parameters are described below.

Table 5.1. Analytical methods suggested for water quality

	Parameters	Analytical Methods
1)[a]	pH	Glass electrode (preferably with the electrode of non-leak iner cell)
2)[a]	EC	Conductivity Cell
3)[a]	Alkalinity	Gran's plot titration method and/or pH4.8 endpoint method; Titration by Burette or Digital Burette with pH Meter
4)	NO_3^-, NO_2^-, PO_4^{3-} and SO_4^{2-}	Ion Chromatography (preferably with suppressor) or Spectrophotometry
5)	NH_4^+	Ion Chromatography or Spectrophotometry (Indophenol blue)[b]
6)	Ca^{2+}, Mg^{2+}, Na^+ and K^+	Ion Chromatography or Atomic Absorption/Emission Spectrometry
7)	Cl^-	Ion Chromatography or Titration
8)	DOC or TOC	TOC analyzer Method or Wet-Oxidation Method
9)	Chlorophyll a	SCOR/UNESCO Method
10)	Total P	Potassium Peroxodisulfate Decomposition Method
11)	Total N	Ultra-violet absorption spectrophotometry method Hydrazinium sulfate reduction Method
12)[a]	SS	1 μm Glass Fiber Filter method
13)	Total dissolved Al	Atomic Absorption Spectrometry with Graphite Furnace, ICP Emission Spectrometry or ICP/MS
14)	Reactive Al	Lumogallion method, spectrophotometry
	COD	Potassium Bichromate Method (or Acidic Potassium Permanganate Method)
15)	DO	DO Meter Method or Winkler-Modified Sodium

		Azide Method

Note: a, applied for non-filtered sample; b, not recommended if the biocide, thymol, is used in sample collection.

Pure water and reagent etc. for analysis should be confirmed to be not interfare with the target analysis matter before analysis. Moreover, the blank value for the target analysis matter should be needed to decrease as possible.

i) Pure water

Pure water for analysis, rinse and dilution for sample should be used less than 0.15 mS m^{-1}.

ii) Standard solution and standard sample

Because the measured values are calculated based on measered results of the collected sample and standard samples, the standard solution and standard matter certified for the traceability shouled be used.

iii) Certified Refference Material

Certified Refference Material (CRM) is used for management of the analysis instrument. For daily management of the instrument, for instance, artificial lake water for the inter-laboratory comparison projects can also be used as working standards.

5.1.1. pH

The pH of a sample is related to the free acid activity as the negative logarithm of the hydrogen ion concentration by the equation.

$$pH = - \log (H^+)$$

where (H^+) is the activity or concentration of free hydrogen ions. The pH of a sample is determined electrometrically, using a standard pH meter with a glass (H^+) electrode in combination with a reference electrode. The glass electrode potential varies as the activity of H^+ ion in solution. The pH meter should be sensitive enough to measure pH up to ± 0.01 pH unit and be calibrated before and after measurement at least at two points (among pH values of 7.0 and 4.0 or 7.0 and 9.0) in the expected pH range. The temperature, which effects on electrometric pH measurements, can be controlled by using instruments that have temperature compensation. Measurement of the standards and samples at 25 ± 0.5 °C in a water bath is recommended.

<u>i) Apparatus</u>

a. A pH meters are commercially available with different specifications and options. A pH meter should have both an intercept and slope adjustment and should be capable of measuring to ± 0.01 pH unit. Two electrodes should be used with the pH meter. A measuring glass electrode is sensitive to hydrogen ions. The reference electrode can be calomel, or silver/silver chloride; other reference electrodes can also be used as long as they have a constant potential. Combination electrodes with both measuring and reference functions are preferable since they require less of the sample to be used.

The pH electrodes should be stored in deionized water (when the room temperature is above 25 °C, it is not recommended to keep an electrode in deionized water for a long time, because electrodes are prone to be attacked by photosynthetic algae), in a 7.4 mS m^{-1} KCl standard, in 10^{-4} M acid (H_2SO_4) solution or in filling solution. Prior to use, the pH electrode should be thoroughly rinsed with deionized water.

One of the chief problems that occur with pH measurements is the aging of the electrode. Reference solutions, which have a known pH and conductivity similar to those of the samples, should be used for checking the pH electrode. These solutions should be stored under refrigeration and replaced when solution pH or conductivity are seen to have changed. If the pH of the reference sample has changed from the previous measurement by more than 0.10 pH unit but conductivity has not changed, the electrode should be checked. The measured pH value is recommended to agree within ± 0.02 pH unit of the expected value. If large differences are observed, a new solution is prepared from a concentrated calibration solution (commercially available standard reference materials). If performance is still inadequate, the electrode must be replaced. If possible, checking pH calibration with a series of dilute mineral acids

(ex. pH 4.0, 4.2, 4.4, 4.6, 4.8, 5.0 with HCl) once in a month is recommended to obtain good pH values. For the preparation of these solutions, dilute 10, 6.3, 4.0, 2.5, 1.6, and 1.0 mL of 10^{-3} mol L^{-1} HCl solution to 100 mL with deionized water, respectively.

b. Thermometer
c. Water bath of 25 °C temperature is recommended. If a temperature controlled water bath is not available, use of water bath without temperature control but containing at least 5 L of water may be considered.
d. Plastic or glass vessel corresponding to the diameter of the cell used

ii) Reagent solution
A commercially available primary standard buffer solution with a pH of 4.01 (4.0), 6.86 (7.0) and 9.18 (9.0) should be used, having guaranteed traceability.

iii) Calibration
a. Switch on the pH meter and install the electrodes and thermometer.
b. Rinse the pH electorodes with deionized water carefully and wipe drops off the electrode with soft paper or something.
c. Use the pH 6.86 (7.0) buffer solution to set the intercept of pH response and temperature control. Then repeat **b**.
d. Use the pH 4.01 (4.0) or 9.18 (9.0) buffer to adjust the slope control of pH response and temperature control. Then repeat **b**.
e. Check the measured pH value to be within 0.02 pH unit of the buffer value.

iv) Measurement procedure
The manufacturer's directions for operation of the instrument should be followed.
a. Place the sample solution in a clean plastic or glass vessel to cover the sensing elements of the electrode.
b. Rinse the pH electorodes with deionized water carefully and wipe drops off the electrode with soft paper or something.
c. Immerse pH electrode in the sample vessel and swirl the sample gently.
d. Allow the electrode to equilibrate and measure the pH of the sample until a constant value is obtained unit. Record the pH value and temperature of the sample.

Notice:
i) The pH electrode in long time dried condition should be filled in the deionized water until achieve equilibrium.
ii) If the pH electrode is unclean, wash it with a cleanser or hydrochloric acid in short time and wash with deionized water enough.

5.1.2. Electric Conductivity (EC or Λ)

Electric conductivity (EC) is recommended to be measured by a conductivity meter at 25 ± 0.5 °C using a water bath. If it is measured at any other water temperature (t °C), its value (EC (t)) is corrected with the following equation to the value (EC (25)) at 25 °C.

$$EC_{(t)} = EC_{(25)} \times [1 + 0.022 \times (t - 25)]$$

The EC of a solution is the reciprocal value of its resistance and can be directly measured using a conductivity bridge with a measuring cell. The conductivity varies with the temperature of the solution and is proportional to the concentration and the species of free ions present in the solution. Since the conductivity also depends on the electrode area and its spacing, the measuring apparatus has to be calibrated to obtain the cell constant or to adjust the meter. A KCl solution of known concentration and conductivity is used for calibration. The conductivity has to be measured before the pH to avoid any possible error due to salt contamination from the pH electrode.

i) Apparatus

a. The conductivity bridge and cell must have a measurement range of 0.1−100 mS m^{-1}. The precision of the conductivity meter has to be within ± 0.5% of the range and have an accuracy of ± 1% of the range.

b. Platinum conductivity cell

c. Thermometer

d. Water bath of 25 °C temperature is recommended. If a temperature controlled water bath is not available, use of water bath without temperature control but containing at least 5 L of water may be considered.

e. Plastic or glass vessel corresponding to the diameter of the cell used

ii) Reagent solution

a. Stock solution A, 0.1 M KCl: 7.456 g of pre-dried (2h at 105 °C) KCl dissolved in deionized water, and diluted to 1000 mL at 25 °C with deionized water.

b. Stock solution B, 0.01 M KCl: 10 mL of 0.1M KCl, dilute to the mark of 100 mL at 25° C with deionized water.

iii) Calibration

Calibration for conductivity measurement is multipoint. With each set of samples, a set of 0.0001 M, 0.0005 M, and 0.001 M KCl solutions should be prepared from 0.01 M KCl stock solution by dilution with deionized water. The conductivity of the deionized water should also be measured. The specific conductivity of known KCl solutions (Table 5.2) should be expressed in a graph. The sample conductivity can then be read directly from this plot of measured conductivity vs. the specific conductivity.

Table 5.2. Conductivity of KC1 solution at 25°C

Concentration	Conductivity
M	mS m^{-1}
0.0001	1.494
0.0005	7.390
0.001	14.700

iv) Measurement procedure

The manufacturer's directions for operation of the instrument should be followed.

a. The samples and the standard solutions are recommended to be measured at 25 °C in a water bath if available.

b. The cell must be rinsed thoroughly with deionized water between measuring each sample and excess water should be shaken off. The conductivity cell must be kept clean.

c. The measured value should be expressed to 0.01 mS m^{-1}. If the temperature of the sample is not 25 °C, correct the measured value to 25 °C (Table 5.3).

Table 5.3. Conductivity of 0.0005 M KC1

Temperature	Conductivity
°C	mS m^{-1}
20	6.68
21	6.82
22	6.95
23	7.10
24	7.24
25	7.39
26	7.54
27	7.69
28	7.84

v) Reporting

EC is expressed as mS m^{-1}

5.1.3. Alkalinity

5.1.3.1. Gran's plot titration method

Gran's plot titration method is recommended for meaurement of alkalinity. Detail theoretical points are explanated in Appendix 4.

i) Gran's ANC and alkalinity

The previous manual has adopted the fixed endpoint titration with an end point of pH=4.8 as a method to measure alkalinity; however, the Gran's plot titration method is recommended to avoid many of the problems encountered with the fixed endpoint titration. Gran's plot titration method is considered to be one of the most precise methods to measure acid neutralizing capacity (ANC). ANC is defined as the sum of base cation equivalent concentration (C_B) minus the sum of strong acid anion equivalent concentration (C_A) as shown in equation (1).

$$ANC = \Sigma C_B - \Sigma C_A \quad (1)$$

where

$$\Sigma C_B = Na^+ + K^+ + NH_4^+ + Ca^{2+} + Mg^{2+} + Al^{n+} + Mn^{2+} + Fe^{3+} \quad (2)$$

$$\Sigma C_A = F^- + Cl^- + NO_3^- + SO_4^{2-} \quad (3)$$

For most of the clear surface water, in which organic acid anion contributes little to ANC, electroneutrality requires that the positive electric charge equals to the negative electric charge in the solution as shown in equation (4).

$$C_B + H^+ = C_A + HCO_3^- \quad (4)$$

Combining equations (1) and (4), ANC can be expressed as

$$ANC = HCO_3^- - H^+ \quad (5)$$

Different from the alkalinity by the fixed endpoint titration, ANC has not only positive value but also negative value, and a negative value of ANC is recognized as a definition of acidification of surface water in many reports.

For most of the clear surface water, the relationship between the alkalinity and ANC is described as,

$$ANC = \text{Alkalinity} - H^+ \quad (6)$$

because the alkalinity measurement by the fixed endpoint titration with an end point of pH = 4.8 is designed to measure HCO_3^-.

Comparison data between pH4.8 method and Gran's plot titration method was shown in Appendix 5.

ii) Equipment and reagent

Gran's plot titration method requires a pH meter with a precise electrode, an automatic titrator or a micro pipette which can dispense 0.5 mL of solution precisely and a magnetic stirrer with a Teflon coated magnet. 0.01N HCl is recommended as an acid titrant. 0.01N H_2SO_4 is also usable.

Since all of the solution including sample solution and standard solution should be kept at the same temperature, a constant temperature room is preferred for this method. When a constant temperature room is not available, heating and coloring devices such as a peltier device are required to keep the temperature of the solution constant.

A pH meter which indicates at least three places of decimals of the pH value or a pH meter which indicates mV below decimal points is required.

iii) Procedure

a. All of the sample solution and the standard solution should be kept at the same temperature within ± 0.5 °C.

b. The pH meter should be calibrated with standard solution with pH values of 4 and 1.68, since the determination of ANC by Gran's plot titration method uses pH region between 4 and 3. At the same time, check the slope of the electrode, theoretically + or – 59.2 mV pH^{-1} at 25°C. If the slope differs more than 10% from the theoretical value (Table 5.4), rinse the electrode according to the manual for the electrode.

Table 5.4. Theoretical slope of a pH electrode

Temperature(°C)	Slope(mV pH^{-1})
10	56.2
15	57.2
20	58.2
25	59.2
30	60.2

c. Put a 50 ml of sample solution into a beaker and stir it gently by a magnetic stirrer.

d. Measure the pH value after the indication has been stabilized, which is the initial pH of the sample.

e. Put a 0.5 mL of 0.01N acid titrant into the solution, and then read the pH value. Repeat adding a 0.5 mL of the acid titrant until the pH value decreases less than 3.4. As far as the pH value is more than 4, it is OK for you to add a 0.5 mL of the acid titrant before stabilizing the indication, because the calculation of ANC by Gran's

plot titration only requires the pH data below 4. When the pH value decreases below 4, careful reading of the pH value is required.

f. If the pH meter does not have an indication of three places of decimals of the pH value, read mV instead of pH, and convert mV into pH according to the relation between the pH and mV obtained in the calibration of the standard solution.

iv) Analysis

a. After the measurement, make a graph indicating the relationship between the volume of the acid titrant added to the solution and H^+ in the solution. Note that the H^+ in the solution should not be expressed in the concentration but an amount of H^+. Take care that the total volume of the sample solution is changed according to the volume of the added acid solution. Table 5.5 shows an example of the observational data of the pH and the amount of H^+ in the solution.

Table 5.5. An example of Gran's plot titration

Va(ml)	E(mV)	pH	$[H^+]$(μmol/l)	Vt(ml)	H^+(nmol)
0	-49.0	7.708	0.02	50	1.0
0.5	-38.1	7.518	0.03	50.5	1.5
1	-29.8	7.373	0.04	51	2.2
1.5	-23.2	7.258	0.06	51.5	2.8
2	-16.4	7.139	0.07	52	3.8
2.5	-1.9	6.887	0.13	52.5	6.8
3	13.7	6.615	0.24	53	12.9
3.5	48.7	6.005	0.99	53.5	52.9
4	60.7	5.795	1.60	54	86.5
4.5	93.2	5.229	5.90	54.5	321.8
5	152.3	4.199	63.28	55	3480.6
5.5	173.4	3.831	147.60	55.5	8191.6
6	184.7	3.634	232.30	56	13008.7
6.5	192.4	3.500	316.42	56.5	17877.6
7	198.2	3.399	399.36	57	22763.3
7.5	202.8	3.318	480.33	57.5	27619.0

Va: the volume of the acid titrant added to the solution
Vt: total volume of the solution

b. Plot the amount of H^+ against the volume of acid titrant added (Va) according to Table 5.5, you will find a linear relationship between both of them near the end of the titration (Fig. 5.1).

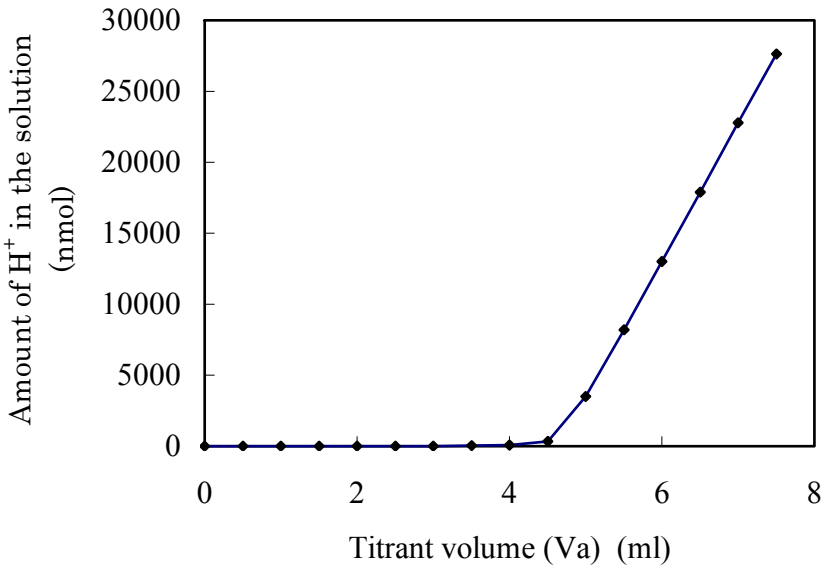


Fig.5.1. The change in the amount of H^+ against the volume of the acid titrant added.

c. Select a linear region to make a linear regression. At least 4 points are required for the regression. The intercept of the titrant volume axis gives you the equivalent point of the titration (Ve) (Fig. 5.2).

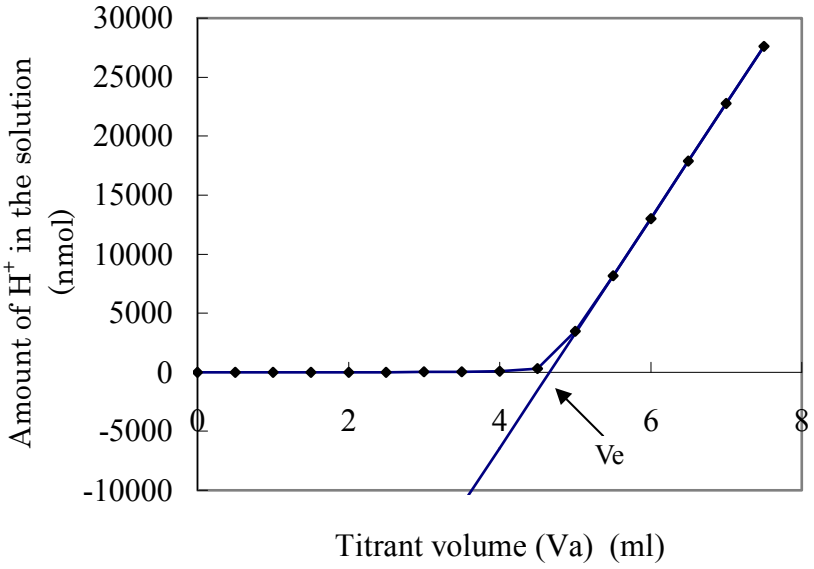


Fig. 5.2. Extrapolated equivalence point volume by a linear regression.

d. ANC (μeq L^{-1}) is calculated by the following formula;

$$ANC = C \times F \times Ve \times 1000/Vs \quad (7)$$

where C is the acid concentration used for the titration, here 10 μeq L^{-1}
F is a factor of the acid,
Ve is the equivalent volume (ml),
Vs is the initial volume of the sample solution, here 50ml

In the case of Table 5.5, Ve was calculated to be 4.66ml as shown in Fig. 5.2.
The factor of the acid titrant (F) used was 1.003 in this case, then,

$ANC = 10 \times 1.003 \times 4.66 \times 1000/50$
$= 935$ μeq L^{-1}

v) Limitation
Gran's plot titration cannot be applied to the water containing high concentration of organic acids. Some of the organic acids still dissociate at pH level of 3.4, and bring error in Gran's plot titration. Gran's plot titration should be applied to surface water with a DOC concentration less than 3 mg L^{-1}.

vi) Reporting
Alkalinity is expressed as meq L^{-1}

5.1.3.2. pH 4.8 endpoint method

Alkalinity is titrated by using a pH meter to the end-point of pH 4.8 and expressed as milliequivalent per liter (meq L^{-1}). The titration with 0.01 M (or 0.001 M) H_2SO_4 is done with a digital burette or burette. The glass electrode of pH meter is immersed in the sample vessel and detects the varying pH as 0.01 M or (0.001 M) H_2SO_4 is added while the titration is going on, the water sample should be gently swirled by magnetic stirrer. Alkalinity X is calculated with the following equation.

$X = a \times (1{,}000 / \text{sample mL}) \times (\text{factor of standard sulfuric acid}) \times 0.02$

a : titration volume (mL), 0.02 : meq / mL 0.01 M H_2SO_4

Preparation of standard 0.05 M Na_2CO_3 solution:
Weigh precisely 1.33 g of Na_2CO_3, which is dried in a muffle furnace at 500 to 600 °C for 40 to 60 minutes and cooled in a desiccator. The Na_2CO_3 should be dissolved and made up with distilled water to 250 mL in a 250 mL volumetric flask.

Preparation of 0.01 M H_2SO_4:
0.05 M H_2SO_4 : Add 3 mL sulfuric acid to a 1 liter reagent bottle fully filled with

distilled water and mix the solution well. The factor (f) of the solution should be determined by the following method. Add 25 mL of the standard solution of 0.05 M Na_2CO_3 to a conical beaker with a volumetric pipet. The 0.05 M H_2SO_4 should be titrated in the beaker to an end-point of pH 4.8 by measuring with a pH meter and by swirling gently with magnetic stirrer. The factor of the 0.05 M sulfuric acid is calculated by following equation.

$$f = 25 / x$$

x = mL H_2SO_4 solution (0.05 M) used

Adding 200 mL of the 0.05 M H_2SO_4 solution to a 1000 mL volumetric flask, the solution is diluted with distilled water to 1000 mL. Similarly, the 0.001 M H_2SO_4 should be prepared by using the 0.01 M H_2SO_4 solution.

5.1.4. NO_3^-, NO_2^-, PO_4^{3-} and SO_4^{2-}

These anions are analyzed by ion chromatography with anion separator and suppressor columns. This analytical method is same as that for wet deposition samples. Colorimetric method is also used, based on Standard Method for the Examination of Water and Wastewater (Greenberg et al., 1992)

Ion chromatography has been widely used in recent years to analyze anions in sample. Sulfate, nitrate, and chloride in sample are separated on an ion exchange column because of their different affinities for the exchange material. The material commonly used for anion separation is a polymer coated with quaternary ammonium active sites. After separation, the anions pass through a strong acid cation exchange column (suppressor column) which exchanges all cations for H^+ ions or an electric suppresser. Chloride, nitrate and sulfate are detected as acids by a conductivity detector. Both isochratic and gradient methods are available for Ion Chromatographic analyses.

Any anions with a retention time similar to that of the main anions could interfere. For example, when NO_2^- exists, it elutes just after Cl^-, which causes the peak to be asymmetric.

The ranges of measured anion concentrations in sample and recommended detection limits are given in Table 5.6.

Table 5.6. Measured Anion Concentration and Recommended Minimum Detectable Amount (MDA)

Anion	Range (μmol/l)	MDA (μmol/l)
SO_4^{2-}	<1 – 200	1
NO_3^-	<2 – 300	1
Cl^-	<1 – 1000	1
NO_2^-	<0.2 – 10	0.2
PO_4^{3-}	<0.1 – 3	0.1
F^-	<1 – 30	1
Br^-	<0.1 – 6	0.1

i) Apparatus

a. Ion chromatograph (example 1: YEW 7000, example 2:DX500) with a conductivity detector

b. Anion separator column (example 1: ICS-A13, example 2: IonPac AG4A-SC or AS11 + AS4A-SC)

c. Anion suppressor column (example 1: HPS-SA-1, example 2:ASRS-1)

d. An integrator is recommended to process the chromatograms.

ii) Reagents and solutions (Isochratic methods)

Example 1

a. Concentrated eluent, 0.4 M Na_2CO_3/0.4 M $NaHCO_3$: dissolve 84.79 g Na_2CO_3 and 67.24 g $NaHCO_3$ in 2 L of hot deionized water.

b. Working eluent, 4 mM Na_2CO_3/4 mM $NaHCO_3$: dilute 40 mL of concentrated eluent to 4 L with deionized water.

c. Regenerant, 15 mM H_2SO_4: dilute previously prepared 1.5 M H_2SO_4 accordingly,

d. Mixed stock solution: 10.4 mmol L^{-1} SO_4^{2-}, 16.1 mmol L^{-1} NO_3^-, and 28.2 mmol L^{-1} Cl^- (often commercially available, otherwise prepare from reagents of high purity)

e. Standard solution A: dilute 5 (or 2) mL of mixed stock solution to 500 (or 200) mL with deionized water (104 μmol L^{-1} SO_4^{2-}, 161 μmol L^{-1} NO_3^-, and 282 μmol L^{-1} Cl^-).

f. Working standard solutions are prepared from standard solutions by diluting 20, 10 and 2 mL of standard solution to 200 mL with deionized water prior to every measurement.

Example 2

a. Concentrated eluent, 0.18 M Na_2CO_3/0.17 M $NaHCO_3$: dissolve 19.6 g Na_2CO_3 and 14.28 g $NaHCO_3$ in 1 L of hot deionized water.

b. Working eluent, 1.8mM Na_2CO_3/1.7mM $NaHCO_3$: dilute 40 mL of concentrated eluent to 4 L with deionized water.

c. Regenerant, 50 mM H_2SO_4: dilute previously prepared 5 M H_2SO_4 accordingly.

d., e., f., same as example l.

iii) Measurement procedures

Example 1

a. Prepare new eluent and regenerator solutions (if required).

b. Set up the chromatograph for most sensitive range

c. Begin to pump the eluent and regenerant (if required) through the columns. Condition instrument for at least 30 minutes.

d. Inject standard solutions through the loop injector (50 or 100 μL) and start analysis. It is preferable that calibration curve will be constructed from at least 5 working standard solutions

e. The injection should start from the highest concentration standard, followed by standards with decreasing concentrations to prevent erroneous high values for the first sample injected after the calibration series. In the case that there is little affect on sample analysis, for example, if injecting deionized water between the standard series and the first subsequent samples injection, injecting standard solution from

lower to higher concentration could be acceptable.

f. Inject sample solution in the same manner as standard solutions.

(when an auto sampler is available)

d. Fill the auto sampler with standards and samples.

e. Turn on the auto sampler to start analyzing samples.

Preparation of a working standard solution of PO_4^{3-}:

The 1 mg L^{-1} PO_4^{3-} working standard solution is parepared by 1.4330 g of KH_2PO_4 with distilled water in 1000 mL volumetric flask. Ordinarily, the working standard solution of PO_4^{3-} is prepared at 1 mg L^{-1} concentration. However, appropriate lower or higher combinations should be prepared according to anion concentration to be determined.

5.1.5. NH_4^+

NH_4^+ is analyzed by ion chromatography with a cation separator column, or by colorimetry with indophenol blue setting the spectrophotometer at a wavelength of 630 nm. These chromatography and colorimetric methods are the same as those for wet deposition samples. Nessler's method (Colorimetric method) is also used.

For measuring ammonium, automated or manual spectrophotometric determination with phenate is used. A sample is mixed with alkaline phenol and hypochlorite to form an indophenol blue complex. A heating bath of 50 °C is used to increase the rate of color formation. The light energy (630 nm wavelength) transmitted through the sample is a function of the concentration of ammonium ion in the sample.

The detection limit of this method is 2 μmol L^{-1} and the concentration range is 2 - 110 μmol L^{-1} as NH_4^+. This range can be extended by sample dilution. The test tubes have to be closed throughout the analysis. Elevated concentrations of ammonium in the laboratory air will result in a positive interference. Wash sample cups with deionized water immediately prior to use and rinse cups again with portion of the standard or sample to be analyzed. It is worth noting that this method requires more skill and training to get good data compared with other instrumental analyses.

i) Apparatus

a. Spectrophotometer with a 630 nm setting
b. Heating bath (50 °C)
c. Glass test tubes with ground-in stoppers

ii) Reagents and solutions

a. Stock solution 5.54 mmol L^{-1}: dissolve 0.2965 g NH_4Cl (dried at 105°C) in deionized water and dilute to 1000 mL.
b. Alkaline phenol: 3.5 g of sodium hydroxide (NaOH) + 8.5 mL phenol + 0.04g sodium nitroprusside in 100 mL deionized water; refrigerate the solution at 4°C for a period not exceeding 1 week; prepare fresh one every week
c. Sodium hypochlorite (NaOCl) solution: dilute 250 mL of 5.25% NaOCl to 500 mL with deionized water

iii) Measurement procedure

a. The spectrophotometer should be switched on for 30 minutes before proceeding
b. Put 5 mL of the sample in the test tube, add 15 mL of deionized water, 0.5 mL of alkaline phenol solution, and 0.5 mL of sodium hypochlorite solution; close the test tube with glass stopper
c. Set the heating bath to 50 °C, leave the sample in the heating bath for 2 hours and measure the sample with the spectrophotometer at 630 nm

5.1.6. Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}

These cations are analyzed by ion chromatography or atomic absorption spectrometer. These are same as those for wet deposition samples.

5.1.6.1. Ion chromatography

Besides anion analyses, ion chromatography has also been widely used in recent years to analyze cations in water sample. The principle is the same as that of anion determination except that different column materials are used and that the suppressor column is often omitted. The material commonly used for cation separation is a cation exchange resin with active surface. Supressor columns are used (example 2:DX500), or sometimes not used (example 1: YEW7000). Sodium, ammonium, potassium, calcium and magnesium ions are detected by a conductivity detector, without changing the eluent when certain columns (example 1: ICS-C25 or C45, example 2: CG14 + CS14 with CSRS-1 recycle supressor) are used. In other columns (example 1: ICS-C15) monovalent cations (Na^{+}, NH_4^{+}, K^{+}) are determined using an eluent and then divalent cations (Mg^{2+} and Ca^{2+}) are determined using another eluent.

Any cations with a retention time similar to that of the main cations could interfere. For example, in samples with high concentration of Na^{+}, the peak of NH_4^{+} becomes asymmetrical and often causes a significant error. In this case, measurement using more dilute eluent could improve the separation of peaks, or gradient methods may be used.

The ranges of measured cation concentrations and recommended detection limits are given in Table 5.7.

Table 5.7. Measured Cation Concentration and Recommended Minimum Detectable Amount (MDA)

Cation	Range (μmol L^{-1})	MDA (μmol L^{-1})
Na^{+}	< 2 - 900	1
NH_4^{+}	< 3 - 1000	1
K^{+}	< 1 - 100	0.3
Ca^{2+}	< 0.5 - 300	0.2
Mg^{2+}	< 1 - 200	0.4

i) Apparatus

a. Ion chromatograph (example 1: YEW 7000, example 2: DX100) with a conductivity detector

b. Cation separator column (example 1: ICS-C25, example 2: CG14 + CS14)

c. An integrator is recommended to process the chromatograms.

ii) Reagents and solutions (Isochratic methods)

Example 1

a. Concentrated eluent, 1 M Tartaric Acid

b. Working eluent, 5 mM Tartaric Acid /1 mM 2,6 PDCA (2,6-Pyridinedicarboxylic acid): Add 0.167g of 2,6 PDCA to 5 mL of concentrated eluent and make up with deionized water to 1 L.

c. Mixed stock solution (a mixture of 1000 mg L^{-1} of each chemical species)

d. Standard solution A

e. Working standard solution

Example 2

a. Concentrated eluent, commercial methane sulfonate (reagent grade)

b. Working eluent, 10 mM methane sulfonate: dilute 0.65 mL of concentrated eluent in 1 L deionized water

c., d. f., the same as example 1

iii) Measurement procedure

a. Prepare new eluent and regenerator solutions.

b. Set up the chromatograph for the most sensitive range.

c. Begin to pump the eluent through the columns. Condition the instrument for at least 30 minutes.

d. Inject standard solutions through the loop injector (50 or 100 μL) and start analysis. It is preferable that calibration curve will be constructed from at least 5 working standard solutions.

e. Turn on the auto sampler switch to start analyzing samples. The injection should start from the highest concentration standard, followed by standards with decreasing concentrations to prevent erroneous high values for the first sample injected after the calibration series. In the case that there is little affect on sample analysis, for example, if injecting deionized water between the standard series and the first subsequent samples injection, injecting standard solution from lower to higher concentration could be acceptable.

f. Inject sample solution in the same manner as standard solutions.

(when an auto sampler is available)

d. Fill the auto sampler with standards and samples.

e. Turn on the auto sampler to start analyzing samples.

5.1.6.2. Atomic absorption (and/or emission)

Atomic absorption spectrometry is a simple and rapid procedure that can be used to determine Na^+, K^+, Ca^{2+} and Mg^{2+} in water sample. Detection limits, sensitivity, and optimum range vary depending on the manufacturer and the atomic absorption spectrophotometer model.

The low concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} in sample requires using very sensitive procedures (see Table 5.8). In flame atomic absorption spectrometry these metals are atomized directly in a flame. A light beam from a lamp, whose cathode contains a specific metal, first passes through the flame containing the atomized sample and then enters a monochromator whose detector measures the current caused by the absorption of light. Since the wavelength of the light absorbed is characteristic for individual metals, the light energy absorbed by the flame is proportional to the metal concentration in the sample measured.

Table 5.8. Range of Measured Cations and Recommended Limits of Detection

Cation	Range (μmol L^{-1})	Detection Limit (μmol L^{-1})
Na^+	0.4 – 90	0.4
K^+	1 – 30	1
Ca^{2+}	0.2 – 80	0.2
Mg^{2+}	0.2 – 20	0.2

Detection Limit is defined as S/N = 2

i) Apparatus

a. Atomic absorption (and/or emission) spectrophotometer

Single- or dual-channel, single- or double-beam instrument having monochromator; photomultiplier detector; adjustable slit; a wavelength range of 190 to 800 nm; a slot burner system, power supply and amplifier; and a suitable recorder of PC

b. Hollow cathode lamps for Na, K, Ca and Mg.

Single-element lamps and multielement lamps can be used. For the determination of Na and K, flame atomic emission measurement can also be used, when the instruments has devices for it.

c. Compressed gases and pressure-reducing valves

Two cylinders of clear acetylene are necessary; the air may be supplied from a laboratory compressor (with a cleaning unit) or a cylinder of compressed air.

ii) Calibration

a. Prepare stock standard solution from high purity chemicals (preferably metals) using deionized water and HNO_3 (conc. spectrograde purity) at a concentration of 1000 mg of metal L^{-1}. Commercially available standard solutions may be used.

b. Prepare a blank (use deionized water and nitric acid- and/or lanthanum nitrate solution) and six calibration standard solutions by diluting the stock metal solution to

various concentrations in appropriate ranges for each set of analyzed samples. The same stock nitric acid-and/or -lanthanum nitrate must be added to both the samples and the calibration solutions.

iii) Procedure

Because of the many differences between atomic absorption spectrophotometers detailed instructions cannot be formulated; the analyst should follow the manufacturer's operating instructions for the particular instrument. In general, operations are as follows:

a. Switch on the analyzer
b. Align the light source for maximum response
c. Ignite the flame
d. Allow the instrument to warm up (at least 15 minutes)
e. Reset the wavelength
f. Optimize and adjust the nebulization rate for maximum response
g. Adjust the burner position for maximum response
h. Prepare a full calibration curve and analyze one standard after every 20 samples

Table 5.9 gives examples of the operating conditions for each metal determined by atomic absorption /emission spectrometry with an air/acetylene slot burner.

Table 5.9. Atomic absorption (AA)/emission (AE) spectrometry of metals

Mode	Na	K	Ca	Mg
	AA or AE	AA or AE	AA	AA
fuel and flame condition	Air-acetylene Stoichiometric	air-acetylene stoichiometric	air-acetylene rich flame	air-acetylene stoichiometric
Analytical line (nm)	589.6	766.5	422.7	285.2

Addition of lanthanum nitrate solution is effective to eliminate the interference of co-existing anions and of unequal ionization ratio in the flame between the sample and standard solutions. It is important to add the correct amount of lanthanum solution so that the lanthanum concentration in both the samples and the working standard solutions are similar.

5.1.7. Cl^-

This anion is analyzed by ion chromatography with anion separator and suppressor columns. Cl^- is also analyzed by titration respectively.

5.1.7.1. Ion chromatography

This analytical method is same as 4) NO_3^-, NO_2^-, PO_4^{3-} and SO_4^{2-}.

5.1.7.2. Titration(argentometric method)

The detail of analytical methods should be referred to Standard Method for the Examination of Water and Wastewater (Greenberg el al., 1992).

When Cl^- is titrated by $AgNO_3$, white AgCl is produced (Formula 1). The excess $AgNO_3$ reacts to CrO_4^{2-} and Ag_2CrO_4 with orange color is produced (Formula 2)

$$Cl^- + AgNO_3 \rightarrow AgCl + NO_3^- \quad (1)$$

$$CrO_4^{2-} + 2AgNO_3 \rightarrow Ag_2CrO_4 + 2NO_3^- \quad (2)$$

i) Apparatus

Burette

ii) Reagents and solutions

a. K_2CrO_4 solution: dissolve 50 g of K_2CrO_4 in 200 mL deionized water, compound with $AgNO_3$ (5w/v%) until a little the red-brown precipitation. After filtered it, fill up by the water to 1L in a volumetric flask.

b. 0.01 mol L^{-1} NaCl solution: dissolve 0.5844 g of oven-dried NaCl (at 500 – 550 °C for 40 – 50 minutes) in deioinized water and fill up by the water to 1 L.

c. 0.01 mol L^{-1} $AgNO_3$ solution: dissolve 1.7 g of AgNO3 in deionized water and fill up to 1 L. The solution is stored in a brown bottle. The 1 mL of this solution is reacted to 0.3545 mg of Cl^-.

d. Determination of the factor (F) of $AgNO_3$ solution:

- Dispense 25 mL of 0.01 mol L^{-1} NaCl solution in a white porcelain dish, add 0.2 mL of K_2CrO_4 solution to the dish, and then titrate the solution by 0.01 mol L^{-1} $AgNO_3$ solution until non-disappeared light orange color; record the volume, mL (a) of the used AgNO3 solution.
- Dispense 45 mL of deionized water and 5.0 mL of 0.01 mol L^{-1} NaCl solution in another white porcelain dish, add 0.2 mL of K_2CrO_4 solution to the dish, and then titrate the solution in the same way; record the volume, mL (b), of the used 0.01 mol L^{-1} $AgNO_3$ solution.
- $F = 20/(a - b)$

iii) Measurement procedure

a. Dispense 100 mL of the sample in a white porcelain dish (volume, 300 mL), add 0.5

mL of K_2CrO_4 solution to the dish, and then titrate the solution by 0.01 mol L^{-1} $AgNO_3$ solution till non-disappeared light orange color; record the volume, mL (c), of the used $AgNO_3$ solution.

b. In the same way, dispense 100 mL of deionized water and 5.0 mL of 0.01 mol L^{-1} NaCl solution, add 0.5 mL of K_2CrO_4 solution to the dish, and then titrate in the same way; record the volume, mL (d), of the used $AgNO_3$ solution.

iv) Calculation and report

Cl^- (mg L^{-1}) = {c – (d – 5/F)} × F × 1000 / sample volume (mL) × 0.3545

Expression of results of ion analysis:

Analytical results are expressed as milligrams per liter (mg L^{-1}) in this manual. The units of milliequivalents per litre (meq L^{-1}) and millimoles per liter (mmol L^{-1}) are valuable for checking analysis by anion-cation balance, as used in Technical Manual for Monitoring Wet Deposition and SI unit. The meq can be expressed also as millimole charge ($mmol_c$), if necessary.

Table 5.10 represents factors for converting the units of ion concentration among milligrams per liter, milliequivalents per liter and millimoles per liter.

Table 5.10 Conversion factors

1 mg L^{-1} = $1/k_1$ meq L^{-1} = $1/k_2$ mmol L^{-1}

	k_1	k_2
Cation		
NH_4^+	18.04	18.04
Ca^{2+}	20.04	40.08
Mg^{2+}	12.15	24.30
Na^+	22.99	22.99
K^+	39.10	39.10
Al^{3+}	8.994	27.00
Pb^{2+}	103.6	207.2
Anion		
NO_3^-	62.00	62.00
NO_2^-	46.01	46.01
SO_4^{2-}	48.03	96.06
Cl^-	35.45	35.45
PO_4^{3-}	31.66	94.98

5.1.8. Dissolved organic carbon (DOC) or Total organic carbon (TOC)

5.1.8.1. DOC

The water sample filtered with 0.45 μm pore size filter is measured by a nondispersive infrared analyzer (TOC analyzer) after removing carbonate and bicarbonate by acidification ($pH \leq 2$) with phosphoric acid and aerating with nitrogen gas.

Otherwise, after add 0.2 potassium peroxodisulfate and 0.5 mL of 0.4 M phosphoric acid solution to the filtered water sample (5 - 10 mL) contained in a glass vial and remove inorganic carbon by aerating with purified oxygen or nitrogen, seal the vial and digest 4 h by heating the sample at temperature between 116 and 130 °C in an autoclave. The resultant carbon dioxide (CO_2) is measured by a nondispersive infrared spectrometry.

i) Appratus

Nondispersive infrared analyzer (TOC analyzer)
Career gas

ii) Reagents and solutions

Pure water (no or minimal content of TOC)
Phosphoric or hydrochroric acid solution(no or minimal content of TOC)

iii) Procedure

a. Prepare the vial of filtered sample and TOC standards
b. Remove inorganic carbon by aerating with purified oxygen or nitrogen
c. DOC is measured after getting stable baseline

iv) Reporting

DOC and TOC are expressed as mg L^{-1}

5.1.8.2. TOC

The sample with no filter is measured as DOC method.

5.1.9. Chlorophyll a: SCOR/UNESCO Method

All photosynthetic algae and cyanobacteria contain chlorophyll-a as one of the pigments in the photosynthetic process. Thus, the chlorophyll-a concentration is a useful and important indicator for estimating the standing crop and production by phytoplankton, and state of trophic level. The method of UNESCO/SCORE is recommended.

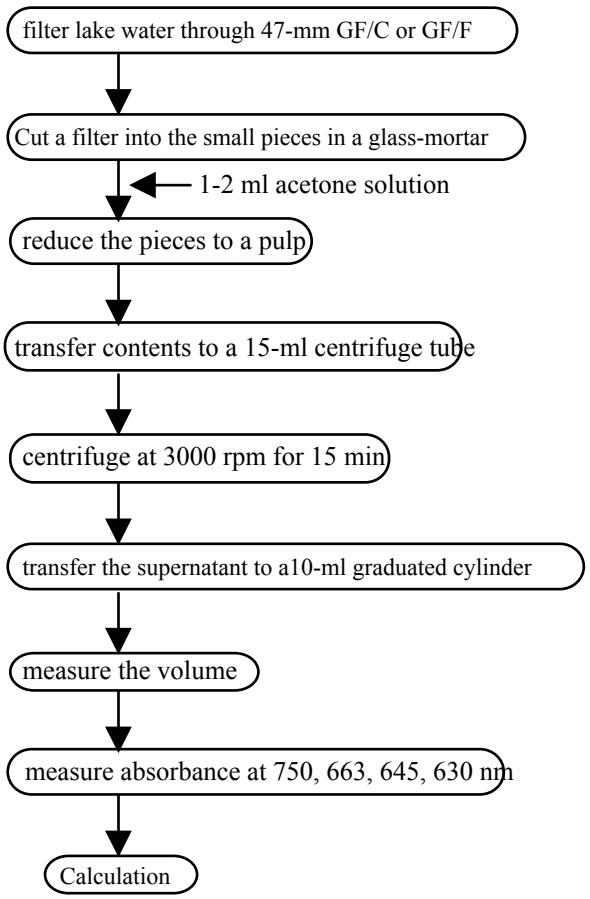


Fig. 5.3 Outline of procedures for chlorophyll determination.

i) Reagents and apparatus

a) 90% acetone solution: Add 100 mL DW to 900 mL acetone. Some add one spoonful of powdered magnesium carbonate per liter

b) Glass-mortars 5 cm in diameter.

ii) Procedure

An outline of the analytical procedure is shown in Fig. 5.3.

a. Filter about 0.2 - 2 L of well-shaken sample water (*V* mL) depending on trophic level through a 47 mm glass fiber filter (GF/C or GF/F).

b. Filtration should be done rapidly to allow swift transport of samples to the laboratory. Where distance is a problem, on-site filtration using a hand-type pump and filtration apparatus is recommended. In this case, filters should be dried and stored in a darkened desiccator and immediately refrigerated or frozen in a deep freezer.

c. Cut a filter with phytoplankton on it into the smallest pieces possible. Place them in a small glass–mortar with 1 or 2 mL of 90% acetone solution and grind vigorously, reducing them to a pulp. Transfer contents carefully with small quantity of acetone solution for rinsing into a 15 mL plastic centrifuge tube. Stopper the centrifuge tube tightly, and store in a cold, dark place (preferably a refrigerator) until centrifugation to fully extract pigments. Centrifuge at 3000 - 4000 rpm for 15 min. After centrifugation carefully decant the green supernatant into a 10 mL graduated cylinder and measure the volume (*Ac* mL).

d. Use a blank of acetone solution and measure the absorbance of samples and blank at 750, 663, 645 and 630 nm, respectively, using *L* cm cells (usually a 1 cm cell). If absorbance at 750 nm exceeds 0.05, centrifuge samples again.

e. Subtract absorbance at 750 nm from 663, 645 and 630 nm, respectively, to correct for turbidity. These are called ab663, ab645 and ab630, respectively.

iii) Calculation

Chl-a ($\mu g\ mL^{-1}$) = $11.64 \times ab_{663} - 2.16 \times ab_{645} + 0.10 \times ab_{630}$

Chlorophyll-a concentration in the sample water, $mg\ m^{-3}$ ($\mu g\ L^{-1}$)

$$= \text{Chl-a} \times Ac \times \frac{1000}{V \times L}$$

See also the *Text Book: Fundamental limnology for inland aquatic environment monitoring on acidification by EANET* (2004).

5.1.10. Total Phosphorus (T-P)

Potassium Peroxodisulfate Decomposition Method

i) Reagents

(1) Potassium peroxydisulfate solution

Dissolve 4 g of potassium peroxodisulfate ($K_2S_2O_8$, nitrogen and phosphorus analysis grade) into 100 mL of deionized water.

(2) Ammonium molybdate-L ascorbic acid mixture solution

Dissolve 6g of 6 ammonium 7 molybdate 4 hydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$), 0.24 g of 2 antimony 2 potassium 3 hydrate ($C_8H_4K_2O_{12}Sb_2\cdot 3H_2O$) in 300 mL of deionized water, add 120 mL of sulfuric acid (1+2) to the solution, and fill up to 500 mL by deionized water (a). Dissolve 7.2g of L-ascorbic acid into 100 mL deionzed water (b). Mix (a) and (b) as 5:1 (v:v).

ii) Procedure

1. Procedure for standard solution

1) Dilute T-P standard solution to prepare sufficient standards and adjust the volume of each to 20.0 mL.

2. Procedure for samples and a blank solution

1) Take 50.0 mL of each sample into a 100 mL-Phosphorus and Nitrogen test bottle. Each sample should contain T-N less than 125 μg.
2) Take 50.0 mL of deionized water into a 100 mL-Phosphorus and Nitrogen test bottle as a blank solution.
3) Add 10.0 ml of potassium peroxodisulfate solution into each 1) and 2) solution before tightening a screw cap, and then mix immediately.
4) Autoclave them at 120 °C and 210 kPa pressure for 30 minutes.
5) After the samples and the blank solution have cooled down, take 20.0 mL of supernatant solution with a care not to agitate the sediments into a 25 mL-test tube with cap.

3. Measurement of ultra-violet absorbance

1) Add 2 mL of ammonium molybdate-L ascorbic acid mixture solution and shake the test tube. Then wait for the reaction for 20 minutes at room temperature.
2) After set the wavelength of the spectrophotometer at 880 nm, take the standard solution with zero concentration (0.0 mg P L^{-1}) into a glass/quartz cell for auto-zero.
3) Then absorbance of the other standards, the blank solution and the samples is measured.

iii) Reporting

T-P is expresses as mg L^{-1}

5.1.11. Total Nitrogen (T-N)

Two kinds of methods were introduced here.

The quantitative analysis ranges are 5.1.11.1 for N, 5~50 μg, and 5.1.11.2 for N 0.33~3.3 μg.

5.1.11.1. Ultra-violet absorption spectrophotometry method [JIS K 0102-1998, modified]

i) Scope and application

This method may be used to determination of total nitrogen in fresh waters in the range from 5 μg to 125 μg.

ii) Summary of the method

All forms of nitrogen, including organic nitrogen, are converted to nitrate by alkaline potassium peroxodisulfate at 120 °C. Ultra-violet absorbance at a wave length of 220 nm is measured to determine nitrate concentration. This method can be applied to the fresh waters containing bromide ion and chrome less than 10 mg L^{-1} and 0.1 mg L^{-1}, respectively.

iii) Reagents

(1) Sodium hydroxide-potassium peroxydisulfate solution
Dissolve 20g of sodium hydroxide (NaOH, nitrogen analysis grade) and 15 g of potassium peroxodisulfate ($K_2S_2O_8$, nitrogen and phosphorus analysis grade) into 500 mL of distilled water.

(2) Hydrochloric acid (1+6)

(3) Hydrochloric acid (1+500)

(4) T-N stock standard solution (500 mg-N L^{-1})
Dissolve 0.722 g of potassium nitrate (KNO_3, dried at 105 - 110 °C for 3 hours) with distilled water and dilute to 200 mL.
Conserve the solution in dark at 0-10 °C.

(5) T-N standard solution (50 mg-N L^{-1})
Quantitatively dilute 20.0 mL of T-N stock standard solution (0.5 mg mL^{-1}) to 200 mL with deionized water.

iv) Procedures

1. Procedure for standard solution

1) Dilute T-N standard solution to prepare sufficient standards and adjust the volume of each to 20.0 mL.

For example, when diluting 0.0, 1.00, 2.00, 3.00, 4.00 and 5.00 mL of T-N standard

solution to 100 mL, 0.00, 0.50, 1.00, 1.50, 2.00 and 2.50 mg L^{-1}, respectively, of standards are obtained. Take 20.0 mL of each to 25ml-test tube.

---

2) Add 4.0 mL of hydrochloric acid (1+500) to the standards to obtain pH 2-3.

2. Procedure for samples and a blank solution

1) Take 50.0 mL of each sample into a 100 mL-Phosphorus and Nitrogen test bottle. Each sample should contain T-N less than 125 μg.
2) Take 50.0 mL of distilled water into a 100 mL-Phosphorus and Nitrogen test bottle as a blank solution.
3) Add 10.0 mL of sodium hydroxide-potassium peroxodisulfate solution into each 1) and 2) solution before tightening a screw cap, and then mix immediately.
4) Autoclave them at 120 °C and 210 kPa pressure for 30 minutes.
5) After the samples and the blank solution have cooled down, take 20.0 mL of supernatant solution with a care not to agitate the sediments into a 25 mL-test tube with cap. Add 4.0 mL of hydrochloric acid (1+6) into the test tubes to obtain pH 2-3.

3. Measurement of ultra-violet absorbance

1) After set the wavelength of the spectrophotometer at 220 nm, take the standard solution with zero concentration (0.0 mg-N L^{-1}) into a quartz cell for auto-zero.
2) Then absorbance of the other standards, the blank solution and the samples is measured.

* Note that a glass cell should not be used for the spectrophotometer since ultra-violet light is absorbed by the glass cell itself.

4. Calculations

Be careful that the procedure for the samples and that for the standards are different in the point that the samples are diluted to 50/60 by addition of 10 mL of sodium hydroxide-potassium peroxodisulfate solution, while the standard solutions are not.

Therefore, the concentrations of the standard solution should be compensated with a ratio of 60/50.

For example, 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1} of the standard solutions should be treated as 0.0, 0.6, 1.2, 1.8, 2.4 and 3.0 mg L^{-1}, respectively, in the calculation in this method.

The absorbance of the blank solution should be subtracted from that of each sample, because the absorbance of the blank solution comes from reagent added to the blank solution and the samples.

Followings are a calculation example of T-N analysis, when absorbance of standard solutions, blank solution and the samples are obtained as shown in Table 5.11 and 5.12.

Table 5.11. Example of absorbance of the standard solutions.

Concentrations of T-N standard solutions (mg L^{-1})	Corrected concentration (mg L^{-1})	Absorbance
0.0	0.0	0.000
0.5	0.6	0.097
1.0	1.2	0.202
1.5	1.8	0.305
2.0	2.4	0.410
2.5	3.0	0.505

Table 5.12. Example of absorbance of the blank solution and the samples.

Sample name	Absorbance
Blank solution	0.025
Sample-A	0.321
Sample-B	0.430

The relationship between absorbance and the corrected concentration of T-N standard solutions is shown in Figure 5.4 with an equation of the linier regression.

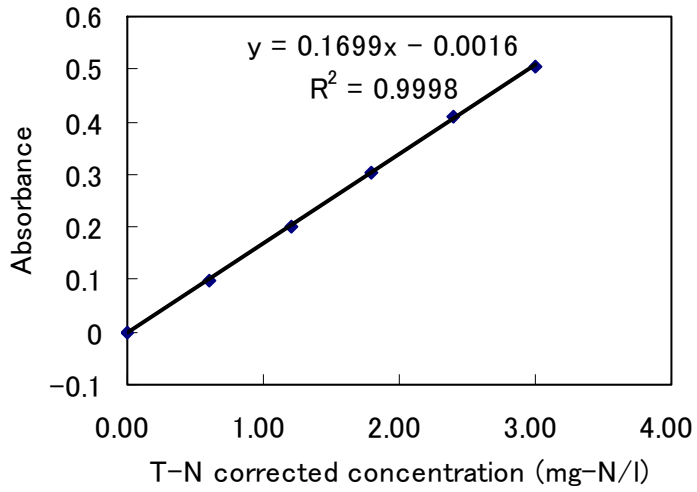


Fig. 5.4. Relation between absorbance and T-N corrected concentration

A linier regression gives the equation;

ABS = 0.1699C-0.0016

where ABS is absorbance and C is T-N concentration.

T-N concentrations are calculated with the equation.

Blank: (0.025+0.0016)/0.1699 = 0.157 mg L^{-1}
Sample A: (0.321+0.0016)/0.1699 = 1.899 mg L^{-1}
Sample B: (0.430+0.0016)/0.1699 = 2.540 mg L^{-1}

After compensation for the blank, the T-N concentrations are obtained as shown in Table 5.13.

Table 5.13. T-N concentration of the samples

Sample name	T-N concentration (mg L^{-1})
Sample-A	1.74
Sample-B	2.38

5.1.11.2. Hydrazinium sulfate reduction Method

i) Reagents

(1) Sodium hydroxide-potassium peroxydisulfate solution
Same as 5.1.11.1) iii) (1)

(2) Hydrazinium sulfate solution
Dissolve 3.5 g of hydrazinium sulfate ($H_4N_2 \cdot H_2SO_4$) into 500 mL, then dilute 10 times.

(3) Copper-zinc solution
Dissolve 0.08 g copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) + 1.76 g zinc sulfate 7-hydrate ($ZnSO_4 \cdot 7H_2O$) into 200 mL distilled water. Then, dissolve its 5 mL into 250 mL distilled water.

(4) 4- Amino-benzenesulfonamide (sulfanilamide)
Dissolve 2 g of sulfanilamide ($C_6H_8N_2O_2S$) and 60 mL of hydrochloric acid into 200ml of deionized water.

(5) *N*-1-naphthyl ethylenediamine solution
Dissolve 0.2 g of *N*-1-naphthyl ethylenediamine dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$) into 200 mL deionized water by colored glass bottle. (Use within 1 week)

ii) Procedure

1. Procedure for standard solution is same as 5.1.11.1)iv)1.

2. Procedure for samples and a blank solution

1) same procedure of 5.1.11.1) iv) 2. 1) ~4)
2) Take the supernatant liquid of 10 mL in a 30 mL test tube with common stopper after cooling the solution

3) Add the copper-zinc solution of 1 ml and hydrazinium sulfate solution of 1 mL
4) Soak the solution in a water bath at 35 °C during 2 hours and cool the solution to laboratory air temperature.

3. Measurement of ultra-violet absorbance

1) Add sulfanilamide of 1ml and shake the test tube.
2) After 5 minutes waiting, add *N*-1-naphthyl ethylenediamine solution of 1 mL and shake the test tube. Then 20 minutes wait the reaction at room temperature.
3) After set the wavelength of the spectrophotometer at 540nm, take the standard solution with zero concentration (0.0 mg-N L^{-1}) into a glass cell for auto-zero.
4) Then absorbance of the other standards, the blank solution and the samples is measured.

5.1.12. Suspended solids (SS): 1μm Glass Fiber Filter method

SS (mg L^{-1}) is defined for a filtered sample water of 1000 mL as a difference of the filter weights by using a precise balance before and after filtering of the sample water.

i) Instruments and apparatus

a. Dryer
b. Balance
c. Glass-fiber filter (45 mm diameter) of 1 μm-pore size

ii) Procedure

a. A glass-fiber filter, which has been filtered with pure water, has been dried at 105 - 110 °C during two hours in a oven and is cooled in a desiccator during two hours.
b. The filter is weighed by a precise balance
c. Sample water (more than 500 mL) is filtered with the filter as the same way above-mentioned, and also has been weighed by a precise balance.
d. Calculation as follow

$$SS = (\text{Weight of filter including SS} - \text{Weight of filter}) \times 1000 / \text{sample volume}$$

iii) Reporting

SS is expressed as mg L^{-1}.

5.1.13. Total dissolved Al

Total soluble Al is analyzed by atomic absorption spectrometry with graphite furnace, ICP Emission Spectrometry or ICP/MS. References on methods (EPA 200.8 Determination of trace elements in waters and wastes by inductively coupled plasma - mass spectrometry; Wetzel, Likens Limnological analyses; 1995)

If total dissolved Al is higher than 0.2 mg L^{-1}, reactive Al is exptected to be analyzed by the lumogallion method according to the procedure 5.1.14).

Total dissolved Al is expressed as mg L^{-1}.

5.1.14. Reactive Al: Lumogallion method

i) Instruments

a. Fluorescence spectrophotometer

b. Water bath

ii) Reagents

a. 4% hydroxylamine hydrochloride ($H_3NO.HCl$)

b. 1% *o*-phenanthroline ($C_{12}H_8N_2$) in 1M HCl

c. 0.01% lumogallion (2,2',4'-Trihydroxy-5-chloroazobenzene-3-sulfonic Acid: $C_{12}H_9ClN_2O_6S$)

d. 20% ammonium acetate (CH_3COONH_4)

iii) Procedure

a. Add a 0.4 mL aliquot of each sample to 7.6 mL deionized water to dilute the samples (20 times dilution).

b. Prepare the standard Al solutions, 0, 20, 100, 200, and 400 μg L^{-1}.

c. Add a 0.4 mL aliquot of 4% hydroxylamine hydrochloride to 8 mL of the diluted samples and the standard solutions, and left the mixture for 30 min.

d. Then, succesively add 0.4 mL of 1% *o*-phenanthroline in 1 M HCl, 0.2 mL of 0.01% lumogallion, and 1 mL of 20% ammonium acetate to the samples, and heat the mixture to 80 °C for 30 min.

e. After the mixture cooled to ambient temperature, measure the fluorescence intensities at λ = 576 nm (Exn. λ = 485 nm) and compare the results with a calibration graph for determination.

iv) Reporting

Reactive Al is expressed as μg L^{-1}.

Note:

Reactive Al analyzed here can be cosidered as an indicator of toxic Al. However, Reactive Al may include Al_{aq}, Al_{ic} and Al_o, but the Al_o is not toxic. Therefore, in case that Reative Al is higher than 100 μg L^{-1}, the following guideline should also be referred for further investigation:

- When the water pH is higher than 5.2: just observe continously (since Al is not toxic independently)
- When the water pH is lower than 5.2: determin concentrations of Al_o and Al_i (Al_{aq} + Al_{ic}). If Al_i is higher than 100 μg L^{-1}, effects on fish should be investigated in detail (guideline for toxicity to fish: $Al_i > 100 – 200$ μg L^{-1}, pH < 5.0 - 5.2.).

Reference:

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5.1.15. Dissolved oxygen (DO)

5.1.15.1. DO meter

DO concentration should be measured in the field by a DO meter

i) Reagents

a. Sulflous sodium solutin for zero caribration
Dissolve sulflous sodium of 1g into 500ml distilled water

b. DO saturate water for span caribration
Aerate clean air by 1 L min^{-1} untill DO saturate

ii) procedure

After caribration of DO meter, DO of the sample is measured with stirring

5.1.15.2. Wikler-Modified Sodium Azide Method

i) Instruments

a. Burette

b. Erlenmeyer flask

ii) Reagents

a. $MnSO_4$ solution (120 g $MnSO_4/H_2O$ in 250 mL DW)

b. Alkaline potassium iodide azide solution (Mix 2 solutions in polyethylene bottle and kept in cool and dark condition:125 g NaOH and 37.5 g of potassium iodide (KI) in 250 mL deionized water, 2.5 g sodium azide in 10 mL deionized water)

c. H_2SO_4 solution (H_2SO_4:water = 1:2)

d. 0.025 N Sodium thiosulfate ($Na_2S_2O_3$)

e. the starch solution

iii) Pre-treatment

a. In the field, inject 0.5 mL of the $MnSO_4$ solution and 0.5 mL of the alkaline potassium iodide azide solution to the 100 mL sample water in the DO test-bottle for stopping a furthermore reaction and mixing fully after putting the stopper to the test-bottle (refer 3.2.1).

b. Carry the sample bottle back to a laboratory by shutting off the sunshine and cooling in cooler box

iv) Measurement procedure

a. Inject 1 mL of the H_2SO_4 solution, stopper the test-bottle and mix it.

b. Titrate the sample in an Erlenmeyer flask by the 0.025 N sodium thiosulfate ($Na_2S_2O_3$) using 1ml of the starch solution.

See detailed measurment of Winkler Method in the Text book: *Fundamental limnology for inland aquatic environment monitoring on acidification by EANET* (2004).

v) Reporting

DO is expressed as mg L^{-1}.

5.2. Plankton and attached algae (diatom)

i) Concentration

Cell density of phytoplankton in natural waters is often very low.We need to concentrate cells to appropriate density for measuring numbers and floral composition analysis.

a. For the samples which need concentration, transfer appropriate sample water fixed with formalin or Lugol' solution on sites into a container (glass graduated cylinder).
b. Settle it for 4 days in the dark.
c. Discard the supernatant to concentrate plankton cells.
d. Decant the sample water to another samll volume of a container,
e. Continue **d** to gain sample water including an appropriate concentrated plankton cells
f. Measure the volume of finally concentrated sample water, divide to two portion, one for diatom analysis, another for other phytoplankton analysis in future (if possible).

ii) Cleaning of concentrated materials

Method 1:

Method 1(Nagumo, 1995) is more convenient for cleaning of diatom samples than Method 2.

a. Add equal volume of half-diluted bleaching solution (sodium hypochlorite (NaClO) solution for home use, detergent free) to the concentrated sample.
b. Stir the solution occasionally for 30 minutes.
c. Allow diatom cells to settle.
d. Decant and discard the supernatant, add distilled water, mix, and allow the materials to settle. Repeat this procedure several times to remove sodium hypochlorite thoroughly from the sample water.

Method 2:

Caution: Treat carefully hot acid, because this reagent gives severe injury to akin and eyes. Need a good ventilation room.

a. Add five volumes of concentrated sulfuric acid to the sample. Heat the mixture in a hood for 10 to 20 minutes until the color of the mixture turn to brown or black. Stop heating and add immediately about 5g of potassium nitrate (KNO_3) to the mixture. Repeat this procedure until the mixture turns to opaque.
b. Allow the material to cool and settle. Stir the mixture well and add distilled water carefully.
c. Decant and discard the supernatant, add deionized water, mix, and allow the materials to settle. Repeat this procedure several times until acid is removed completely from the sample.

iii) Slide preparation

1. Drop a small volume (200 to 500 μL) of well mixed sample onto microscopic coverslips and allow them to dry slowly. Then dry them on a hot plate.
2. Invert the coverslips and place onto mounting media of high refractive index (Preurax or Mount media; Wako Chem. Co. Ltd., Hyrax) on a microscopic slide.
3. Warm to evaporate the solvent from mounting media and make the specimen permanent.
4. Label the slide (name of lake, sampling station, date etc.) .

iv) Count of diatom cell

1. Identify and count the diatom cells at species level by a microscope. Count at least 500 cells and show relative rate (%) of each species.
2. Results are used for making a database and predictive models for pH.
   See detailed describtion of diatom model, **8.1.2.**

5.3. Sediment

After a core sample of lake sediment is centrifuged (refer 3.2.4 and 4.2), as much as possible without being touched by air, the pore water of the sediment is taken with syringe as the supernatant liquid for chemical analysis. Then the following ions should be analyzed:

・SO_4^{2-}, NO_3^- and NH_4^+

In addition, the core samples of lake sediment itself in separate strata should be analyzed for Pb by atomic absorption spectrophotometry with graphite furnace after acid extraction or ICP Emission Spectrophotometry or ICP-MS.

・Pb, Pb-210 for estimating the date of accumulation

Analytical methods suggested for lake sediment and their pore water are summarized in Table 5.14.

Table 5.14　Analytical methods suggested for lake sediment and their pore water

Parameter	Analytical Methods
NO_3^-	Ion Chromatography or Spectrophotometry
NH_4^+	Ion Chromatography or Spectrophotometry
SO_4^{2-}	Ion Chromatography
Pb, Pb-210	Atomic Absorption Spectrophotometry with Graphite Furnace after Acid Extraction, ICP AES or ICP-MS

5.3.1. SO_4^{2-}, NO_3^- and NH_4^+

The same method as water 5.1 after the extraction.

5.3.2. Pb

After Pb is extracted by acids (**EPA 200.2** "*Sample preparation procedure for spectrochemical determination of total recoverable elements*") the extracts is analyzed by atomic absorption spectrophotometry with graphite furnace (**EPA 7010** "*Graphite furnace atomic absorption spectrophotometry*").

If the accuracy of measurement is not satisfactory due to low concentration of Pb in the sediment, or if the quality assurance is not sufficient due to interfering substances, the analysis of Pb should be the done by the solvent extraction method or by ICP-MS (**EPA 200.8** "*Determination of trace elements in waters and wastes by inductively coupled*

plasma - mass spectrometry"). ICP-MS analysis may be done according to methods **EPA 6020 - 6020A** "*Inductively coupled plasma-mass spectrometry*", **USGS Method I-5020-05** "*Determination of Elements in Natural-Water, Biota, Sediment, and Soil Samples Using Collision or Reaction Cell Inductively Coupled Plasma–Mass Spectrometry*".

Sample preparation is recomended to be done according to **EPA 3050B** "*Acid digestion of sediments, sludges, and soils*" or **EPA 3051A** "*Microwave assisted acid digestion of sediments, sludges, soils, and oils*" or **EPA 3052** "*Microwave assisted acid digestion of siliceous and organically based matrices*".

Pb is expressed as mg L^{-1}

6. Quality assurance/quality control (QA/QC) program

6.1. Introduction

It is desirable to assess synthetically whether evidence of acidification of inland water are observed or not, by evaluating chemical and biological characteristics of the inland water, quantity of atmospheric deposition and other parameters. Some lakes are considered to reveal slow change in acidification, since the sensitivity to acid deposition differs from lake to lake. Therefore, it is necessary to collect reliable data to detect exactly the sign of acidification.

The objectives of QA/QC activities are to obtain reliable data that can be comparable among the countries of the East Asian region, as well as with other networks by ensuring data accuracy, precision, representativeness and completeness in acid deposition monitoring.

To assure specific data quality, QC activities should be implemented for all the steps of the measurement activities, from sample collection to data reporting. These QA/QC programs should cover all QA/QC activities, including the activities of Network Center (NC), the National Centers and the sampling/chemical analysis organizations.

6.2. Data quality objectives (DQOs)

The required data quality objective (DQO) values can be different, depending on the objectives of programs. The DQO values define the desirable levels of accuracy and precision required by the program. The main objectives of the monitoring are to accumulate baseline data and to detect possible impacts on inland aquatic environment. Then, based on the accumulated data, it will be possible to understand trends in the inland aquatic environment and/or to validate models on water quality. For these objectives, DQO values shown in Table 6.1 are applied. The participating countries are expected to make efforts to meet these DQOs.

Table 6.1. Data quality objective values in monitoring on inland aquatic environment

a) Required accuracy and precision (unit: %)

Accuracy[1)]	Precision[2)]
± 15	15

[1)] Accuracy is calculated by the following formula:

$A = [(\text{certified values})-(\text{analytical values})]\times 100/(\text{certified values})$

[2)] Precision (S_i) is calculated by the following formula:

$S_i = (\Sigma d_i^2/2N_i)^{1/2}\times 100/Av$

where d_i and Av denote, the difference between the duplicate analyses and mean, respectively, and N_i is the number of sample pairs in the reporting period.

b) Detection limits and determination limits

Items	Detection limits		Determination limits	
	μmol L^{-1}	mg L^{-1}	μmol L^{-1}	mg L^{-1}
SO_4^{2-}	0.3	0.03	1.0	0.10
NO_3^-	0.5	0.03	1.5	0.10
Cl^-	0.5	0.02	1.5	0.05
NH_4^+	0.8	0.01	3.0	0.05
Na^+	0.3	0.01	1.0	0.03
K^+	0.3	0.01	1.0	0.04
Ca^{2+}	0.2	0.01	0.6	0.03
Mg^{2+}	0.3	0.01	1.0	0.03
Alkalinity	5			
pH	Replicate measurement of RM should agree to within ± 0.05 pH value of RM.			
EC	Replicate measurement of deionized water (EC: less than 0.15 mS m^{-1}) should be agree with ± 0.02 mS m^{-1}.			

RM: Reference material can be used as a laboratory working standard for inland water analysis.

6.3. Standard operating procedures (SOPs)

6.3.1. SOPs

SOPs are the procedures used in all the processes of the monitoring system, i.e. in the field, laboratory, and data management areas. Each sampling and chemical analysis organization (laboratory) should make effort to prepare SOPs that meet the actual conditions of respective organizations, taking account of the technical manuals and the national QA/QC programs. SOPs provide a method to ensure that all personnel follow the same procedures to avoid variance of data quality between personnel in charge, and that they conduct their works with good understanding of QA/QC. In preparing SOPs, it is important that they are sufficiently specific and easy to understand, and that they should be reviewed and updated on the basis of latest information and circumstances.

6.3.2. Major Items to be Included in SOPs in Monitoring on Inland Aquatic Environment

Standard operating procedures (SOPs) should be prepared in accordance with the inland aquatic environment monitoring manual to minimize errors by persons in charge for all elements of operation from sample collection through data reporting. It is important SOPs be complied with in actual operations. Even if the samplers or analytical

instruments used are conformed to the monitoring manual, its makers and/or types may be different in each sampling organization or analytical laboratory. Therefore, SOPs should be prepared taking into account the actual condition of each organization laboratory. Individual SOPs should clearly describe scope of application, nomination of responsible supervisor and the person, in charge, and reporting form and so on. In the following table, the necessary items in individual SOPs on the monitoring are listed, but the additions and/or exclusion are needed according to the actual condition of each laboratory in preparing domestic SOPs.

List of items to be included in SOPs

1. Sampling
1.1. Appointment of sampling staff and their supervisors
1.2. Check of possible changes around the sampling sites
1) Local situation (new construction of emission and contamination sources etc.)
2) On-site situation
1.3. Sampling methods
1) Sampler (involving the documentation of check and maintenance)
2) Sampling interval (sampling dates)
3) Cleaning of vessels with sample water
4) Duplicate sampling

2. Sample transportation and custody
2.1. Transportation of samples
2.2. Sample custody

3. Measurement and chemical analysis
3.1. Appointment of analysis staff and their supervisors for each item
3.2. Development of training plan
3.3. Deionized water
1) Daily maintenance
2) Documentation of maintenance
3.4. Measurement by instruments
1) Measuring conditions of instruments
2) Calibration
3) Performance tests (sensitivity, stability, interference and its removal, documentation of repair)
4) Calculation of lowest detection limits and lowest determination limits
5) Documentation of maintenance
3.5. Operating procedures for measurements
1) Preparation of calibration curves
2) Measurement/analysis of samples

3) Repeated measurements/analyses
4) Check of sensitivity fluctuation
3.6. Treatment of measurement results
1) Calculation of concentrations
2) Measurement of sensitivity fluctuation
3) Repeat measurements/analyses
4) Calculation of ion balances
5) Comparison of measured and calculated conductivity

4. Quality assurance and quality control
4.1. Evaluation of sample collection
1) Comparison of precipitation amount with standard rain gauge
2) Evaluation of ion balance
3) Evaluation of conductivities
4.2. Evaluation of reliability
1) Evaluation of sensitivity fluctuations
2) Evaluation of repeated measurements/analyses
3) Evaluation of field blanks
4) Comparison between measured data and lowest detection and lowest determination limits
4.3. Evaluation of results
1) Representativeness of sampling sites
2) Evaluation of sample validity
3) Evaluation of completeness for the sampling period
4) Determination of total precision

5. Management of sampling instruments, laboratory, measurement/analysis instruments and reagent / glass ware
5.1. Management of sampling instruments
1) Appointment of management staff and their supervisors
2) Documentation of names of manufactures, types, manufacture dates and operation methods
3) Daily and regular maintenance and inspection methods (including trouble shooting, parts supply and recording)
5.2. Laboratory management
1) Appointment of management staff and their supervisors
2) Daily and regular maintenance and inspection methods (including items and recording format)
5.3. Management of measurement/analysis instruments
1) Appointment of responsible staff for each instrument, and overall measurement
2) Documentation of names of manufactures, types, manufacture dates and operation

methods

3) Daily and regular maintenance and inspection methods (including trouble shooting, parts supply and recording)

5.4. Management of reagents, standard materials, etc.

1) Appointment of management staff and their supervisors

2) Receiving and disposal of reagents (recording format of dates, manufacture names, dealers, purity, degree of standard and valid period)

5.5. Management of glassware and polyethylene vessels

1) Appointment of management staff and their supervisors

2) Cleaning methods

3) Storage

4) Confirmation of cleanness

6. External audit

1) Check of sampling sites

2) Measurement of field blank values

3) Operation check of samplers

4) Evaluation of the results of quality control

5) Evaluation of the measured results

6.4. QA/QC for field operations

6.4.1. Sampling sites

Sampling for inland aquatic environment should be done at the same sites with soil and vegetation, in particular in acid deposition vulnerable areas where acid deposition causes or is likely to cause significant adverse effects.

Sampling should be conducted in one of the sites that can be clearly defined in one of the two categories.

6.4.2. Criteria for site selection

It is recommended to select the sites which meet the site selection criteria described in 2.1.2

6.4.3. Representativeness survey of sampling sites and time

It is recommended to check the representatives of sampling sites/points according to the descriptions in 2.1.2.

6.4.4. Documentation of conditions of concerned water bodies

The information on characteristics of the water bodies concerned (refer to 2.2) should

be periodically reported to the NC through the National Center.

6.4.5. Collection and handling of samples

a. Registration of the personnel in charge of collection of samples and their supervisors
Each organization in charge of collecting samples will appoint persons to collect samples and their supervisors. The persons to collect samples should be well trained. The persons who collect samples at each site and their supervisors should be registered to the National Center. The name of the person who collects each sample should be recorded in the on-site record table.

b. Sample collection (refer to 3.2)
Surface water should be sampled directly by a clean polyethylene bucket or a dipper at the representative point of the lake. The duplicate samples should be collected at the same point. The water sample should be taken full up in well washed 2 L polyethylene or polypropylene bottle without air after washing by sample water.

c. Sampling frequency
The monitoring on inland aquatic environment should be carried out with the frequencies as shown in 2.3.

d. Handling and transportation of samples (refer to Chapter 4)
Transportation of samples from sampling sites to chemical analysis laboratories must be done in cooler boxes filled in freezer packs.

6.4.6. On-site measurement (refer to 3.1)

Water temperature should be measured on site and should be recorded on the field reporting form. Air temperature, transparency and outward appearance such as color are recommended to be measured on site and should be recorded on the field reporting form. EC and pH are recommended to be measured in the water bath at 25°C in the laboratory. If a temperature controlled water bath is not available, use of water bath without temperature control but containing at least 5 L of water may be considered. However, on-site measurement of pH and electric conductivity are useful as reference data.

6.5. QA/QC for laboratory operations

6.5.1. Measurement parameters

Measurement parameters are shown in 5.3 above.

6.5.2. Measurement procedures (refer to Chapter 6)

Electric conductivity (EC) and pH of the samples are recommended to be measured in

the water bath, which is thermostated at 25 °C immediately after arriving to the laboratory. If a temperature controlled water bath is not available, use of water bath without temperature control but containing at least 5 L of water may be considered. Alkalinity should be measured by titration with a 0.01 mol L^{-1} or 0.001 mol L^{-1} sulfuric acid.

If the measurement and analysis are done more than several days after arrival at the laboratory, the samples should be stored between − 20 °C to − 40 °C. All chemical analysis should be finished within 1 week.

6.5.3. Fundamental measurement and analysis matters

Freedom from contamination of the apparatus, materials and reagents using in measurement and analysis must be confirmed beforehand: blank values of substances should be as low as possible. Measurement and analysis should be executed by persons who are well trained. To maintain high analytical quality, SOPs must be prepared for the management apparatus, materials and reagents.

6.5.4. Adjustment of analytical instruments

Each of the analytical instruments must be calibrated when they are used, and they should be adjusted as appropriate. Before sample analysis, confirmation of the concentration of Reference Material (RM) sent by NC is highly recommended for the chemical analysis every time

a) pH meter

For the Quality Control, the pH should be measured at 25 ± 0.5 °C. The vessel which was filled with certified solution should be soaked in a temperature-controlled water bath; after calibration of the pH meter, tests of reproducibility and linearity are to be carried out to assure reliable measurement. It should be confirmed that the attached thermometer can also be reliable by comparison with a certified thermometer. It should be confirmed that the water bath can control the temperature fluctuation in the water bath to within the allowable range (± 0.5 °C).

The measurement of pH at the site should be carried out by using a portable electrometric pH meter with glass electrode. Before the determination at the site, the pH meter must be calibrated by using standard solutions for the instrument.

b) EC meter

For the Quality Control, the EC should be measured at 25 ± 0.5 °C. It should be confirmed before the measurement that reliable data can be obtained with the calibration of the EC meter in a temperature-controlled water bath, with tests of reproducibility and linearity. It should also be confirmed that the attached thermometer

can be reliable by comparison with a certified thermometer, and it should be confirmed that the constant-temperature water bath can control the temperature fluctuation in the water bath to within the standard (25 ± 0.5 °C).

The measurement of EC at the site should be carried out by using a portable EC meter. Before the determination at the site, the EC meter must be calibrated by using standard solutions for the instrument. When EC is measured at the water temperature of the site, it should be corrected to the value at 25°C. The equation for correcting is as follows:

$$(EC_t) = (EC_{25}) \times [1 + 0.022 \times (t - 25)]$$

where, t: water temperature at the site.

c) Ion chromatograph
After setting up the components of working eluent and condition of flux, and regulating the conditions under which target ions can be separated well, it should be then confirmed that the response is stable and the prescribed sensitivity is achieved.

d) Atomic absorption spectrometer
After setting up conditions of the current of midair cathode lamp, the height of the burner, the fluxes of fuel gas and combustion-supporting gas, measuring the wavelength and slit range, it should be confirmed that the response is stable and that the prescribed sensitivity is achieved. If there is a possibility of optical interference, then, must be made to achieve sufficient reliability.

e) Spectrophotometer
Regarding target ions, it should be confirmed that the absorption is stable and the prescribed sensitivity is achieved. If there is a possibility of optical interference, then, must be made to achieve sufficient reliability.

f) Digital burette with pH meter
For the measurement of alkalinity, digital burette with a pH meter should be used where possible. The pH meter attached to digital burette should be calibrated by same methods as a).

6.6. Data control

6.6.1. Introduction

There are three purposes concerning quality assurance of data control.
1) Assure that all sample data will be stored in database in an adequate manner.
2) Mark with flags the data, whose accuracy and representative are doubted.
3) Recognize and describe samples, that were measured without standard methods, i.e. with contamination, instrument trouble, bulk sampling, etc.

Quality assurance and quality control in data control should be carried out in analysis organizations, the National Centers, and NC, individually.

6.6.2. Data check

a. Treatment of abnormal and unrecorded data

When the sensitivity of instruments is not stable, when the results of duplicate analyses or re -measurements are significantly different, or when the ratio of a theoretical value to that for determined data in ion balance and EC is significantly different from 1, measurement should be repeated since reliability is low. In addition, when samples seem to be obviously contaminated, these data should be treated as unrecorded data.

These problems will waste much labor, time, and expense. In addition, abnormal or unrecorded data can corrupt research results. Therefore, careful checks are needed to avoid data of inadequate quality. When abnormal or unrecorded data appear, the process should be carefully reviewed to prevent the occurrence of the same problem in the future.

b. Analyses of Non Detected and Lowest Determination Limit

In determining the detection and determination limits for the respective methods (Ion chromatography, Spectrophotometry, Atomic absorption spectrometry) used in measuring the concentrations of several ions in inland water samples, a standard solution with concentration near the lower determination limit should be measured 5 times.

On methods (Ion chromatography, Spectrophotometry, Atomic absorption spectrometry) used in measuring the concentration of several kinds of ion in inland water samples, the certified solution in lowest concentration (near the Lowest Determination Limit) for making the calibration curve should be measured in 5 times with prescribed operation.

Based on the standard deviation “s” of the repeat analyses above, 3 times the value is defined as Non Detected, and 10 times the value is defined as the Lowest Determination Limit (the unit of “s” is the same as that used for the concentration in water).

$$\text{Not Detected} = 3\ s\ (\mu mol\ L^{-1})$$

$$\text{Lowest Determination Limit} = 10\ s\ (\mu mol\ L^{-1})$$

Because the Lowest Determination Limit is different among analytical instruments used and analytical conditions vary, LDL should be determined whenever analytical conditions are established or changed. It should be confirmed that LDL is below the DQO values described in Table 6.1 b)

c. Cation and anion balance

All measured data should be checked for following R_1 and R_2 values. If necessary, re-measurement should be carried out and the adequacy of data should be checked. If the concentration or standard deviation of concentration of an ion differs greatly from the long term average concentration or standard deviation of concentration, respectively, during a given period, then this cause should be carefully considered.

Calculation of ion balance (R_1)

1) Total anion (A) of equivalent concentration (μeq L^{-1}) is calculated by summing the concentration of all anions (c: μeq L^{-1}) and alkalinity (ALK: μeq L^{-1}). Alkalinity considered to be corresponded to bicarbonate ions (HCO_3^-).

$$A\ (\mu eq\ L^{-1}) = c\ (SO_4^{2-}) + c\ (NO_3^-) + c\ (Cl^-) + (ALK)^*$$

* If PO_4^{3-}, NO_2^- etc were measured, add next fomula, {+ c (PO_4^{3-}) + c (NO_2^-)+…}.

2) Total cation (C) equivalent concentration (μeq L^{-1}) is calculated each concentration of cations (C: μeq L^{-1}).

$$C\ (\mu eq\ L^{-1}) = 10^{(6-pH)} + c\ (NH_4^+) + c\ (Na^+) + c\ (K^+) + c\ (Ca^{2+}) + c\ (Mg^{2+})^*$$

* If Fe^{2+}, Mn^{2+} etc were measured, add next formula, {(+ c (Fe^{2+}) + c (Mn^{2+})+…}.

3) Calculation of ion balance (R_1)

$$R_1 = 100 \times (C-A) / (C+A)$$

4) R_1, which is calculated using the above equation, should be compared with standard value in Table 6.2. When R_1 is not in the range, re-measurement, check with standard samples, or inspection of standard curve are necessary, or the flags which indicate unsatisfied data should be marked in the database.

When the concentration (C) of ion is shown as (mg L^{-1}), it should be changed in equivalent concentration (μeq L^{-1}) as follows:

$$\mu eq\ L^{-1} = mg\ L^{-1} \times (1000/\text{equivalent weight})$$

thus the total of anions and cations are shown as follows:

$$A\ (\mu eq\ L^{-1}) = \{c\ (SO_4^{2-})/48.03 + c\ (NO_3^-)/62.01 + c\ (Cl^-)/35.45^*\} \times 1000 + (ALK)$$

* If PO_4^{3-}, NO_2^- etc were measured, add next formula: {+ c (PO_4^{3-})/26.32 + c (NO_2^-)/46.01 +}

$$C\ (\mu eq\ L^{-1}) = 10^{(6-pH)} + \{c\ (NH_4^+)/18.04 + c\ (Na^+)/22.99 + c\ (K^+)/39.10 + c\ (Ca^{2+})/20.04 + c\ (Mg^{2+})/12.16^*\} \times 1000$$

* If Fe^{2+} , Mn^{2+} etc were measured, add next formula : {+ c (Fe^{2+})/27.92 + c (Mn^{2+})/27.47 +…}

Table 6.2. Allowable ranges for R_1 in different concentration ranges

(C + A) μeq L^{-1}	R_1
< 50	± 30
50 - 100	± 15
> 100	± 8

The flags should be marked as follows:

999	Missing measurement, reason not specified.
899	Measurement not defined, reason not specified.
781	Below detection limit.
701	Less accurate than usual, reason not specified .
699	Mechanical problem, reason not specified.
599	Contamination not specified.

Comparison between calculations and measurement in electrical conductivity (R_2)

1) Total electric conductivity (Λcalc) should be calculated as follows:

$$\Lambda calc\ (\mu S\ cm^{-1}) = 349.7 \times 10^{3-pH} + \{80.0 \times c\ (SO_4^{2-}) + 71.4 \times c\ (NO_3^-) + 76.3 \times c\ (Cl^-) + 73.5 \times c\ (NH_4^+) + 50.1 \times c\ (Na^+) + 73.5 \times c\ (K^+) + 59.5 \times c\ (Ca^{2+}) + 53.0 \times c\ (Mg^{2+}) + 44.5 \times (ALK)\}/1000$$

c: equivalent concentration (μeq L^{-1}) of ions in the parenthesis, each constant value is ionic equivalent conductivity at 25 °C (see Table6.3). Alkalinity considered to be corresponded to bicarbonate ions (HCO_3^-).

For SI unit system , convert Λcalc (μS cm^{-1}) into Λcalc (mS m^{-1}) following

$$\Lambda calc\ (mS\ m^{-1}) = \Lambda calc\ (\mu S\ cm^{-1}) \times (1/10)$$

Table 6.3. Equivalent weight of cations, anions and ionic equivalent conductivity

Ion	Equivalent Weight	Ionic Equivalent Conductivity λ (S cm^{-2} eq^{-1})	Ion	Equivalent Weight	Ionic Equivalent Conductivity λ (S cm^{-2} eq^{-1})
H^+	1.008	349.7	NO_3^-	62.01	71.4
NH_4^+	18.04	73.5	SO_4^{2-}	48.03	80.0
Ca^{2+}	20.04	59.5	Cl^-	35.45	76.3
K^+	39.10	73.5	HCO_3^-	61.02	44.5
Mg^{2+}	12.16	53.0	$HCOO^-$	45.0	54.6
Na^+	22.99	50.1	CH_3COO^-	59.1	40.9
Fe^{2+}	27.92	54	F^-	19.00	55.4
Fe^{3+}	18.62	68	Br^-	79.90	78.1
Mn^{2+}	27.47	53.5	NO_2^-	46.01	71.8
			PO_4^{3-}	26.32	92.8

W. H. Haynes (Edition-in-Chief) (2011) CRC Handbook of Chemistry and Physics, Ed 92nd, 5-77, 78, CRC Press.

2) Ratio (R_2) of calculations to measurements(Λ_{meas}) in electric conductivity should be

calculated as follows:

$$R_2 = 100 \times (\Lambda_{calc} - \Lambda_{meas})/(\Lambda_{calc} + \Lambda_{meas})$$

3) R_2, which is calculated using the above equation, should be compared with standard value in Table 6.4. When R_2 is not in the range, re-measurement, check with standard samples, or inspection of standard curve are necessary, or the flag which indicate unsatisfied data should be marked in the database.

Table 6.4. Allowable ranges for R_2 in different concentration ranges

Λ_{meas} (mS m^{-1})	R_2
< 0.5	± 20
0.5 - 3	± 13
> 3	± 9

(1 mS m^{-1} = 10 μS cm^{-1})

The flags should be marked as follows:

999	Missing measurement, reason not specified.
899	Measurement not defined, reason not specified.
781	Below detection limit.
701	Less accurate than usual, reason not specified.
699	Mechanical problem, reason not specified.
599	Contamination not specified.
477	Inconsistency between measured and estimated conductivity.

d. Duplicate sample collection and replicate analysis

Duplicate sampling and analysis should be carried out in the laboratories. Double measurement (duplicate sampling and analysis) of inland water provides a measure of overall monitoring precision.

To estimate the contribution of sampling and analytical variability, duplicate sampling and replicate analyses of three times for each sample should be performed. It should be confirmed that the obtained relative standard deviation is below the Required Precision shown as Table 6.1. The standard deviation between the two samples should be calculated, and if the relative standard deviation is larger than 15%, it must be sampled once more and analyzed again.

6.7. Site performance audit and laboratory audit

For assurance of accuracy and precision of a field sampling and a laboratory measurement, a site performance audit and laboratory audits should be carried out periodically by the National Center. A site performance audit ensures that no major

physical change has taken place at the site, includes reviews of on-site measurement, sampling, sample transportation and data reporting, and provides field training and exchange of information. A laboratory audit consists of inspection and advice on sample handling, capacities of instruments, preparation and implementation of SOPs, and other QA/QC activities and their records. The National Center should prepare the SOP for site audits. The results of each audit should be documented and stored at the National Center.

6.8. Training programs

It is necessary to improve knowledge and expertise related to inland aquatic environment monitoring. Therefore, NC and local technical training should be provided. Details of the training will be given in a separate document.

6.9. External quality assurance program

To review the accuracy of chemical analysis, NC should send 2 bottles of artificial inland water for inter calibration to all chemical laboratories, evaluate statistically the results of the analysis of the samples and prepare reports about the results. 1 bottle is for this program and another should be used for usual chemical analysis as Working Standard (Reference Material).

As one of other external quality assurance programs, there is the intercomparison test for The international Cooperative Programme (ICP) on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes supplied by Norwegian Institute for Water Research.

7. Data control and reporting

7.1. Data control

Quality assurance and quality control in data control should be carried out in analysis organizations, National Centers, and NC, individually. Data checking should include activities such as: 1) treatment of abnormal and unrecorded data, 2) judgment of valid data, for example, calculation of ion balance (R_1) and comparison between calculation and measurement in electrical conductivity (R_2).

For more detailed methods about data evaluation, "QA/QC program" should be referred to.

7.2. Data reporting

Each organization responsible for field sampling and each analytical laboratory should record, control, submit and store the information as follow:

7.2.1. Information about sampling sites

Efforts should be made to obtain the following information.
(1) Information concerning watersheds/catchments of sampling lakes
(2) Information concerning characteristics of lakes
(3) Information concerning sediment in sampling lakes
(4) Information concerning living organisms in sampling lakes

7.2.2. Matters related to collection of samples

The records of on-site information (lake name, sampling point, name in charge, sampling time, air and water temperature, out-looking of sample, transparency, climate, etc.), information about sample collection instruments (pictures of instrument and design diagrams, model name, manufacturer and manufactured date, etc.) if used and/or used tools should be reported to National Center.

7.2.3. Matters related to analytical procedures

(1) Calibration and measurement procedures for analytical instruments
(2) Miscellaneous other values required to obtain measurements

7.2.4. Local quality control activities

Each organization responsible for the field sampling and each analytical laboratory will record the following information and will store them with the data:
(1) Performance of all procedures prescribed in SOPs
(2) Routine instrument check and maintenance, record of instrument adjustment

(calibration of instruments)

(3) Names of producers and traceability of standard materials etc., institution of measurement conditions of analytical instruments and its results

(4) Results of analysis of lowest detection limits and lowest determination limits

(5) Results of duplicate analysis

(6) Evaluation of cation and anion balance and conductivity difference

(7) Results of site performance audit

Reporting digit and/or decimal places will be clarified. The example of report format of Inland Aquatic Environment is shown as follow,

Notice

*Double measurement data

In data reporting, double measurement data should be reported respectively as well as average data of them.

*On-site measurement data

The pH and EC of on-site measurement data are recorded as reference data. In data reporting, the data measured in the laboratory (25 °C) should be submitted. And the pH and EC of on-site measurement data should not be used in the calculation of R_1 and R_2.

Reporting forms:

Inland Aquatic Environments (prepare for every survey)

1) Site

Site type	1.lake 2.river(stream), 3.other( )
Site name	
Location	

2) Sampling method

Water sampling method and sampling device	method: device/manufacture: model:
On site filtration	1.do(material of filter:), 2.don't
Temperature at the Shipping	1.uncontrolled, 2.cooling(°C), 3.freezing
Mean time from sampling to analysis	day(s)
Name of sampling organization and reporter	

3) Results of analysis(surface water at the center of aquatic system)

Describe in analytical results (Inland aquatic environment) :Form(Inland A)

Form (Inland A-1) Analytical results of inland aquatic sample

Site name: Date of sampling:

Date of analysis: Sample No.:

Name of laboratory: Name of reporter:

Items		Sample No.1						Sample No.2						Ratio of Mean	Mean
		1	2	3	Mean	RSD(%)	flg	1	2	3	Mean	RSD(%)	flg	(No.1 / No.2)	Value
W.T. (^{o} C)															
pH	On-site														
	at 25^{o} C														
EC	On-site														
mS m^{-1}	at 25^{o} C														
Alkalinity meq L^{-1}															
SO_4^{2-} mg L^{-1}															
NO_3^{-} mg L^{-1}															
Cl^{-} mg L^{-1}															
NH_4^{+} mg L^{-1}															
Na^{+} mg L^{-1}															
K^{+} mg L^{-1}															
Ca^{2+} mg L^{-1}															
Mg^{2+} mg L^{-1}															
NO_2^{-} mg L^{-1}															
PO_4^{3-} mg L^{-1}															
(Fe^{2+} mg L^{-1})															
(Mn^{2+} mg L^{-1})															
R_1															
R_2															

Note: R_1:cation and anion balance(100 × (cation−anion)/(cation+anion)), R_2:specific conductance value (100 × ($\Lambda_{calc}-\Lambda_{meas}$)/($\Lambda_{calc}+\Lambda_{meas}$)). If the R_1 and R_2 do not meet the criteria, measurement of additional ions, such as Fe^{2+} and Mn^{2+} should be considered.

Form (Inland A-2) Analytical results of inland aquatic sample

Site name: Date of sampling:

Date of analysis: Sample No.:

Name of laboratory: Name of reporter:

Items	Sample No.1						Sample No.2						Ratio of Mean	Mean
	1	2	3	Value	RSD(%)	flg	1	2	3	Mean	RSD(%)	flg	(No.1 / No.2)	Value
DO (on-site or laboratory)														
transparency (m)														
water color														
DOC (COD) mg L^{-1}														
D-Al mg L^{-1}														
Reactive Al, µmol L^{-1}														
T-N mg L^{-1}														
T-P mg L^{-1}														
SS mg L^{-1}														
River discharge (m^3 sec^{-1})														

Form (Inland A-3) Analytical results of inland aquatic sample

Site name: Date of sampling:

Date of analysis: Sample No.:

Name of laboratory: Name of reporter:

<table>
<tr><td>Sample</td><td colspan="3">River bottom ( ), Lake bottom ( ), Lake water ( )</td><td></td></tr>
<tr><td rowspan="4">Bottom bed dominancy</td><td colspan="2">Mud</td><td>( )</td><td rowspan="4">Comments:</td></tr>
<tr><td colspan="2">Sand and small stones (<5 cm in diameter)</td><td>( )</td></tr>
<tr><td colspan="2">Stones (5-20 cm in diameter)</td><td>( )</td></tr>
<tr><td colspan="2">Stones (>20 cm in diameter)</td><td>( )</td></tr>
<tr><td rowspan="2">Bottom bed cover</td><td colspan="2">Overview cover of particulate organic material (leaves and stems) (%)</td><td></td><td rowspan="2">Comments:</td></tr>
<tr><td colspan="2">Overview cover of mosses on the bottom stones (%)</td><td></td></tr>
<tr><td rowspan="5">Diatom</td><td rowspan="3">Depth of sediment cm - cm
Sampled area cm × cm
Number of sample</td><td rowspan="3">Depth of water m- m</td><td rowspan="3">Total cell count</td><td rowspan="3"></td></tr>
<tr></tr>
<tr></tr>
<tr><td colspan="2">Taxon list Relative abundance (%)</td><td rowspan="2">Comments:</td></tr>
<tr><td colspan="3"></td></tr>
<tr><td colspan="4">if possible, pH preference groups by Hustedt classification (%)</td></tr>
<tr><td rowspan="2">Other organisms</td><td colspan="3">Taxon list (with density) of attached algae, phytoplankton, zooplankton, fishes, macrophytes, benthic invertebrates</td><td rowspan="2">Comments:</td></tr>
<tr><td colspan="3"></td></tr>
</table>

( ): Choose one (Partially reffered by ICP-Waters)

Form(Inland A-4) Analytical results of Sediment sample

Site name: Date of sampling:

Date of analysis: Sample No.:

Name of laboratory: Name of reporter:

		Sampling point: sampling depth: m					
Items		Sediment sample					
		1	2	3	Value	RSD(%)	flg
Upper layer	NH_4^+ (mg L^{-1})						
	NO_3^- (mg L^{-1})						
	SO_4^{2-} (mg L^{-1})						
Middle layer	NH_4^+ (mg L^{-1})						
	NO_3^- (mg L^{-1})						
	SO_4^{2-} (mg L^{-1})						
Bottom layer	NH_4^+ (mg L^{-1})						
	NO_3^- (mg L^{-1})						
	SO_4^{2-} (mg L^{-1})						
The kind and name of sediment sampler		Kind:			Name		
Core diameter and total length of sample		Diameter: mm			Total length: mm		
The centrifuge		Name:		Used revolution: rpm		Used centrifugal acceleration: g	
		Centrifugal time: min.		Max revolution: rpm		Max centrifugal acceleration: g	
Sample depth of analysis		Upper: ~ mm		Middle: ~ mm		Bottom: ~ mm	
W.T. and DO above sediment		W.T.: (depth m)			DO: (depth m)		
		Kind of thermometer:			Kind of DO analysis:		
Note:							

4) Meteorological condition (reported year)

Month / Items		1	2	3	4	5	6	7	8	9	10	11	12
Tempera-ture(°C)	Monthly mean												
	max.daily mean												
	min.daily mean												
Humidity (%)	Monthly mean												
	max.daily mean												
	min.daily mean												
Mean wind speed ($m\ s^{-1}$)													
Most appearance wind Direction (bearings)													
Precipitation amount ($mm\ month^{-1}$)													
Sunshine duration ($hours\ month^{-1}$)													
Solar radiation ($MJ\ m^{-2}\ month^{-1}$)													

Hourly data of temperature, humidity, wind direction, wind speed, and daily precipitation amount and daily sunshine time, etc. prefer to be reported by the data files of computer and it's printout sheets. In case of reported by data files of computer and it's printout sheets, the description in above column can be omitted.

5) Others

Note:

8. Future direction

8.1. Bio-indicators including diatoms, invertebrates and fishes

8.1.1. Introduction

Acidification of inland waters, lakes and rivers, has been largely influenced on aquatic organisms at sensitive regions in Europe, Canada and North America. In these areas, biological monitoring including experimental assessments has been carried out as well as monitoring of surface water chemistry attributable to atmospheric pollution since 1980'. Numerous ecological studies and monitoring results conducted in acidified areas, concerning the effects of acidification on aquatic organisms, have been revealed that what kind of species was sensitively reacted, what nutritional structures are existed, what substances harmfully affected (Haines 1981; Shindler 1988; Gorham 1998).

In EANET areas, monitoring activities of inland aquatic environments including surface water chemistry and living organisms mainly diatom species started since 1998. The latter item is recommended as one of optional parameters to be measured in an EANET technical document with an insufficient explanation except a diatom part.

No clear trend was observed in pH at water bodies in EANET monitoring sites during 2000 to 2004, whereas the lowest annual average of pH 6.12 was observed in a Chinese site, although the frequency of pH values found in rain samples gathered from all over the EANET region are similar to sets provided by the monitoring networks in Europe and United States (EANET 2006). This might mean gradual acidification of surface water in sensitive area in future, like as reported in some of Japanese rivers (Nakahara et al., 2009; Matsubara et al. 2009).

In this section, a basic feature of biological indicators (bio-indicators) for acidification of aquatic environments will be described and a future direction for biological monitoring in the EANET areas will be discussed. The Programme manual of ICP-Waters (Norwegian Institute for Water Research 1996) is partially referred.

8.1.2. Biological indicator species for acidification

Two categories showing the effects of acidification on aquatic organisms have been developed, sensitive and tolerant species or taxonomic groups for acidic condition. The distribution of these species is usually restricted to geographical regions where environmental factors, such as temperature, light length, predators etc., assure their life. In EANET countries located from the tropical to the frigid zone, we will need peculiar taxonomic sets for acidic condition in each countries or climatologically classified regions, since it is impossible to find out the universal species distributing over the EANET areas.

1) Algae

In phytoplankton and attached algae, two taxon, Chrysophyceae which includes some acid sensitive species and produce species-specific siliceous cysts and scales, and Bacillariophyceae which have historically conservative hard valves made from silicate and contains species with wide range of pH habitats including acid sensitive species, have been used as biological indicators in paleolimnological and inferable studies.

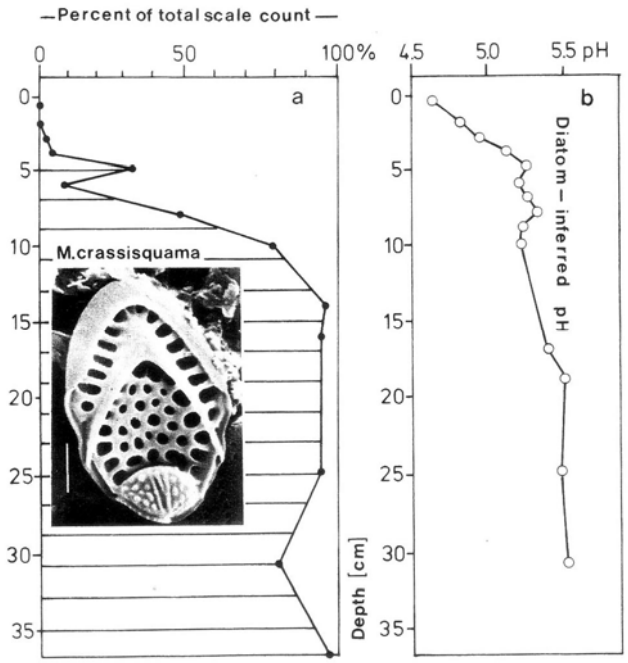

Fig. 8.1. a) Relative abundance of *Mallomonas crassisquama* scales with respect to

total *Mallomonas* scales in a sediment core of the Kleiner Arbersee, b) pH values inferred from diatom flora from the same sediment core. From Steinberg and Hartmann (1986).

Chrysophytes

Steinberg and Hartmann. (1986) indicated lowering rate of *Mallomonas crassisquama* in total *Mallomonas* numbers in sediments of Lake Kleiner Arbersee (FRG), and good consistency with diatom inferred pH trend (Fig. 8.1). The advanced chrysophyte-inferred pH models are given in Wilkinson et al. (1999), Dixit et al. (2002).

Diatoms

Diatoms belong to the algal class Bacillariophyceae and have narrow optima or tolerance for pH. Many acidophilous or acidobiontic species are known (see last version of EANET Manual). The former are the diatom species which grow well in acidic conditions and the latter only in acidic habitats.

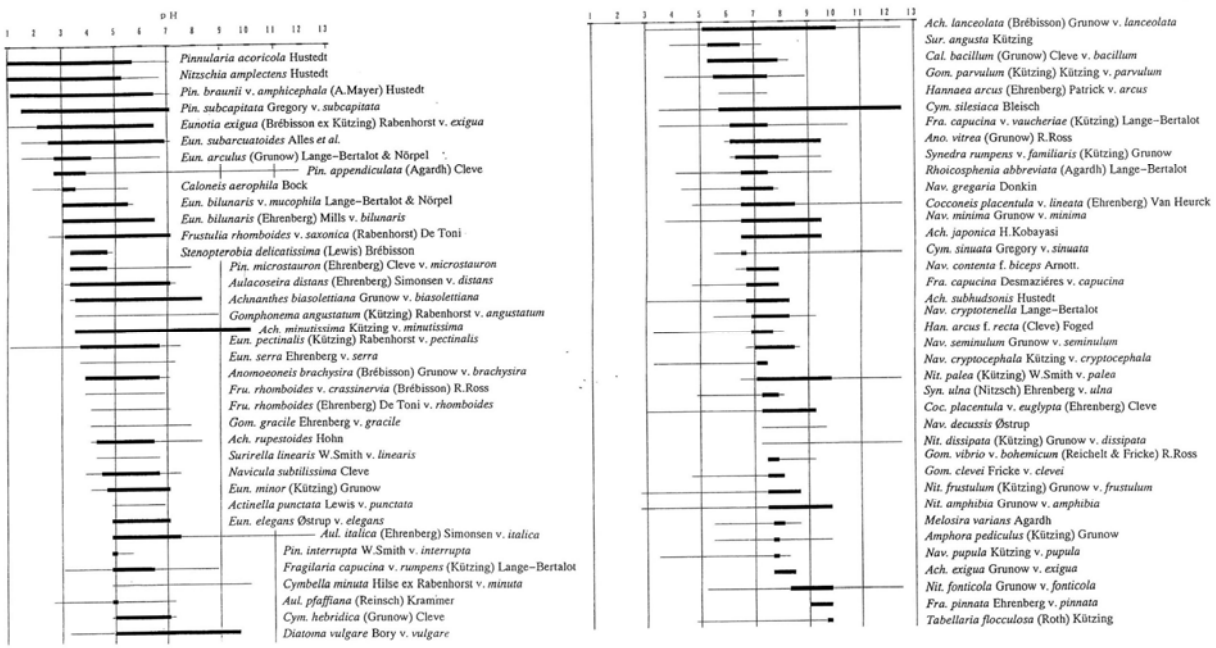

Fig. 8.2. Distribution of diatom species along the pH garaduents in Japan. Bold lines indicate pH values at smpling sites where the taxa were dominant. From Watanabe and Asai (1999).

In Japan, distribution of diatom species along the pH garaduents is presented by Watanabe and Asai (1999) (Fig.8.2), based on the investigation of many water bodies including lakes, rivers, hot springs etc.

Diatome species are classified to five groups as followings;

(1) Acidobiontic: occurring at pH values < 7 with optimum distribution at pH 5.5 and under

(2) Acidophilous: occurring at pH about 7 with widest distribution at pH < 7

(3) Circumneutral (Indifferent): equal occurrence on both sides of pH 7

(4) Alkaliphilous: occurring at pH about 7 with widest distribution at pH > 7

(5)Alkalibiontic: occurring at pH values > 7

(From Hustedt's classification)

Diatome inferred pH

Cell walls of diatomes are composed of SiO_2 and are well preserved in lake sediment. From those characteristics, diatom assemblages have been successfully used as to infer trends of water pH resulting from lake acidification, using an equation obtained beforehand. This is culculated using the Index B method of Renberg and Hellberg (1982).

$$\text{Index B} = \frac{\%\,\text{circ} + 5 \times \%\,\text{acp} + 40 \times \%\,\text{acb}}{\%\,\text{circ} + 3.5 \times \%\,\text{alk} + 108 \times \%\,\text{alkb}}$$

Here, circ = circumneutral, acp = acidophilous, acb = acidobiontic, alk = alkaliphilous, alkb = alkalibiontic

Estimated equation;

$$pH = a - b \times \text{Log (Index B)}$$

Yoshikawa et al. (2000) obtained an equation for diatom-inferred pH using a data-set

from the Adirondacks, USA, and adopted to a Japanese small pond to estimate past pH changes using a original list of diatoms with pH category.

Inferred pH = 6.91 – 0.85 × Log (Index B)

He succeeded to indicate the acidification process of L. Sawanoike in Kyoto, from the late of 1940' to 1980' (Fig.8.3).

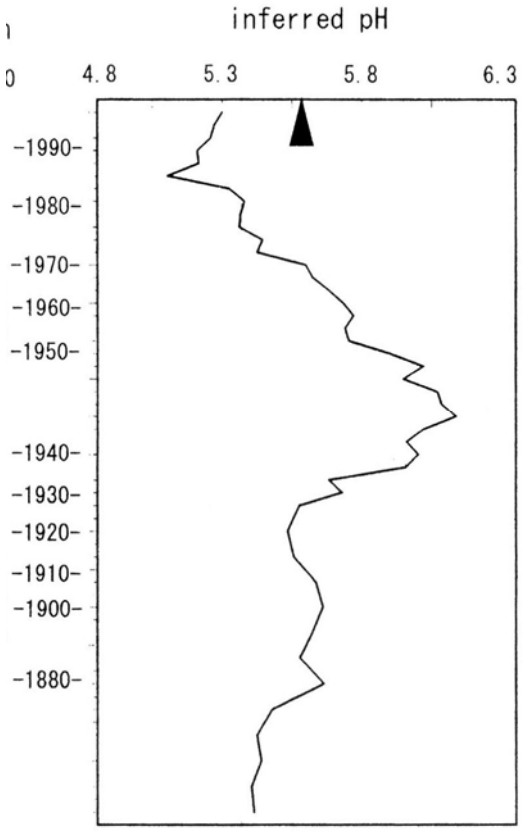

Fig.8.3. Profile of the inferred pH calculated from Index B in a core from L. Sawanoike. Arrow indicate mean air-equilibrated pH at present day. From Yoahikawa et al. (2000)

It is nessesarry to mind in heart that the coefficiets (a, b) of the equation differ from regions or countries (e.g.: Inferred pH = 6.40 - 0.85 x Log (Index B) for southwest Sweden lakes (Renberg and Hellberg 1982), Inferred pH = 6.3 - 0.86 x Log (Index B) at southwest Scotland (Flower 1986)), and the diatom species belongging to the above groups (1)-(5) differ also.

Thus, we need to make preparations in advance an apporopriate regrretion equation between observed pH and Log (Index B) to estimate past pH memorized in sediments, using data sets of Index B determined from the diatom composition in many surficial sediment or water samples with their habitat pH values, and to have a categorized list of diatoms at a given region.

2) Invertebrates

Aquatic invertebrates play an important part as indicators for inland water acidification. The acid sensitive species or groups disappear successively depending on their acid sensitivity, while others are more tolerant. A representative and plainly demonstrated figure indicating sensitivity of aquatic organisms along pH for acidification including invertebrates is shown in Fig. 8.4.

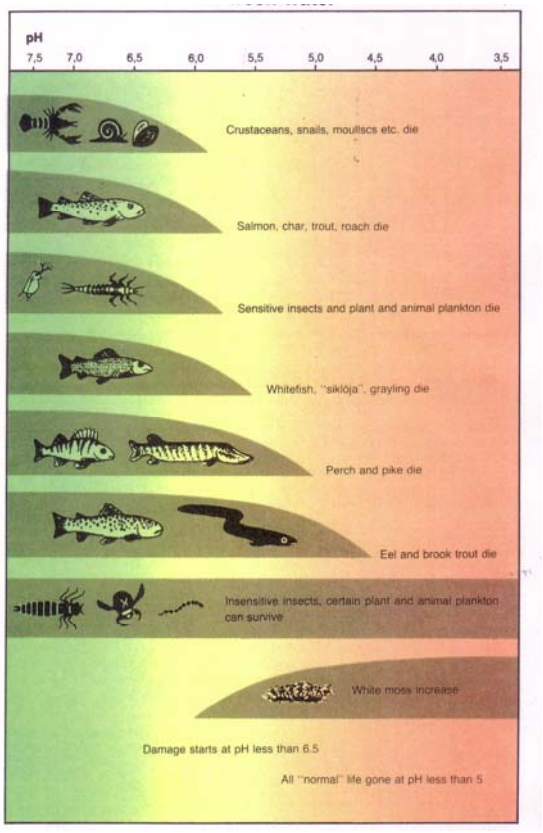

Fig.8.4. The sensitivity of aquatic organisms to lowered pH fresh water in Sweden (Ministry of Agriculture Environment '82 Committee 1982). Approximate borderlines for various animals and plants in fresh water are shown.

Generally, it is known as follows what happen in an invertebrate community during progressing acidification of surface water (Baldigo et al. 2009).

(1) The density of benthic macroinvertebrates may or may not change.

(2) Species richness often decreases.

(3) Certain acid-sensitive species are replaced by more tolerant species.

(4) One or two functional feeding groups tend to predominate communities in response to acidification and associated changes in the abundance of predators (fish) and (or) primary food supplies.

And we can add next item.

(5) The biomass of invertebrate community may mostly decrease.

Zooplankton and macroinvertebrates

Of the Cladocera species, which are cosmopolitan, daphnids and chydorids exhibit low tolerance to acidity in lakes. Mollusks are highly acid sensitive. Snail is not generally found below pH 5.6. It has been known well *Gammaarus lacustris* is not found below pH 6, whereas we have not this species in EANET areas.

Aquatic insects

Almost all species of mayfly (Ephemeroptera) are very sensitive indicators for acidic condition. Many species of stonefly (Plecoptera) and caddis fly (Trichoptera) disappear also in acidified water. The genera including sensitive species are *Ephemera, Ephemerella, Heptagenia, Baetis, Caenis* in mayflies, *Isoperla, Diura* in stoneflies, *Hydropsyche, Apatania* in caddis flies. On the contrary, some taxa are acidophilous or acidobiontic ones telling good indicators in strongly acidified water, such as *Protonemura* ・*Nemoura* ・ *Capnia* (Plecoptera), *Rhyacophila* (Trichoptera), *Chironomus*・ Orthocradinae (Chironomidae) (Fig. 8.5).

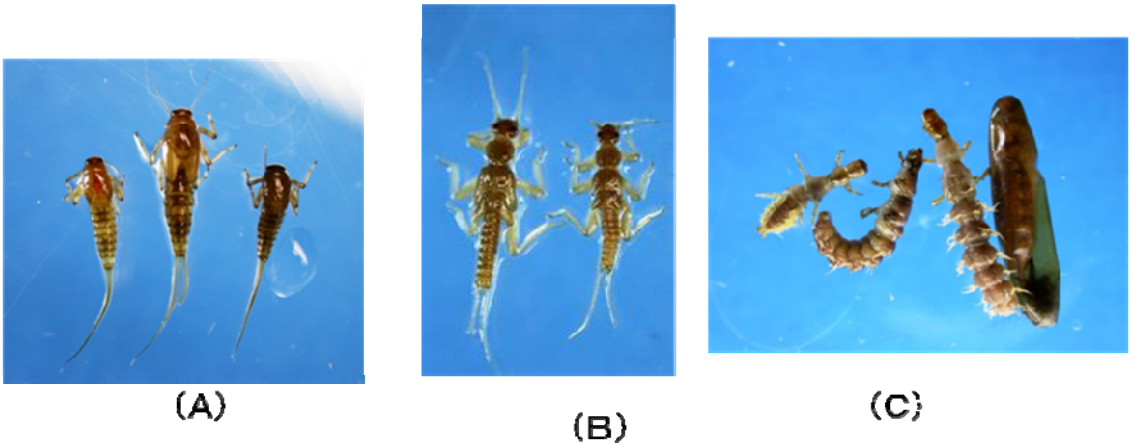

Fig. 8.5. Typical acidosenstive (A) and acidotorelant (B and C) species in naturally acidified water. (A) *Baetis* sp. (Ephemeroptera), (B) *Protonemura* (Plecoptera), (C) *Rhyacophila* (Trichoptera).

Acidification numbers (Acidity Index)

In order to compare the degree of water acidity, a common index "Acidification numbers "used in different regions and countries with different invertebrate fauna is presented and often used in ICP-Waters (See in detail the ICP-Waters Manual) .

Indicator species with the same tolerance to acidity are assigned the same number or "acidification score". Four categories, indicator species and acidification score are given in Table 8.1.

This score is given to each sampling site in a target water body and an average score at several sampling sites will be calculated for a representative score of the water body. The lowest score, 0, shows highly acidified and score 1 unacidified water. Some phases of the biological recovery of invertebrates in ICP-Waters areas using Acidification numbers (Acidity Index) are given in ICP/Water report 87/2007. A list of macroinvertebrates with sensitivity levels in western Norway is recorded by Raddum and Fjellheim (1984), and in German by Braukmann and Biss (2004).

Table 8.1. Categories, indicator species and acidification score for calculating Acidification numbers (Acidity Index). Species/Group are illustration for Norway. From ICP-Waters manual

Category	pH tolerance	Species/Group	Acidification score
A	Indicators extinct at pH5.5-6.0	*Gammarus* spp., Gastropods, *Baetis* spp.	1
B	Indicators extinct at pH5.0-5.5	*Daphinia* spp., *Apatania* spp., *Hydropche* spp.	0.5
C	Indicators that tolerate acidity pH4.7	Small mussels, *Pisidium*	0.25
D	Indicators that tolerate acidity pH<4.7	*Corixa* spp., Odonata, Coleopterans	0

3) Fishes

Atlantic salmon (*Salmo salar*) that the disappearance was first reported by acid water in Norwegian over 90 years ago is known as the historical and one of symbolic indicators for water acidification. Thereafter, tolerance pH levels for many freshwater fishes have been determined in Europe, USA and Canada (Johnson 1982; Magunuson et al.1984). The lower limit of pH tolerance of fishes varies depend on species and water quality; many species start to disappear at pH 5.5. Juvenile or young fishes are intolerant of much higher pH values. There may be two causes of fish disappearance or decrease of standing crops from rivers and lakes by water acidification, the direct and indirect causes, from the researches on mainly salmonoid fishes. The direct cause contains inhibition of wonder of anadromnus fishes, toxic effect of hydrogen ion on avoidance and feeding behavior of juvenile fishes, lowering of sperm activities, lowering of survivor rate of eggs and juveniles, longevity of duration of hatch, lowering of hatching rate, inhibition of normal development of embryo and juvenile growth, ion balance in blood plasma such as Na^+ or Cl^-. Aluminum ion give also severe toxic effects on regulation of osmotic pressure by injuring gills of almost all freshwater fishes. Bearing in mind the aluminum ions influence generally at pH 5.0-5.5 above about 0.1mg/L, higher values than hydrogen ion concentration of pH 4.5.

Biomass and population density of fishes which are usually occupy higher level of a food chain, are received indirectly the disappearance or lowering density of food items such as insect larvae or other invertebrates that are more sensitive to acidity than the target fish species.

As most acid tolerant fish species, the Japanese dace (Cypridae; *Tribolodon hakonensis*) inhabiting at around pH 3.5 in L. Osorezanko, Japan and the eastern mudminnow (Umbridae; *Umbra pygmaea*) distributing in USA, are known well.

4) Others

Aquatic macrophytes are also affected by acidification of waters(farmer 1990). Changing of flora to *Sphagnum* in riverbeds has been observed in progress of acidification. Benthic filamentous algae (mainly *Zygnema, Zygogonium, Mougeotia)* increase their standing crops at pH less than 5. Characeae disappears clearly in acidified water below about pH 5.

8.1.3. Aluminum toxicity

Inorganic monomeric Al (the sum of aquo, hydroxyl and inorganically complexed forms) in total Al is highly toxic to aquatic organisms. Al is released to streams or lakes from soils of acidified watersheds or lake sediments depending on pH (Fig. 8.5). Inorganic Al concentrations (Al_i in Fig.8.5) increase generally as pH decreases exponentially below 5.5 except case of high concentrations of DOC or organic acids.

Though the threshold concentrations of inorganic Al harmful to aquatic organisms differ from taxa and chemical condition especially Ca concentration, pH of 5.5 (or 6.0) and an inorganic Al of 2.0 μmol L^{-1} is critical levels for general aquatic ecosystem ((Baldigo et al. 2009)

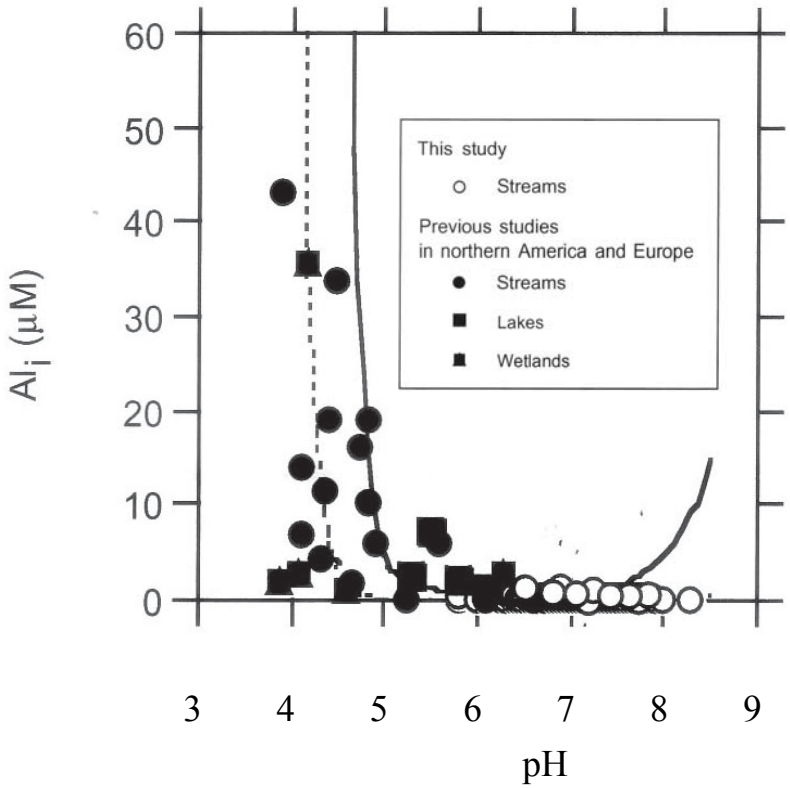

Fig. 8.5. Variations of Al_i concentration as a function of pH in surface water in Japan, America and Europe (Koshikawa et al. 2007). The solid line represents theoretical solubility of amorphous $Al(OH)_3$ and the dotted line theoretical solubility of gibbsite.

8.1.4. Criterion of pH value changing the aquatic organisms and ecosystem

We cannot indicate strictly “a critical pH value” affecting faunal and floral changes and influencing overall aquatic ecosystem by progressing acidification, because the effects are depending the specific for species, their life cycle stages, physiological conditions of each species, the food-web structures by constitutes etc., and also water chemicals affect largely on acidic condition or toxicity for organisms with their complex combinations.

But we may present a pH of 6.0 and 6.5 as “apparent level” and “alert level” for biological changes by acidification under the considerations of many papers concerning the pH-sensitivity of aquatic organisms, such as: “a majority of field and laboratory studies show that aquatic insects and crustaceans are relatively tolerant to acidic conditions until pHs fall below 6.0. ….unaffected above pH 6.4 slightly impacted at

pHs 5.7-6.4 (Baldigo et al. 2009), in Sweden Fig. 8.4 is indicating the start of damage at pH less than 6.5, in China pH 6.5 is critical level for fishes (Li & Tang 1998), pH 5.7 in Northern Britain and Europe for impoverished stream fauna (Haworth 1990), 6.0 in Canada and USA (Howells, 1990). Holt and Yan (2003) recommended pH 6 as a biological recovery goal based on the studies of crustacean zooplankton in Killarney Park, Ontario.

In a Japanese naturally acidified river receiving the volcanic effects with unacidified tributaries (ranging pHs 2.7-6.9), density and compositions of macroinvertebrates changed drastically at boundary of pH about 6 (Fig. 8.6). The density (also biomass), especially of ephemeropterans, decreased below pH 6 and plecopterans including only two species predominated at acidic pH range.

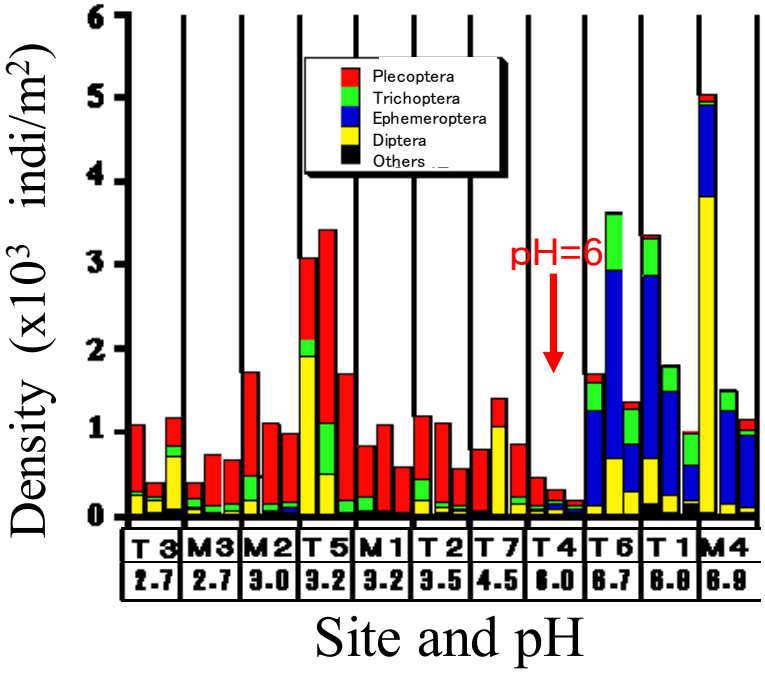

Fig. 8.6. The density of macroinvertebrates along pH gradient in naturally acidified rivers with unacidified tributaries in Fukushika district (Fukuhara et al. unpublished data). Samplings were done at May, July and September (from left column)

We will recommend that biological monitoring in surface water should be started at pHs

6.5 - <7 levels before no or little effects on aquatic life.

8.1.5. Future research direction

(1) Taxonomical studies and a list up of indicator species in EANET countries

Since aquatic organisms are inhabiting at geographically characteristic habitats except cosmopolitan species, basic taxonomical studies of phytoplankton including crysophytes (Chrysophyceae) and diatoms (Bacillariophyceae), zooplankton including cladocerans (Cladocera) and chydorids (Chydoridae), benthic invertebrates and fishes to make their inventories are important in each country or climatological units. Fortunately, numerous researches conducted at ICP-Water areas on tolerance or sensitivity of aquatic organisms to surface water acidification have been revealed many representatives in species or genus levels. Referring to this information, species level identification and determination of sensitive organisms to acidity is necessary in each area with making inventory data.

(2) The diatom inferred pH

Working group for diatom indicator (2000) reported, (1) diatoms are an useful indicator group for monitoring lake acidification, (2) planktonic and attached diatoms are effective for monitoring recent acidification of lake water, whereas diatom samples from sediment are effective for estimating long-term acidification, (3) for determining a diatom inferred pH equation effective to pH 5-7, at least 30 set data (observed pH : Index B) should be available with Hustedt's classification, (4) need of more taxonomic studies and a key with photographs (a catalog book) to identify species, (5) preparation of an equation to be adopted to Japan or east Asia district.

A list by Yoshikawa et al. (2000) and Fig. 8.2 in this section will provide information to categorize diatoms. Note that common species in diatoms are largely distributed in East Asia.

Recently, Watanabe (2005) published a picture book of freshwater diatoms containinng about 1500 taxa from Japan, East Asia, Europe, America and Africa, describing pH

tolerance to each taxon based on his original five classes.

The top-bottom method is useful to know the information about recent acidification in lakes. Two sets of diatom floral composition from surficial sediment (top) and baseline or pre-industrial depth bottom sediments should be compared to know snapshot picture of environmental change (Wilkinson et al. 1999).

(3) New index for acidification process

Other than Acidity Index for benthic invertebrates, some indices, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness, acid BAP (Biological Assessment Profile) Index, PMR (Percent Mayfly Richness), ATI (Acid Tolerant) Index, are presented for estimation of acidification process (Baldigo et al. 2009). Other indices, BMWP (Biological Monitoring Working Party Biotic Score) Index, EPT/(EPT+C,C=Chironomidae), ASPT (BMWP average score per taxon) are also compared for the assessment of the input of AMD (Acid Mine Drainage) (Gray and Delaney 2008). Baldigo et al. (2009) showed the new index, acid BAP derived from PMR and ATI, was strongly correlated with concentrations of inorganic Al, pH, ANC, and base cation surplus (BCS) in the researches on the streams of the western Adirondack Mountains.

Schneider and Lindstrom (2009) presented a new index, AIP (acidification index periphyton), based on non-diatomaceous benthic algae,. They say that the AIP is most sensitive between mean annual pH values of approximately 5.5 and 7.0 and can be especially useful in detecting the first signs of an acidification trend.

These indices will become also useful in future at EANET areas, although region-specific basic data-sets of inventory are inevitably necessary to adopt.

(Haruo Fukuhara)

8.2. Catchment/watershed-scale analysis

1) Inland water as the output of biogeochemical processes

Inland water can be considered as the output of biogeochemical processes in the watershed/catchment (see Fig. 1 of the chapter 1). Rainwater precipitated within the catchment area flows into the stream through hydrological processes in the catchment, although a part of the water returns to the atmosphere by evapotranspiration or retains in plant bodies or soil layers for a while.

Dissolved materials/ions are transported with the water flow in the catchment through biogeochemical processes, such as plant uptake, microbial consumption/transformation, cation/anion exchange on clay mineral surface, and mineral weathering. Ion constituents of wet and dry depositions are also applied in the biogeochemical processes. Therefore, water chemistry of streams (and lakes) must reflect such processes. Effects of acid deposition on inland water should be evaluated taking the biogeochemical processes on the watershed/catchment-scale into account. At least, basic information including geology, soil, and vegetation type should always be taken into account for evaluation of inland water.

2) Qualitative and quantitative evaluation of effects

Terrestrial ecosystems consist of many components, including atmosphere, plant, soil, and stream. So far, most monitoring activities on acid deposition have been promoted for each component independently, namely monitoring on wet deposition, dry deposition, soil and vegetation, and inland aquatic environment. This approach may be useful to accumulate baseline data for spatial and temporal trend analysis for each component. However, precise discussion on relationship between components may need more integrated approach for both qualitative and quantitative evaluation.

Therefore, integrated monitoring including atmospheric deposition, soil, vegetation, and inland water, is required to evaluate effects of acid deposition on ecosystems qualitatively and quantitatively. Catchment-scale analysis may be one of solutions for this requirement. The integrated approach taking biogeochemical processes into account

should be promoted in a catchment scale.

The catchment-scale monitoring may allow us to discuss relationship between seasonal or annual changes in stream water chemistry (concentrations or material/elemental fluxes) and those in atmospheric deposition. Material/elemental input-output budget in the catchment can be calculated based on the atmospheric deposition chemistry, stream water chemistry, and water balance. Meteorological data and the data on biogeochemical processes may be helpful for a comprehensive analysis to understand relationship between stream water chemistry and atmospheric deposition. For example, effects of excess N depositions on ecosystems (so-called N saturation) should be evaluated basically in the catchment scale. Moreover, catchment-scale biogeochemical model may allow evaluation of the current status and future projection of soil and river water chemistry and material/elemental cycles.

3) Promotion of catchment-scale analysis in East Asia

The methodologies of catchment-scale biogeochemical analysis have been developed firstly by the long-term monitoring in Hubbard Brook Experimental Forest, New Hampshire, U.S. (Likens & Bormann, 1994). Then, similar programs and research activities have been promoted in Europe (e.g. by ICP Integrated Monitoring, UNECE), Japan (by several institutes and universities), and China (e.g. IMPACTS). However, in East Asia, catchment-scale analysis has not been enough promoted in other countries than Japan and China.

Strategy Paper on Future Direction of Soil and Vegetation Monitoring of EANET, which was adopted at 2nd Session of the Scientific Advisory Committee of EANET in 2002, suggested promoting case studies of the catchment-scale analysis to develop monitoring methodologies applicable to the East Asian region. The case studies have been implemented by NC and scientists in the EANET countries in the Sakaerat Silvicultural Research Station (SRS) site in Thailand, the Danum Valley Conservation Area (CA) site in Malaysia, and the Kajikawa study site in Japan. Moreover, the regular catchment-scale monitoring has just started in the Lake Ijira catchment, Japan, where

acidification of the catchment area was suggested. In fact, acidification mechanisms in the Lake Ijira catchment are being clarified gradually by the catchment-scale analysis (Nakahara et al., 2010).

The guideline for catchment-scale monitoring, which was developed based on experience through the case studies above in the East Asian region, was endorsed by Scientific Advisory Committee of EANET at its Tenth Session (SAC10). It is expected that catchment-scale monitoring will be promoted in the participating countries. Moreover, development of the catchment-scale biogeochemical model is highly expected for future projection of impacts on terrestrial ecosystems and inland water.

8.3. Research needs

Based on a preliminary audit of the document, it should be noted that developed and now used Technical Manual for Inland Aquatic Environment Monitoring adequately reflects all the works carried out when studying the surface waters in the frame of EANET, though it requires some refinement, in particular, as concerns the methods used for analysis. In order to optimize studies and direct them into the practice, besides the annual reports, it would be useful to conduct every 4-5 years **the Surface Water Workshop** to discuss summarized results obtained, which show the state of the water ecosystems chosen for investigation in the frame of the EANET.

The analysis should include the influence of anthropogenic factors, in particular for the flux of acidifying components on the drainage area, examination of bottom sediment cores, and variations in the species of phytoplankton populations. Based on the results obtained, the managers must give suggestions for further researches. If there are high acid loads in the watershed and, at the same time, the aquatic ecosystems are highly stable to these loads (other words, there are high buffering capacity and stabile chemical composition of the water masses), it may be recommended to choose a new study object (lake, river) whose ecosystem is more sensitive to acid loads.

So far, actual effects of acid deposition on aquatic organisms have not enough been studied in the EANET countries. In particular for the issue, the following topics can be considered:

- To study indicator species for preparation of the method for biological monitoring: Taxonomical studies of aquatic organisms, especially diatom and aquatic insects, in each EANET country for list up of tolerant or sensitive species for acidification.
- To study toxic mechanisms of Al or H^+ on aquatic organisms (eg. physiological activities, activity of enzyme, etc.)

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Appendix 1. Acid neutralizing capacity of soil

Acid input into soils is neutralized by 1) carbonate and bicarbonate in soil and/or saprolite, 2)exchangeable basic cations in soil, 3） weathering of secondary minerals (clay minerals) and primary minerals forming rocks.

Most of the acids loaded from atmosphere are sulfuric acid, nitric acid and hydrochloric acid. Ammonium salts input to the soils are converted eventually into nitric acid. They are all strong acids, which dissociate almost completely in water. For instance, when nitric acid is added to soils containing calcium carbonate, the following reactions proceed.

$$CaCO_3 + H^+ + NO_3^- = Ca^{2+} + NO_3^- + HCO_3^-$$

and

$$CaCO_3 + 2H^+ + 2NO_3^- = Ca^{2+} + 2NO_3^- + H_2O + CO_2$$

Since these reactions proceed almost quantitatively, acids input into soil are neutralized as long as calcium carbonate exists in soil and/or saprolite. However calcium carbonate is consumed for neutralizing acids, and neutralizing capacity of the soil/saprolite declines.

If all carbonate is consumed, soil pH drops rapidly to around 5 and next step of neutralization will start. Neutralizing reactions in this pH range (<pH 5) involve cation exchange reactions and dissolving reaction of minerals in soil and/or saprolite containing alkaline and alkaline earth metals. The negative charge responsible to the cation exchange in soil are derived from 1) isomorphous substitution in clay mineral, 2) dissociation of proton from OH on the broken edge of clay minerals, and 3) dissociation of proton from COOH and phenolic OH contained in so-called humus. Thus capacity of absorption and adsorption of cations is dependent on quality and quantity of clay minerals and soil humus, and the proportion of basic cations occupying the exchange sites is called base saturation (%). When H^+ is added to the soil solution, it exchange for those cations, mainly of Ca, Mg and K, on the exchange sites of clay minerals and organic matter.

$$Ca\text{-}Clay + 2H^+ + 2NO_3^- = 2H\text{-}Clay + Ca^{2+} + 2NO_3^-$$

The H^+ absorbed on clay intrudes into clay mineral structure, and aluminum ion

dissolved from the inside structure is absorbed in the exchange site as an exchangeable cation.

Another acid neutralizing process is by the weathering of rock forming primary minerals. In this reaction acid neutralizing capacity is equal to the amounts of alkaline and alkaline earth minerals contained in the minerals. An example of the reactions is weathering of olivine. Dissolution of olivine by nitric acid proceeds in the following formula and magnesium nitrate and monosilicic acid are produced.

$$MgSiO_3 + 2H^+ + 2NO_3^- + H_2O = Mg^{2+} + 2NO_3^- + H_4SiO_4$$

In this reaction silicic ion accepts protons and metal ion is released. Therefore acid neutralizing capacity of the silicate minerals is equal to the amount of silicic ion, and also to the amounts of alkaline and alkaline earth metals contained.

After most of the exchange sites are occupied by H^+ or Al^{3+} or the amount of weatherable minerals declines, next buffering range begins to appear due to dissolution of alumino-silicate such as kaolinite.

$$Al_2Si_2O_5(OH)_4 + 6H^+ + 6NO_3^- = 2Al^{3+} + 6NO_3^- + 2H_4SiO_4 + H_2O$$

Aluminum hydroxides also function in the same manner.

$$Al(OH)_3 + 3H^+ + 3NO_3^- = Al^{3+} + 3NO_3^- + 3H_2O$$

In the buffering range where alumino-silicate minerals start to dissolve, however, soil pH is maintained at an unfavorably low level to the growth of plants and microorganisms and concentration of aluminum ion, which is toxic to plant, becomes high. Water percolated from the soil has similar characteristics.

As mentioned above magnitude of acid neutralizing capacity in the pH range favorable to plants and microbes depends on the amounts of carbonate, weatherable minerals and exchangeable basic cations. Consequently different soils and geologies develop different magnitudes of neutralization capacity. Ones of the most common carbonate minerals are calcite ($CaCO_3$) of limestone and dolomite ($CaMg(CO_3)_2$) of dolostone. Those geological regions have very high Acid neutralizing capacity, and the stream

water exhibit high pH. Although acidic soils mainly under humid climate contain little carbonate, soils develop under arid to semi-arid climate such as Calciosols, Chernozems, Kastanozems, and Gypsisols (FAO 1988) contain secondary precipitated calcium carbonate, and neutralize acids effectively. The more alkaline and alkaline earth metals are contained in the rock forming minerals, the higher the acid neutralizing capacity becomes in saprolite layers. Therefore, silicic rocks such as granite, rhyolite, and dacite have lower acid neutralizing capacity, meanwhile rocks as gabbro, basalt and ultrabasic rocks (peridotite and serpentinite) rich in colored minerals are high in neutralizing capacity. Andesyte, diolite and porphyriteare are the intermediates.

Soils also contains weatherable primary minerals such as feldspar, feldspathoids, ferromagunesian minerals, glass, micas, zeolites and apatite derived from the parent materials. Their amounts differ depending on the inherent contents of the minerals of the parent materials from which the soils are derived, and on the degree of their loss during weathering processes. The lattice clay minerals such as smectite and vrmiculite found in soils as secondary minerals also contain base elements in the crystal structure by isomorphous substitution, and acids are neutralized when those base elements are hydrolyzed. Meanwhile 1:1 lattice clays as kaolinite, which are formed in the more advanced weathering stages than 2:1 lattice clays or from more silicic parent materials, have lower neutralizing capacities because of the lower contents of base elements.

The 2:1 lattice clay minerals are formed in the temperate in general, and they are subjected to leaching under humid tropical climate to form 1:1 lattice clay minerals and oxides of iron and aluminum. Even in the temperate, however, when 1:1 lattice clay formed from silicic parent materials is dominant, soils have lower neutralizing capacity. Leaching of base elements from soils is inhibited and the base saturation becomes higher under the drier climate, which results in higher acid neutralization capacity. As a whole, acid neutralizing capacity is higher in the drier temperate, and lower in the humid tropics.

The soils as Ferralsols and Acrisols developed as the result of strong weathering under humid and hot environments contain little weatherable minerals, 1:1 lattice clay is dominant clay mineral, and soil organic contents are maintained low due to rapid decomposition. Thus those soils are characterized by low CEC (cation exchange capacity) and base saturation, and the neutralization is solely due to aluminum dissolution in the strongly acid pH range. However, the soils such as Lixisols developed

in monsoon tropics accompanied with pronounced dry seasons are comparatively higher in neutralization because of higher base saturation, though the CEC are low. The young soils mainly in humid temperate as Cambisols have wide range of characteristics, and show wide variations in neutralization depending on the parent materials and magnitude of leaching of bases. But in general the Cambisols are more acid and less saturated in bases than are Luvisols, which are characterized by high CEC due to 2:1 lattice clay and high base saturation.

Reference

FAO 1988; World Reference Base for Soil Resources. World Soil Resources Report 84, FAO, Rome

Appendix 2. River system in tropical areas

For the explanation on river systems in tropical regions, there is not so much difference between the system in tropical areas, except on the pollution level, water quality and water discharge. Factors in tropical areas are to be paid attention to are such:

1. River characteristics

a. Lower order of selected river

b. Unpolluted river condition

c. River located in conservation area. This is important so that river is not polluted in future.

d. River should not be located in peat land.

e. River should not be closely located to active volcano.

f. Upstream area not exploited for sand and rock mining.

2. Characteristics of River Catchment

a. Area of river catchment should not be too large.

b. Geologically, rock characteristics should not be too acid and basic.

c. If river is not located in the conservation area, upstream part of the catchment area is not allowed to be used for human activities which can impact to the quality of flow in downstream.

d. Soil in the catchment area should not be strongly acid and basic.

3. Characteristics of Water Quality and Quantity in monitored location

a. pH less than 7.5, and more than 6

b. EC less than 10 mS m^{-1}

c. Suspended solid less than 40 mg L^{-1}

d. BOD less than 2 mg L^{-1}, and COD less than 6 mg L^{-1}

e. River bottom should be visible.

f. Difference between river discharge in the wet season and dry season less than 30.

4. Factors to be considered during water sampling implementation

a. Recording of river discharge and water level in wet, dry and transition season

b. Sampling time should be the regularly conducted.

c. Water sampling is not to be done too early in the morning or too late in the afternoon.

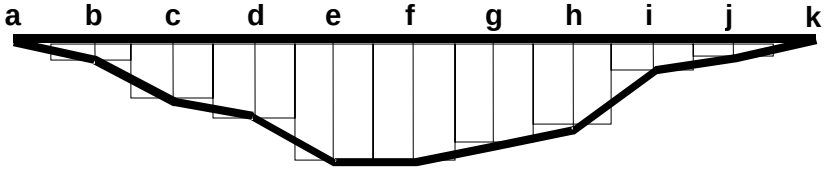
d. Water sampling is to be conducted 12 times per year.

e. Sampling location is to be situated center of flow.

Appendix 3. Measurement of water discharge (an example)

1. Equipments
   - Current meter
   - Tape measure (- 1-meter ruler)

2. Divide into several sections along horizontal direction.



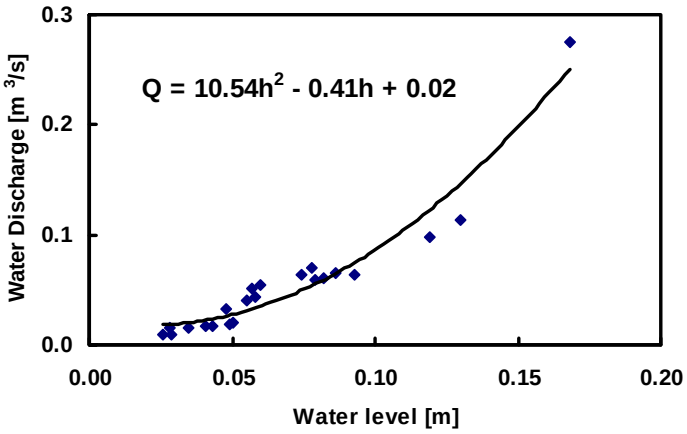
App-Fig.1.1. Cross-sectional map of river flow

This method is the summation of the products of the subsection areas of the stream cross-section and their respective average velocities.

3. Measure water level and velocity by using tape measure and current meter, respectively, in each section.
   - Water velocity is measured in 60% of water depth.
   - Water velocity is measured in 20% and 80% of water depth.

4. Sum up water discharge in each section.
   - Discharge is usually expressed in cubic meters per second, that is [$m^3\ s^{-1}$].

Continuous water discharge (Q) is estimated based on water level and water discharge.



App-Fig. 1.2. Estimated curve based on water level and water discharge (an example)

Appendix 4. Definition and meaning of the parameters

Parameters	Definition	Meaning
pH (power of Hydrogen exponent)	Cologarithm of hydrogen ion concentration in water	An indicator of acidic, neutral and alkali states in water.
Electric Conductivity (EC)	Reciprocal of electric resistance in water	An indicator of inorganic ion concentration in water. A value of EC is generally low at less concentration of inorganic ions in water.
Alkalinity	Contents of basic elements in water such as Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}	An indicator of ability for neutralizing acidity in water, especially by soil, rock and plants in the watershed
Major cations: NH_4^{+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}	Concentrations of the ions in water	Indicators required to estimate the ion balance, to calculating EC and to know inorganic nutrient salts in water
Major anions: SO_4^{2-}, NO_3^{-}, Cl^{-}	Concentrations of the ions in water	Indicators required to estimate the ion balance, to calculating EC and to know inorganic nutrient salts in water
Dissolved organic carbon (DOC) or Total organic carbon (TOC)	DOC: Dissolved organic carbon content in water TOC: Particulate organic carbon and DOC content in water	An indicator of organic pollution in water
Other anions: NO_2^{-} and PO_4^{3-}	Concentrations of the ions in water	Indicators required to estimate the ion balance, to calculating EC and to know inorganic nutrient salts and trophic status in water
Chlorophyll a	Chlorophyll a content in water, green-color pigment of photosynthetic plants and algae (phytoplankton and periphyton (attached algae))	An indicator of algal biomass in water, such as a trophic state
Total P(Total Phosphorous)	Dissolved and particulate phosphorus content as a nutrient	An indicator of trophic state in water

	in water	
Total N (Total Nitrogen)	Dissolved and particulate nitrogen content as a nutrient in water	An indicator of trophic state in water
Suspended solids (SS)	Particulate matter contet in water; composed of soil particle, microorganisms and detritus	An indicator of water condition, such as turbid water, eutrophication, sedimentation, etc.
Total dissolved Al	Dissolved aluminum content in water	An indicator of harmful to organisms. Aluminum becomes leached out after alkali metals were leached from soil layer and sediment.
Reactive Al	Reactive aluminum content in the dissolved Al: sum of aquo and hydroxy Al (Al_{aq}), inorganically complexed Al (Al_{ic}) and organically complexed Al (Al_o) by the lumogallion method	An indicator of toxic Al; the reactive Al should be measured if total dissolved Al > 200 μg L^{-1} in the water.
Chemical oxygen demand (COD)	Oxygen content required for decomposing organic matter in water	An indicator of organic pollution in water
DO (Dissolved Oxygen)	Dissolved oxygen content in water	An indicator of aerobic or anaerobic condition in water
Pb or Pb-210	Lead content in sediment	An indicator for estimating the accumulation date of core sample of lake sediment to separate strata by relating the existence time to diatom in the strata.
Stable isotope of S	Ratio of ^{32}S : ^{33}S : ^{34}S : ^{36}S in sediment	An indicator for relating the lake sediment to the origins of acidifying matter

Appendix 5. Comparison data between the pH 4.8 methods and Gran's Plot titration method for alkalinity

The fixed endpoint titration with an end point of pH = 4.8 has an innate error. When 1 litter of pure water whose pH is 7 is titrated with acid to an end point of pH = 4.8, 15.7μeq of acid is required to decrease pH as shown in the following equation.

$$10^{-4.8} - 10^{-7.0} = 15.7 \times 10^{-6} \text{ (mole)} \qquad (8)$$

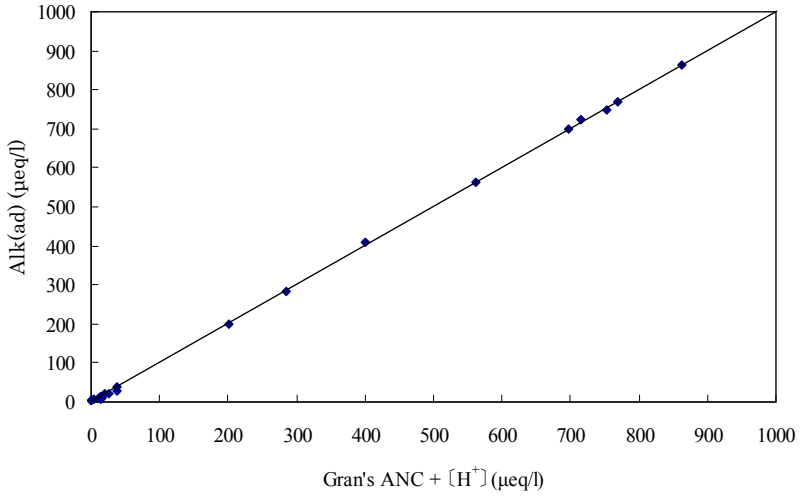
This is equivalent to 15.7 μeq L^{-1} of alkalinity; however, since pure water should have an alkalinity of 0, the alkalinity measured by this method is overestimated.

For the surface water which has plenty of alkalinity, typically more than 200 μeq L^{-1}, this error is negligible, but it becomes significant for acidic surface water such that the alkalinity is less than 50 μeq L^{-1}. Therefore, the data obtained by the pH 4.8 method for acidic surface water should be adjusted by using equation (9).

$$Alk_{(ad)} = Alk - (10^{-4.8} - 10^{-pH}) \qquad (9)$$

where $Alk_{(ad)}$ is an adjusted alkalinity, and pH is the initial pH of the sample.

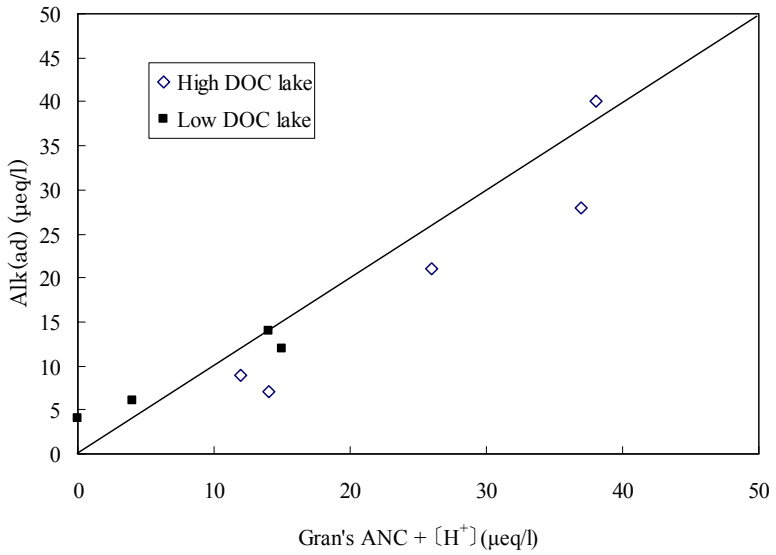
App-Fig. 5.1 shows the comparison between $Alk_{(ad)}$ and Gran's ANC of 19 lakes in Japan. As discussed in the main text, since alkalinity is equivalent to (ANC + $[H^+]$), the x-axis of the figure is expressed in (ANC + $[H^+]$). $Alk_{(ad)}$ and Gran's ANC + $[H^+]$ were identical in the region of high ANC. However, some ANC + $[H^+]$ data disagreed with $Alk_{(ad)}$ in the low ANC region as shown in Fig. App-Fig. 5.2.



App-Fig.5.1. Comparison between Alk(ad) and Gran's ANC of 19 lakes in Japan

App-Fig. 5.2 also shows the effect of DOC on Gran's ANC for the lakes with low ANC. All of the high DOC lakes plotted in App-Fig. 5.2 as open squares are classified into dystrophic lake and contain more than 3mg L^{-1} of DOC, while low DOC lakes are oligotrophic. Gran's ANC gives higher value for the high DOC water than that from the pH 4.8 method except one case.

Gran's plot titration should be applied to surface water with a DOC concentration less than 3 mg L^{-1}.



App-Fig.5.2. The effect of DOC on Gran's ANC