04 CONCEPTS OF SAMPLING, STORAGE AND ANALYSIS: SEAWATER AND SEDIMENT

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Introduction

The hydrological cycle is the process driven by the energy of the sun in which water travels from the Earth's surface to the atmosphere and back to the ground. About 97% of the Earth's water is contained in the oceans. And the rest of it is in the form of ice sheets and glaciers. Hence, it is very important to preserve the quality of water in this water cycle. Water enters the atmosphere as water vapour through evaporation, transpiration and sublimation. Water vapour high in the atmosphere forms into clouds through condensation. Water eventually returns to earth through precipitation as rain, snow, sleet and hail. When precipitation occurs over land, some water seeps into the ground as groundwater, a small amount is taken up by plants and animals, and the rest will return to rivers and streams as surface run-off to begin its journey back to the oceans.

The hydrological cycle involves the following processes and their flow path determines the ocean water quality

Surface runoff is the precipitation that falls on land (Fig. 1) and flows downhill towards stream channels which join rivers and eventually reach the oceans. Only about one third of precipitation falling on land will return to rivers and oceans. The rest will be soaked into the soil as groundwater, evaporated or transpired. Some of the water from precipitation will soak into the soil and rocks as groundwater. A varying proportion of groundwater stays in the shallow soil layer, and will move slowly towards streams and rivers. When groundwater soaks deeper into the soil it refills the underground aquifers, where it can stay for long periods of time or be used by humans through drilling wells into aquifers. Melting, freezing, boiling, evaporating and condensing are always reversible changes and can be reversed by heating or cooling but the water quality depends on the how well the ecosystem is protected from anthropogenic sources of contamination.

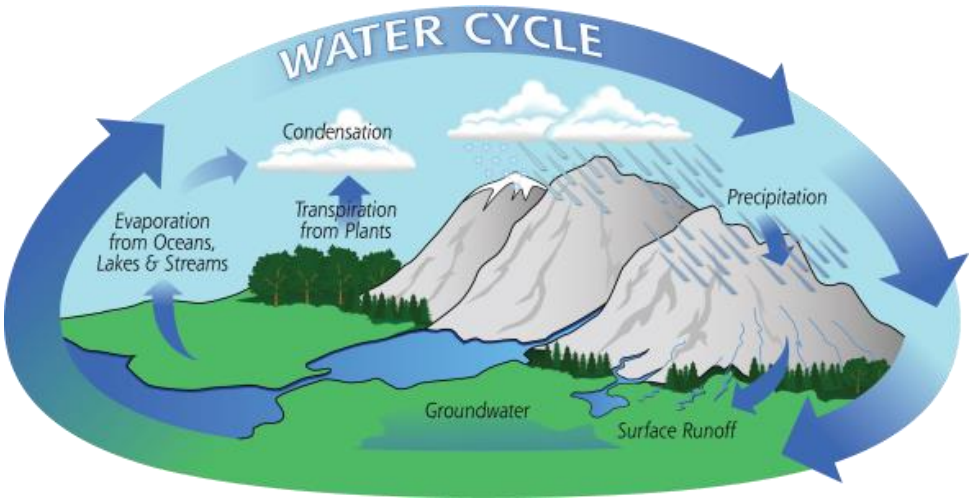

Fig. 1 Schematic diagram of the water cycle

Site selection for sea sampling

Factors to be considered

In order to fix the sites for continuous monitoring of the water quality a general idea of the area will aid in better analysis of the data collected. Details like length of the coastline, districts, drainage system or watershed of the place, major industries, ecological sensitive areas, places of special interest (e.g tourism, SEZ etc), major and minor ports and fishing activities. Budget available will also decide the frequency and the selection of parameters for analysis of sea water will depend on the hypothesis to be tested.

Seawater sampling methods

Physical characteristics of seawater viz. temperature, light penetration and pressure are assessed by making *in situ* measurements. For the estimation of most of the physicochemical parameters of seawater, different types of water samplers are used to draw samples from the specific depths. In oceanographic studies seawater samples are drawn from the following internationally defined 'standard depths'(Table1)

Table 1: Internationally defined standard depths

Serial number	Depth (m)	Serial number	Depth (m)
1	0 or Surface	18	900
2	10	19	1000
3	20	20	1100
4	30	21	1200
5	50	22	1300
6	75	23	1400
7	100	24	1500
8	125	25	1750
9	150	26	2000
10	200	27	2500
11	250	28	3000
12	300	29	4000
13	400	30	5000
14	500	31	6000
15	600	32	7000
16	700	33	8000
17	800	34	9000

Types of water samplers

Different types of water samplers are used for the collection of seawater ranging from shallow to deeper depths on board vessels.

Meyer's water sampler

It is the simplest sampler for the collection of water samples from any desired depth of shallow systems like the nearshore waters, estuaries and mangroves, where there is no appreciable hydrostatic pressure. Meyer's water sampler consists of an ordinary glass or Perspex bottle of about 1-2 l capacity and is enclosed with a rubber cork. It is weighted below with a lead weight and there are two strong nylon ropes, one tied to the neck of the bottle and other to the cork . While operating, the corked –up (closed) bottle is let down to the desired depth using the neck-rope) where the stopper is jerked open by a strong pull of the cork rope. The water flows into the bottle. When the bottle is full, which is known by the disappearance of air bubbles, the cork rope is released to keep the cork closed. Afterwards, using the neck rope, the bottle containing the water sample is taken out of the water column. This sampler is suitable up to 20m depth.

Friedinger's water sampler

This is a most commonly used water sampler for collection onboard vessels. It has a cylindrical portion made of plexiglass or Perspex and is with two hinged covers (Fig. 2). During operation, the sampler is sent down in an open state to the desired depth and can be closed by a drop weight messenger (a weight drilled out, so that, it will slide down the wire rope in order to remove or attach it, either hinged or slotted) which falls down inside a sliding rope and closes the covers and makes the bottle water-tight.

Fig. 2 Friedinger's water sampler

Nansen's reversing water sampler

A **Nansen bottle** is a device for obtaining samples of seawater at a specific depth It was designed in 1910 by the early 20th-century explorer and oceanographer Fridjof Nansen and further developed by Shale Niskin.The bottle, more precisely a metal or plastic cylinder, is lowered on a cable into the ocean and when it has reached the required depth, a brass weight called a "messenger" is dropped down the cable. When the weight reaches the bottle, the impact tips the bottle upside down and trips a spring-loaded valve at the end, trapping the water sample inside. The bottle and sample are then retrieved by hauling in the cable.

A second messenger can be arranged to be released by the inverting mechanism, and slide down the cable until it reaches another Nansen bottle. By fixing a sequence of bottles and messengers at intervals along the cable, a series of samples at increasing depth can be taken.

The sea temperature at the water sampling depth is recorded by means of a reversing thermometer fixed to the Nansen bottle. This is a mercury thermometer with a constriction in its capillary tube which, when the thermometer is inverted, causes the thread to break and trap the mercury, fixing the temperature reading. Since water pressure at depth will compress the thermometer walls and affect the indicated temperature, the thermometer is protected by a rigid enclosure. A non-protected thermometer is paired with the protected one, and comparison of the two temperature readings allows both temperature and pressure at the sampling point to be determined.

Handling of seawater samples

Different hydrographic parameters differ in their storage conditions and time limits, it will be useful to subdivide water sample into aliquots which are added in different containers each receiving appropriate storage treatment as given in the Table 2.

Table 2: Seawater storage and sample holding time

Parameter	Preservative	Sample Holding Time	Sample Container Size	Type of Container
Alkalinity	4°C	14 days	100 ml	Plastic or Glass
Ammonia	4°C, H_2SO_4 to pH < 2	28 days	100 ml	Plastic or Glass
Chloride	None	28 days	50 ml	Plastic or Glass
Chlorophyll a	4°C	12 hrs	500 ml	Plastic or Glass
Color	4°C	48 hours	50 ml	Plastic or Glass
Conductivity	4°C	28 days	100 ml	Plastic or Glass
Nitrate	4°C	48 hours	100 ml	Plastic or Glass
Nitrate-Nitrite	4°C, H_2SO_4 to pH < 2	28 days	100 ml	Plastic or Glass
Nitrite	4°C	48 hours	50 ml	Plastic or Glass
Odor	4°C	24 hours	200 ml	Glass
Orthophosphate	Filter immediately, 4°C	48 hours	50 ml	Plastic or Glass
Particulate Organic matter	4°C	12 hours	2 l	Glass
pH	None	Immediately	25 ml	Plastic or Glass
Silica	4°C	28 days	50 ml	Plastic
TDS	None	7 days	100 ml	Plastic or Glass
Temperature	None	Immediately	1 l	Plastic or Glass

In order to cover the range of parameters which need to be sampled and analysed a variety of sample containers are required as given below in Table 3. For collecting microbiological samples, bottles to be washed and sterilized. This can be carried out by placing them in an oven at 170°C for at least two hours.

Sample recording and labeling

Consequent to sampling, the sample bottles should be labeled and given a unique code number to identify the source and type of sample along with the date and time of sampling. A brief note on the local site conditions, such as weather, human activity on the banks, state of waterbody, etc., at the sampling site will be useful in analysis of data.

Table 3. Specification of quantity of sample and container material.

Parameter	Container
Pesticides and phenols	1000 ml glass (or Teflon) bottles with Teflon lined caps
Metals (except mercury)	500 ml polyethylene bottles
Mercury and phosphorus	100 ml glass bottles
All other chemical parameters	1000 ml polyethylene bottles
Microbiological analysis	Strong thick-walled glass bottles of at least 300 ml

Basic equipments needed for sampling and analysis

1. Physical parameters: CTD (conductivity, temperature and depth)

Thermosalinograph

It is a measuring instrument mounted near the water intake of ships to continuously measure sea surface temperature and conductivity while the ship is in motion. The thermosalinograph uses a conductivity cell to measure conductivity, which can then be translated into a value of salinity. Also a thermistor cell measures the temperature of the surface water, which when combined with the conductivity can be used calculate the density of the water and the sound velocity within it.

Multi-parameter probes

Presently multi-parameter probes are available for *in-situ* measurement using different sensors to measure various parameters especially a combination of physical chemical and biological parameters. The sensors need to be periodically calibrated to the site conditions for better accuracy in results. The parameters such as temperature, salinity,pH, dissolved oxygen, nutrients (N, P, Si), and biological parameters (chlorophyll a) can be obtained in single consolidated probes as shown in Fig.3

Fig.3 Multiparameter probe

2. Chemical parameters estimation in seawater the essential instruments

Precision weighing balance

Precision balances are a necessity in any laboratory. This will aid on displaying sample measurements on backlit LCD screen. Utilizing highly precise, gold plated ceramic capacitance sensors, these balances tare and stabilize quickly and provide reliable weight measurements of chemical required for standard preparations for chemical analysis of seawater within 0.0001 grams. The smaller capacity models (320g or 500 g) which include a glass draft shield with 3 sliding doors for easy access to the weighing chamber will be suitable.

Spectrophotometer

The spectrophotometer (fig. 4) is an essential instrument to determine the chemical parameters and pigments in seawater. This measures the amount of light that a sample absorbs. The spectrophotometer works by passing a light beam through a sample to measure the light intensity of a sample. These instruments are used in the process of measuring colour.

Fig.4 Spectrophotometer

Spectrophotometer instrumentation and principle

A spectrophotometer is made up of two instruments : a spectrometer and a photometer. The spectrometer is to produce light of any wavelength, while the photometer is to measure the intensity of light. The spectrophotometer is designed in a way that the liquid or a sample is placed between spectrometer and photometer. The photometer measures the amount of light that passes through the sample and delivers a voltage signal to the display. If the absorbing of light changes, the voltage signal also changes. Spectrophotometers come in a variety of shapes and sizes and have multipurpose uses to them. The different types of spectrophotometers available are all different from one another, based on their application and desired functionality. The most popular spectrophotometers are 45 degrees, sphere and multi-angle spectrophotometers.

The basic spectrophotometer instrument consists of a light source, a digital display, a monochromator, a wavelength sector to transmit a selected wavelength, a collimator for straight light beam transmission, photoelectric detector and a cuvette to place a sample.

The intensity of light is symbolized as l_0 measure the number of photons per second. When the light is passed through the blank solution, it does not absorb light and is symbolized as (l). Other important factors are Absorbance (A) and Transmittance (T).

$$T = l/l_0$$

$$A = -\log_{10} T$$

Here, we need to measure the intensity of light that passes a blank solution, and later measure the intensity of light passing a sample. Calculate the transmittance and the absorbance. The number of protons transmitted and absorbed totally depending on the length of the cuvette and the concentration of the sample.

The transmittance and absorption relation is:

$$\text{Absorbance } A = -\log_{10} T = -\log(T) = -\log(l_t/l_0)$$

The transmittance of an unknown sample can be calculated using the formula given below.

Transmittance (T) = l_t/l_0

Here,

l_t – Light intensity after passing via cuvette

l_0 -Light intensity before passing via cuvette

Further, there are several varieties of spectrophotometer devices such as UV Spectrometry, atomic emission spectrophotometry and atomic absorption spectrophotometry. It can also be classified into two types based on the range of light source wavelengths (fig.5) like IR spectrophotometer and UV-visible spectrophotometer Table 3 gives the wavelength range for estimation of various parameters in sea water by standard methods.

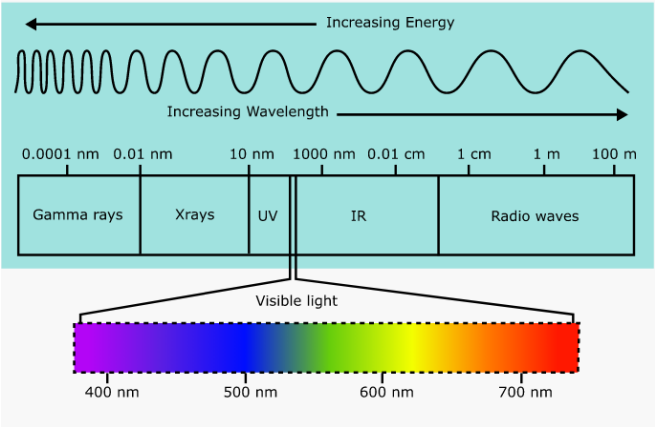

Fig. 5 Wavelength range for spectroscopy

Table 3: wavelength range for estimation of various parameters in sea water

Parameters	Wave length range in nm
Reactive phosphorous	885
Reactive silicate	810
Reactive Nitrite/Nitrate	543
Ammonia	640

3. Biological parameters:

Chlorophyll pigments

The rapid chemical method for estimating living plant matter in the particulate organic matter of sea water is to determine the characteristic plant pigments- the chlorophylls, carotenes and Xanthophylls. These are three crucial types of pigments found in plants and algae, responsible for absorbing light energy and playing vital roles in photosynthesis. Chlorophyll is found in all photosynthetic organisms, Carotenes and Xanthophylls are found in plants, algae, and some bacteria.

Chlorophyll: Green pigment the two main types are Chlorophyll a and Chlorophyll b
Absorbs blue and red light (430-450 nm and 650-700 nm) and Reflects green light (520-560 nm)

Carotenes:Yellow-orange pigments , Absorb blue-violet light (400-500 nm) and Reflect yellow-orange light (500-600 nm)

Xanthophylls: Yellow pigments Absorb blue-violet light (400-500 nm) and reflect yellow light (500-600 nm)

Analysis Methods: Spectrophotometry, Chromatography (HPLC, TLC)

The chlorophyll pigments a, b and c are seen in the marine phytoplankton. Of these the chlorophyll 'a' is important as it is used to represent the biomass of phytoplankton in the sea. Moreover, chlorophyll 'a' measurement is required to calculate assimilation number, i.e. the physiological index of the photosynthetic efficiency of phytoplankton.

Principle

Chlorophyll bearing organisms present in known volume of water sample is filtered and dissolved in a solvent (Acetone 90% v/v). The pigment content dissolved in unit volume of acetone is measured spectrophotometrically. Since on an average, primary production in the ocean bears a fairly constant relation to the chlorophyll content, measurement of these pigments is also used as an index of productivity.

Requirements

Glass fiber filter paper (GF/C),vacuum filtering unit (fig. 6), measuring jar, centrifuge and centrifuge tubes with caps

Fig. 6 Vaccum filtering unit

Sampling procedure and sample storage

Adequate sampling of the euphotic zone or detrital layers for phytoplankton is a subject which is outside the scope. Generally 500 ml – 5 L in volume sample is filtered through a small piece of clean 0.3 mm mesh nylon netting to remove the larger zooplankton. For open sea samples filtration of small volumes through a 0.15mm mesh net will still not retain significant amounts of phytoplankton.

Two or three drops (0.1 – 0.2 ml) of magnesium carbonate suspension is added. The sample may be stored in a cool dark place for a maximum of about 8hr. It is desirable, that samples may be filtered through a membrane filter at the time of collection.

Reagents

1. Distill reagent grade acetone over 1% of its weight of both anhydrous sodium carbonate and anhydrous sodium sulphite. Collect the fraction boiling at a constant temperature near 56.5°C (uncorrected). 100ml of water is pipette out into a volumetric flask and acetone added to make the volume to exactly 1000 ml. the redistilled acetone should be stored in a tightly stoppered dark bottle and the 90% reagent prepared in moderately small amounts (say 1 liter at a time) for use. This reagent is conveniently dispensed from a polythethylene wash bottle which should be kept nearly full. If good quality reagent acetone is available, it should be shaken with a little granular anhydrous sodium carbonate and decanted directly for use.
2. Magnesium Carbonate Suspension:
   Add approximately 1 g of finely powdered magnesium carbonate (light weight of Levis grade) of analytical reagent quality to 100 ml of distilled water in a stoppered Erlenmeyer flask. Shake vigorously to suspend the powder immediately before use.

Procedure

- Water samples collected for chlorophyll pigments must be passed through a coarse filter 0.2 mm mesh to remove zooplanktons. Thoroughly mix the sample and known volume (500ml) of the sample was filtered through a 47 mm dia Millipore AA filter or 4.5 cm Whatman GF/C glass filter paper.

Note

- If not added previously, introduce about 1 ml of magnesium carbonate suspension to the last few hundred milliliters of sample being filtered. The magnesium carbonate is added at this stage to ensure that the phytoplankton chlorophyll is prevented from becoming acid with the resulting decomposition to give phaeophytin pigments. If not added previously, introduce about 1 ml of magnesium carbonate suspension to the last

- Millipore filters have the advantage that they dissolve in acetone completely, give no complications at the centrifugation stage, and require no particular precautions during filtration. However, unless great care is taken, undesirably high blanks will occur when using Millipore filters, making the determination of small concentrations of carotenoids difficult. These filters are expensive. Glass filters are cheaper and their use results in practically no blank. They are recommended if a cell grinding step is required to give better extraction, although care must be taken when filtering samples through the comparatively glass filters and trouble is experienced at the centrifugation stage.

- Drain the filter thoroughly under suction before removing it from the filtration equipment and if a Millipore filter is used trim away the peripheral excess of the unstained membrane with clean scissors.
- Place the filter in a 15 ml stoppered centrifuge tube. If a Millipore filter was used add approximately 8 ml of 90% acetone, stopper the tube, and dissolve the filter by shaking the tube vigorously. If a glass filter paper was used add approximately 0 ml 90% acetone, stopper the tube, and disperse and disintegrate the paper by shaking the tube vigorously. Allow the pigments to be extracted by placing the tube in a refrigerator in complete darkness for about 20 hrs (It is good practice to shake the tubes vigorously once more after they have been 1 or 2 hr in the refrigerator).
- Remove the tubes from the refrigerator and let them warm up in the dark nearly to remove temperature. Add 90% acetone to make the extracts from the Millipore filters upto exactly 10 ml.
- The extract is centrifuged (4000 rpm) for 10 mts. Decant the clear supernant into a 10 mm path length cuvette and measure the extinction at the following wavelengths (750, 665, 645 and 630) against a cell containing 90% acetone. The amount of pigments in the sample is calculated using the revised formula of Jeffery and Humphrey (1975). Strickland and Parsons Equation

$$C \text{ (chlorophyll a)} = 11.6\, E_{665} - 1.31\, E_{645} - 0.14\, E_{630}$$

$$C \text{ (chlorophyll b)} = 20.7\, E_{645} - 4.34\, E_{665} - 4.42\, E_{630}$$

$$C \text{ (chlorophyll c)} = 55\, E_{630} - 4.64\, E_{665} - 16.3\, E_{645}$$

Where

- E stands for the absorbance at different wavelengths obtained above and corrected by the 750 nm reading.
- Chlorophyll a, b and c are the amounts of chlorophyll

- Calculate the concentration of pigments in sea water from the equation:

$$mg\ pigment/m^3 = \frac{C \times 10}{V}$$

Where C is a value of obtained from the Strickland and parsons equations and
V is the volume of seawater filtered in litres.

Sediment sampling and analysis

Factors to be considered for site selection

The shape of rivers changes through time as erosion, deposition and transportation of sediment occurs. Local base level changes due to landslides, damming of irrigation and drinking water purposes or hydroelectric power projects also lead to long term shifts in equilibrium of the landscape. The sediment load from a drainage basin depends on the prevailing slope and the characteristics of the channel which determine the velocity of the flowing water. More recently complex computer models are used to understand landscape evolution through a balance between tectonic uplift (mountain building) and denudation (erosion) processes. Hence site selection will depend on the hypothesis to be tested, drainage system or watershed of the place, ecological sensitive areas or habitats,places of special interest (e.g tourism, SEZ etc), major and minor ports and fishing activities. All season accessibility to the site determines the periodicity of sampling.

Sediment sampling

Sediment sample is collected from the sea / river using the grab (Fig.7). Sample should be representative of the area sampled. For this, collect samples from at least 4 sites in an area. Pool these samples in wide basin. The pooled sediment is mixed thoroughly. Then quartering is done, removing the opposite quarters and till sizeable quantity i.e., around 500 g sediment is obtained. Collect in heavy-duty plastic bags.

Fig. 7 Peterson grab used for sampling from the sea

Sediment processing

Do not store the sediment wet for more than 1 day. Wet samples should be kept under refrigeration if storage is needed for more than 1 day. Then air dry the sediment in shade in well ventilated places. To reduce the drying time, oven drying can be done at 50-60°C (Temperature should not be increased above 60°C, since it will cause loss of nutrients). Then pulverize the sediment gently by breaking clods using mortar and pestle. (Do not over grind the sediment so as to break the sand particles, which will cause errors in textural analysis). Then sediment samples can be stored in well labeled, capped plastic bottles or heavy-duty plastic bags. At this stage, it is ready for analysis. While storing, store in a dry place.

Sediment Analysis:

1. Physical parameters:
Grain size: sieving or sedimentation.
2. Chemical parameters:
Elemental analysis: AAS, ICP-MS, or chromatography.
Organic matter: loss-on-ignition or elemental analysis.
3. Biological parameters:
Benthic organisms: microscopy or DNA analysis.

-Sediment community structure: statistical analysis.

International Standards and Guidelines

1. International Oceanographic Commission (IOC).
2. National Oceanic and Atmospheric Administration (NOAA).
3. International Council for the Exploration of the Sea (ICES).
4. American Society for Testing and Materials (ASTM).

SUGGESTED READINGS

"Methods of Seawater Analysis" (Grasshoff et al., 1999)
"Standard Methods for the Examination of Water and Wastewater" (APHA, 2017)
"Sediment Sampling and Analysis" (National Research Council, 2001)

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